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The influence of the menstrual cycle on depressive symptoms and stress

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ABSTRACT

Background: The risk of developing a depressive disorder is twice as high for women as for men. This sex disparity emerges in puberty and ends at menopause, a time frame during which most women have a menstrual cycle. This suggests that the menstrual cycle and fluctuating ovarian hormones might contribute to the development of depressive disorders. In turn, ovarian hormones interact closely with one of the two major physiological stress regulators, the hypothalamus-pituitary-adrenal (HPA) -axis. Therefore, the interaction between all three entities - the menstrual cycle, depression, and stress markers – is close at hand and object of this dissertation.

Methods: Firstly, the relationship between the menstrual cycle and depressive symptoms is explored by investigating if and how depressive symptoms change across the menstrual cycle in participants with and without a depressive disorder (study 1) and whether menstrual cycle irregularity, specifically irregularity in length (study 2) and anovulation (study 3), interacts with depressive symptoms. This was investigated in multiple samples (adults and adolescents) through longitudinal, smartphone-based ambulatory assessments. Secondly, possible differences in biological stress marker concentrations between menstrual cycle phases were investigated. Thereby, two meta-analyses (study 4 and 5) compared cortisol as a marker for basal HPA axis activity (study 4) and its reactivity in response to acute stressors (study 5) between cycle phases. Lastly, perceived stress was investigated as a moderator of the association between ovarian hormone fluctuations and depressive symptom changes in a sample of peripubertal females using weekly measures of stress, depressive symptoms, and hormone concentrations (study 6).

Results: The results indicated that depressive symptoms fluctuate in some hormone sensitive individuals, with varying intensities and patterns (peri-menstrual or mid-cycle increase of symptoms, study 1). This cycle-related fluctuation differed between single depressive symptoms, highlighting the importance of a symptom-based approach (study 1-4).

Furthermore, higher depressive symptoms were observed in irregular menstrual cycles with respect to cycle length (study 2) and anovulation (study 3). When investigating differences in biological stress markers across the menstrual cycle, meta-analytic comparisons revealed small-sized effects of higher basal cortisol concentrations (study 5) and lower cortisol reactivity (study 6) in response to stressors in the follicular phase compared to the luteal phase. Finally, subjective stress moderated the direction of the association between hormone fluctuations and depressive symptoms. Specifically, in a high-stress context, hormone surges were linked to symptom increases whereas in a low-stress context, hormone withdrawals were linked to symptom increases.

Discussion: This dissertation presented a collection of interconnected studies that highlight that hormone fluctuations across the menstrual cycle are interconnected with stress and depressive symptoms. Possible biological explanations include a differential sensitivity of $GABA_A$-receptors to allopregnanolone, but this needs further investigations. Implications for future research involve the improvement of screening tools for individuals sensitive to hormone fluctuations and the development of treatment options for premenstrual exacerbation of depression. The generalizability of the results might be limited, as only one cycle per individual was investigated for relatively small samples in the primary studies. This was compensated by highly frequent assessments throughout the cycle and meta-analyses to increase power. Further strengths of this dissertation include a variety of investigated age groups, samples from multiple countries, and a multimethodological approach across the six studies, including primary studies using biological markers and psychological assessments, meta-analyses, and guidelines for future studies. Additionally, the studies were preregistered, analyses scripts were made openly accessible, and existing guidelines were adhered to.

In conclusion, it is essential to include the menstrual cycle and ovarian hormone fluctuations and their interconnection to stress in future research on depression. Identifying female-specific

risk factors of depression is crucial to provide individualized and more effective treatment options and to improve the overall understanding of stress-related mental disorders.

Kurzzusammenfassung (Abstract in deutscher Sprache)

Hintergrund: Das Risiko, eine depressive Störung zu entwickeln, ist für Frauen doppelt so hoch wie für Männer. Dieser Geschlechtsunterschied beginnt in der Pubertät und endet mit der Menopause und umfasst damit einem Zeitraum, in dem die meisten Frauen einen Menstruationszyklus haben. Dies deutet darauf hin, dass der Menstruationszyklus und fluktuierende Sexualhormone zur Entwicklung depressiver Störungen beitragen könnten. Sexualhormone wiederum stehen in enger Wechselwirkung mit einem der beiden wichtigsten physiologischen Stressregulatoren, der Hypothalamus-Hypophysen-Nebennieren-Achse (HHNA). Daher ist die Interaktion zwischen allen drei Einheiten (Menstruationszyklus, Depression und Stressmarkern) naheliegend und Gegenstand dieser Dissertation.

Methoden: Zunächst wurde die Interaktion zwischen Menstruationszyklus und depressiven Symptomen beleuchtet. Dabei wurde bei Teilnehmerinnen mit und ohne depressive Störung untersucht, ob und wie sich depressive Symptome über den Menstruationszyklus hinweg verändern (Studie 1). Zusammhängend wurde untersucht, ob Zyklusunregelmäßigkeiten mit depressiven Symptomen zusammenhängen, insbesondere Unregelmäßigkeiten bezüglich Zykluslänge (Studie 2) und Anovulation (Studie 3). Dies wurde in mehreren Stichproben (Erwachsene und Jugendliche) durch longitudinale, Smartphone-gestützte Ambulatory Assessment Studien untersucht. Darüber hinaus wurden mögliche Konzentrationsunterschiede von biologischer Stressmarkern zwischen Zyklusphasen untersucht. Dabei wurden in zwei Meta-Analysen (Studie 4 und 5) Cortisol als Marker für die basale Aktivität der HHNA (Studie 4) und deren Reaktivität auf akute Stressoren (Studie 5) zwischen den Zyklusphasen verglichen. Schließlich wurde subjektiver Stress als Moderator des

Zusammenhangs zwischen Sexualhormonschwankungen und Symptomschwankungen in einer Stichprobe peripubertärer Teilnehmerinnen untersucht. Dafür wurden wöchentlich Stress, depressive Symptome und Hormonkonzentrationen gemessen (Studie 6).

Ergebnisse: Die Ergebnisse deuten darauf hin, dass depressive Symptome bei einer Subgruppe von hormonsensitiven Personen im Menstruationszyklusverlauf schwanken. Hierbei gibt interindividuelle Unterschiede in Schwankungsintensität und Schwankungsmustern (Verstärkte Symptome perimenstruell oder in der Mitte des Zyklus, Studie 1). Diese zyklusbedingten Schwankungen unterschieden sich auch zwischen den einzelnen depressiven Symptomen, was die Bedeutung eines symptomspezifischen Ansatzes unterstreicht (Studie 1-3, 6). Darüber hinaus wurden bei unregelmäßigen Menstruationszyklen in Bezug auf Zykluslänge (Studie 2) und Anovulation (Studie 3) höhere depressive Symptome beobachtet. Bei der meta-analytischen Untersuchung von Phasenunterschieden bei biologischen Stressmarkern ergaben sich kleine Effekte, die höhere basale Cortisolkonzentrationen (Studie 4) und geringere Cortisolreaktivität (Studie 5) in der Follikelphase im Vergleich zur Lutealphase zeigen. Darüber hinaus moderierte subjektiver Stress die Richtung des Zusammenhangs zwischen Hormonschwankungen und depressiven Symptomen (Studie 6). Insbesondere wurden bei hohem Stress Hormonanstiege mit einer Symptomverschlechterung in Verbindung gebracht, während bei niedrigem Stress Hormonabfälle mit einer Symptomverschlechterung in Verbindung gebracht wurden.

Diskussion: In dieser Dissertation wurden sechs miteinander verbundener Studien vorgestellt, die zeigten, dass Hormonschwankungen während des Menstruationszyklus mit Stress und depressiven Symptomen zusammenhängen. Zu den möglichen biologischen Erklärungen dafür gehört eine unterschiedliche Sensitivität von $GABA_A$-Rezeptoren gegenüber des Progesteronmetabolits Allopregnanolon, was jedoch in zukünftigen Studien vertieft untersucht werden muss. Für zukünftige Forschung sind die Verbesserung von

Screening-Instrumenten für hormonsensitive Personen und die Entwicklung von Behandlungsmöglichkeiten für die prämenstruelle Exacerbation von Depressionen essentiell. Mögliche Limitationen der Generalisierbarkeit der Ergebnisse entstehen, da in den Primärstudien nur ein Zyklus pro Person bei relativ kleinen Stichproben untersucht wurde. Dies wurde durch sehr häufige Messzeitpunkte während des gesamten Zyklus und Meta-analysen zur Erhöhung der statistischen Power kompensiert. Zu den weiteren Stärken dieser Dissertation gehören die Vielfalt der untersuchten Altersgruppen, Stichproben aus mehreren Ländern und ein multimethodischer Ansatz in den sechs Studien, einschließlich Primärstudien mit biologischen Markern und psychologischen Erhebungen, Meta-Analysen und Leitlinien für künftige Studien. Darüber hinaus wurden die Studien präregistriert, die Analyseskripte offen zugänglich gemacht und bestehenden Leitlinien eingehalten.

Zusammenfassend lässt sich sagen, dass der Menstruationszyklus und die Schwankungen der Sexualhormone, sowie ihr Zusammenhang mit Stress, in zukünftiger Depressionsforschung unbedingt berücksichtigt werden müssen. Die Identifizierung frauenspezifischer Risikofaktoren für Depressionen ist von entscheidender Bedeutung für die Entwicklung individualisierter und wirksamerer Behandlungsmöglichkeiten und für ein verbessertes Verständnis stressbedingter psychischer Störungen.

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CHAPTER 1

1. Introduction

The menstrual cycle has played a role in many legends and myths since antiquity. The Greek philosopher Pythagoras believed that women[1] menstruated to excrete a nutrient overdose from eating too much. The Roman Gaius Secundus proclaimed that the blood of menstruation was toxic and that women were only able to survive because they became immune to it over the years. Such conceptions were common in the ancient world, the Middle Ages, and the Renaissance period (Künzel, 2004). Even into the 20th century, the so-called "toxin of menstruation", menotoxin, was still being examined by scientists, although it is no longer a relevant research topic due to a lack of evidence for its existence (Finn, 1996).

Over the last three decades, socio-cultural and political changes have gained significant momentum in addressing the menstrual cycle and related health issues (Critchley et al., 2020): Since the start of the 21st century, menstrual cycle-related topics in the public and global realm have focused on menstrual health and hygiene in adolescents, specifically those in low- and middle-income countries, with programs such as the "WASH (water, sanitation, hygiene) in schools" agenda or books devoted to menstrual hygiene management and education in puberty (Critchley et al., 2020). Moreover, high-income countries have more publicly targeted the issue of menstrual equity, defined as "the affordability, accessibility and safety of menstrual products" (womensvoices.org, 2023), through public campaigns. Such campaigns have focused public attention on removing taxes on sanitary products, e.g. in Germany (Heute im

[1] The terms "women" and "girls" are often incorrectly restricted to individuals with the female biological sex, while neglecting the female gender. Not all women with the female gender have the biological female sex and therefore menstruate (e.g. trans women). Conversely, some men or non-binary people have the female biological sex and menstruate. Therefore, menstruation is not exclusive to women and cannot be generalized to all women. In this dissertation, I address the subgroup of women who menstruate while acknowledging that some results of this thesis cannot be applied to women who do not menstruate, and that research is lacking for people who menstruate and are not women.

Bundestag, 2019), or on making them more easily available, for example for homeless people in the USA (Rep. Meng, 2022). In some countries, a focus on menstrual cycle-related health has resulted in regulations and laws. Most prominently, in February 2023, Spain became the first country in the world to approve paid menstrual leave of absence for women with debilitating period symptoms ("Press releases - Congreso de los Diputados," 2023). These political and socio-cultural changes have also yielded measurable economic effects, reflected, for instance, in the increasing number of period tracking apps, with at least 200 million downloads by 2016 (Earle et al., 2021), and increased interest in and use of menstrual cups ("Why are menstrual cups becoming more popular?," 2018) or absorbent period underwear (Kale, 2021).

In summary, cultural, sociological, and economic changes in the last decades demonstrate a growing interest in the menstrual cycle and related health issues among the general population.

CHAPTER 2

2. Theoretical background

2.1 Hormonal changes across the female lifespan

Paralleling the aforementioned socio-cultural and political changes, scientific investigations of the menstrual cycle are also growing in number. So far, fundamental biological research has led to a more thorough understanding of the critical role of the menstrual cycle in reproductive mental health, and how it evolves across the lifespan. Fluctuations in ovarian hormones have been investigated in females from menarche, i.e. the first menstrual cycle, to menopause, i.e. the last menstrual cycle, and examined in terms of their association with mental health.

The first menstrual cycle, menarche, occurs on average between the ages of 12 and 13 years in high-income countries such as Germany or the USA and slightly later in others (e.g. Haiti;15.37 years) (American Academy of Pediatrics et al., 2006). The menarche is preceded by the adrenarche – marked by increases in hypothalamic-pituitary-adrenal (HPA) axis activity – and the associated production of androgens. Subsequently, the hypothalamic-pituitary-gonadal (HPG) axis is activated (gonadarche), which stimulates a drastic rise in concentrations of ovarian hormones such as estrogens (i.e. estradiol, estrone), in turn stimulating the maturation of secondary sex characteristics (Biro et al., 2014). The first menstrual cycles are often irregular, with high variability in length (up to 61 days), low rates of ovulation (0-45%; Gunn et al., 2018) and low mean ovarian hormone concentrations (e.g. progesterone; Carlson and Shaw, 2019). It can take up to six years until a regular menstrual cycle is established (American Academy of Pediatrics et al., 2006).

2.1.1 Biological mechanisms of the underlying cycle

A regular menstrual cycle lasts on average 28 days, but deviations between 22 and 36 are in the normal range (Fehring et al., 2006). The HPG axis finely coordinates the menstrual cycle through ovarian hormones, specifically estradiol, progesterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Typically, the first day of the cycle is defined as the first day of menstrual bleeding. Starting on this first day, multiple follicles mature through exposure to LH and FSH, which is stimulated by gonadotrophin-releasing hormone (GnRH) in the hypothalamus. These growing follicles synthesize and distribute estradiol, which in turn inhibits LH and FSH and slows down the maturation of the follicles. Once one dominant follicle is fully matured, it is released into the fallopian tube, where it can be fertilized (ovulation). The remains of the dominant follicle in the ovary are transformed into the corpus luteum, which produces progesterone and estradiol to initiate several biological functions in preparation for a possible pregnancy. If no fertilization occurs, this is excreted along with the inner uterine wall – menstruation – and the cycle begins anew (Bale and Epperson, 2017; Schmalenberger et al., 2021; Siekmann, 2016) .

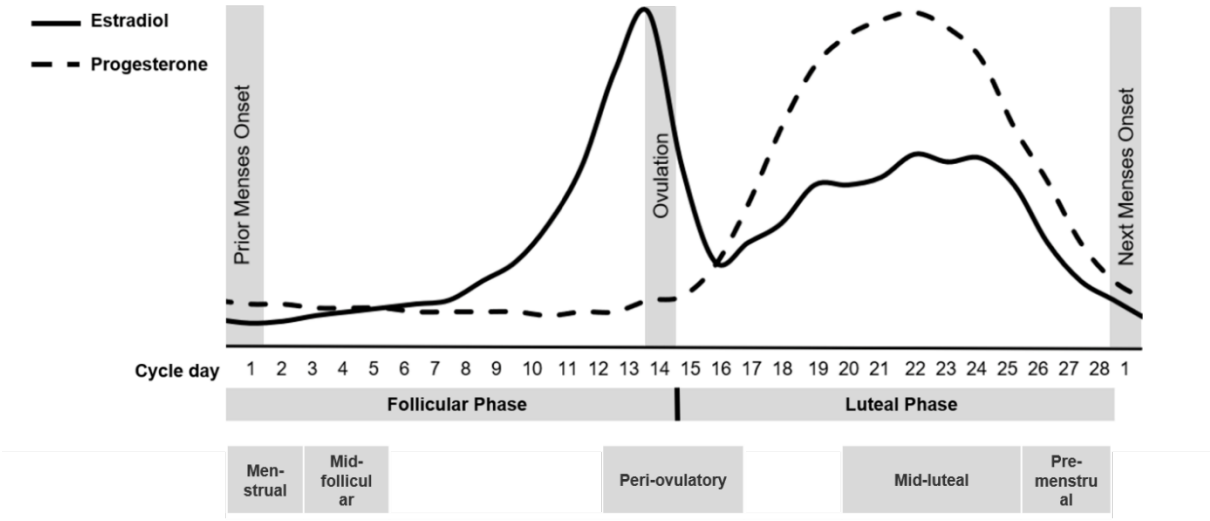
The menstrual cycle can be broadly divided into the follicular phase (days 1 to 14[2], starting at menstruation) and the luteal phase (days 15 to 28), but recent guidelines recommend a more precise and finer-grained distinction (Schmalenberger et al., 2021) in order to reflect hormone fluctuations within the two broader phases. In the mid-follicular phase (days 4 to 7[2]), estradiol and progesterone concentrations are low. In the periovulatory phase (4 days surrounding ovulation), a strong surge and subsequent decrease in estradiol concentrations occurs, while progesterone concentrations slowly increase. In the mid-luteal phase (days -9 to -5 before menstruation onset[2]), progesterone and estradiol concentrations are high. In the premenstrual phase (-3 days before menstruation onset), estradiol and progesterone concentrations drop,

[2] Example of an average 28-day cycle in which ovulation occurs

before reaching a nadir in the menstrual phase. The premenstrual and menstrual phase can also be summarized as the perimenstrual phase (6 days surrounding menstruation onset; Schmalenberger et al., 2021). Figure 1 depicts the hormone fluctuations and the corresponding cycle phases.

Figure 1

The menstrual cycle (figure adapted from Schmalenberger et al., 2021)



The menstrual cycle is naturally suppressed during pregnancy and postpartum breastfeeding. Upon less frequent breastfeeding, the cycle resumes, but might initially be irregular, with lower progesterone production. The duration of breastfeeding-related amenorrhea (absence of menstruation) is highly variable (McNeilly, 2001). The final cessation of menstrual cycles, menopause, typically occurs between the ages of 45 and 50 years, although the menopausal transition begins around three years earlier and lasts until about one year after the final menstrual cycle. This transition is marked by drastic endocrine changes that are related to the decreasing number of available ovarian follicles. FSH concentrations increase in the early follicular phase as the number of follicles decline (Burger, 2002). Estradiol concentrations fall sharply during the late menopausal transition, contributing to possible

symptoms such as hot flashes, night sweats, sleep problems, and mood and cognitive problems (National Institute of Health, 2005). In general, hormone fluctuations and menstrual cycles during the menopausal transition are characterized by high variability and irregularity. The hormone concentrations become more stable after menopause, which marks the end of the reproductive stage (Burger, 2002).

This cycle-related fluctuation in hormones across the reproductive lifespan not only accounts for biological changes but has also been attributed to and investigated regarding psychological changes such as fluctuations in depressive symptoms.

2.2 The menstrual cycle and affective disorders

Depressive symptoms are associated with the menstrual cycle in multiple ways. First, the prevalence of depression is twice as high in females as in males (Van de Velde et al., 2010). This sex discrepancy first emerges in puberty, when hormone fluctuations over the course of the menstrual cycle first develop with menarche, and ends after menopause, when menstrual cycling ends as well. This suggests an underlying role of the menstrual cycle and fluctuating hormones (Angold et al., 1999; Schiller et al., 2016). Second, specific affective disorders that are characterized by symptom changes across the menstrual cycle have been observed, underlining a possible influence of the menstrual cycle on affect and emotions. These disorders include premenstrual dysphoric disorder (PMDD), premenstrual syndrome (PMS), and premenstrual exacerbation (PME) of mental disorders (Eisenlohr-Moul, 2019).

PMDD is characterized by cyclical fluctuations in affective symptoms (depressed mood, affective lability, irritability, anxiety, diminished interest, low energy, difficulties concentrating, changed appetite, feeling overwhelmed, sleep problems) and physiological symptoms (e.g. breast tenderness, joint pain, feeling bloated). A diagnosis of PMDD according to the DSM-5 criteria requires the presence of at least five of these symptoms (one of which must be a “core emotional symptom”, referring to the first four symptoms in the list above) in

the premenstrual week, which remit in the postmenstrual week (American Psychiatric Association, 2013). Uniquely, the DSM-5 criteria include the method for diagnosing the disorder, which requires daily symptom ratings for at least two menstrual cycles, as retrospective ratings have been shown to substantially bias diagnoses (Eisenlohr-Moul, 2019; Marván and Cortés-Iniestra, 2001). The lifetime prevalence of PMDD is 5-8% in menstruating individuals (Dennerstein et al., 2012).

PMS represents a subclinical, less impairing form of PMDD. However, it is not an officially classified disorder in the DSM-5 or ICD-10 or ICD-11. The American College of Obstetricians and Gynecologists define PMS as at least one psychological and physiological symptom that appears during the five premenstrual days and is not present in the first half of the menstrual cycle (ACOG Committee on Practice Bulletins--Gynecology, 2001). Depending on the definition of PMS, a prevalence of 13-18% in menstruating individuals is reported in the research (Halbreich et al., 2003).

PME occurs when existing mental disorders significantly worsen during the perimenstrual phase. Research on PME is sparse, but studies have indicated its occurrence in disorders such as bipolar disorder, bulimia nervosa, borderline personality disorder, agoraphobia, schizophrenia, substance abuse, and posttraumatic stress disorder (reviews from Eisenlohr-Moul, 2019; Handy et al., 2022; Kuehner & Nayman, 2021; Pinkerton et al., 2010). Due to a lack of epidemiological data, it is not possible to provide prevalence rates, and the precise biological underpinnings of PME in these disorders are yet to be determined. Only PME in major depressive disorder has been investigated in slightly greater depth. To date, two prospective studies have investigated PME in depressive disorders (Fakhari et al., 2011; Hartlage et al., 2004), These studies suggest an overall perimenstrual worsening of individual symptoms (Hartlage et al., 2004) or higher overall sum scores for depressiveness (Fakhari et al., 2011) in some participants. Both of these studies discussed the probable role of hormone

fluctuations, and specifically the increased hormone sensitivity of individuals affected by PME in depressive disorders. This concept of hormone sensitivity is addressed further in the following.

2.3 Hormone sensitivity as explanatory factor for higher prevalence of depression

Fluctuating hormones have been investigated as a potential cause of menstrual cycle-related changes in affective symptoms for several decades (Eisenlohr-Moul, 2019; Schmidt et al., 1998; Wei et al., 2018). Such affective symptom changes have also been examined in relation to other reproductive events that are accompanied by drastic hormonal changes, for example pregnancy (Schiller et al., 2022) or menopause (Gordon et al., 2015). Disorders that are related to changes in ovarian hormones are commonly summarized as reproductive mood disorders (Schweizer-Schubert et al., 2021). It has become clear that it is not absolute hormone concentrations per se that differ between people with and without reproductive mood disorders (Wei et al., 2018); rather, changes in hormone concentrations have been shown to precede symptom changes in affected individuals (Schiller et al., 2022; Schmidt et al., 2017, 1998; Wei et al., 2018). These symptom changes, which follow normal changes in hormone concentrations, were found to only affect some women with increased susceptibility, leading researchers to propose the pathophysiological paradigm of "hormone sensitivity" (Schweizer-Schubert et al., 2021). According to the current definition, some susceptible women show an abnormal affective response to normal hormone fluctuations. This can apply to both ovarian hormone withdrawal (Bloch, 2000) and surges (Schmidt et al., 2017). Moreover, there are indications that individuals who are susceptible to hormone fluctuations during one reproductive event (e.g. the menstrual cycle) are at greater risk of developing other reproductive mood disorders, e.g. during/after pregnancy or in the menopausal transition (Payne et al., 2009). However, rigorous research on the longitudinal relationship between

hormone fluctuations and reproductive mood disorders is lacking (Schweizer-Schubert et al., 2021).

Some studies have sought to determine biological explanations and psychosocial risk factors that contribute to hormone sensitivity (Girdler et al., 2012; Schweizer-Schubert et al., 2021). Identifying such risk factors and biological underpinnings is crucial in order to improve our understanding of reproductive mood disorders, to specify diagnostic procedures, and ultimately to enable precision treatment and prevention programs for these disorders. Possible biological underpinnings of hormone sensitivity (for example increased sensitivity of $GABA_A$ receptors to the progesterone metabolite allopregnanolone; ALLO) have been investigated but are not yet fully understood (Girdler et al., 2001; Martinez et al., 2016; Schiller et al., 2016; Walker et al., 2004; Wei et al., 2018). Besides research on biological mechanisms that drive hormone sensitivity, there is some research examining psychological risk factors: For instance, preexisting mental disorders such as anxiety (Breaux et al., 2000; Perkonigg et al., 2004), posttraumatic stress disorder (Pilver et al., 2011), or major depressive disorder (Breaux et al., 2000) have been associated with an increased risk of reproductive disorders related to hormone sensitivity. Furthermore, trauma exposure and adverse childhood experiences have shown correlations with the occurrence and severity of PMDD and PMS, suggesting a potential modulating role in hormone sensitivity (Pilver et al., 2011, 2011; Yang et al., 2022), although some prospective studies yielded heterogenous findings regarding the relation of trauma exposure with the occurrence and severity of PMDD and PMS (Eisenlohr-Moul et al., 2016; Segebladh et al., 2011). Moreover, research focusing on the influence of stressful life events and perceived stress on PMDD found that individuals with PMDD or PMS reported increased daily life stress (Beddig et al., 2019; Hoyer et al., 2013; Namavar Jahromi et al., 2011; Perkonigg et al., 2004), high perceived chronic stress (Kleinstäuber et al., 2016), and high arousal negative affect toward stressors (Beddig et al., 2019).

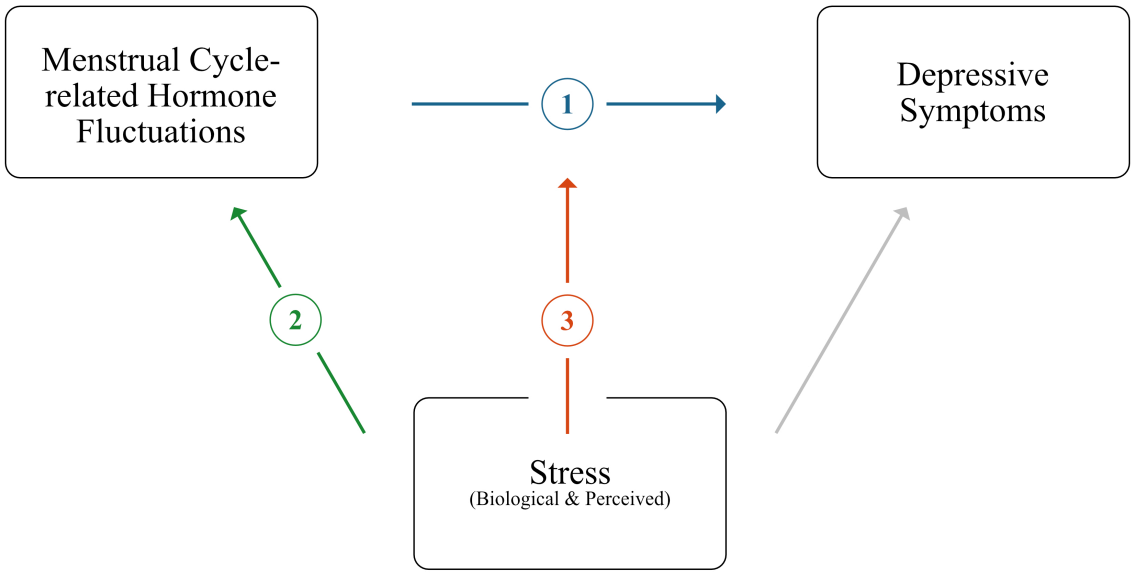
These risk factors and possible biological underpinnings of hormone sensitivity in PMDD can inform investigations on influencing factors of PME of depression, but an equivalent impact of each individual factor should not be assumed. The role of risk factors such as stressful life events or perceived stress for PME of depression has not yet been investigated. To fill this gap, a heuristic model of stress as a moderating factor for hormone sensitivity to depressive symptoms is introduced in the following, and is developed further with the results of this dissertation.

2.4 Heuristic model of stress as moderator of hormone sensitivity to depression

The proposed heuristic model investigates the associations between depressive symptoms, menstrual cycle-related hormone fluctuations, and biological and subjective stress parameters. Figure 2 visualizes this model. The model aims to provide a better understanding of the multifaceted etiology of the menstrual cycle-related influence on depression.

Figure 2

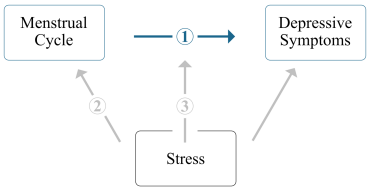
Heuristic model of stress as moderating factor of hormone-mood sensitivity



Note. The theoretical background of the paths will be examined as follows: Path 1: section 2.4.1, Path 2: section 2.4.2, Path 3: section 2.4.4.

In the following sections (2.4.1 – 2.4.4), the state of research regarding the associations depicted in the model is presented (Path 1: Menstrual cycle-related hormone fluctuations & Depressive symptoms, Path 2: Menstrual cycle-related hormone fluctuations & Stress, Path 3: Stress as a moderator of the hormone – symptom relationship). Based on this, open research questions are derived and the related studies that were conducted within the scope of this dissertation are introduced (section 2.5). It should be noted that the extensively studied direct relationship between stress and depressive symptoms is not investigated in this dissertation; however, to complete the model, a brief theoretical background is provided in chapter 2.4.3.

2.4.1 Path 1: Menstrual cycle-related hormone fluctuations and depressive symptoms



As described in section 1.2, research has demonstrated associations between depressive symptoms and the menstrual cycle. These assumptions have been traced back to the sex disparity during the time frame between puberty and menopause on the one hand, and cycle-specific affective disorders such as PMDD, PMS, and PME on the other hand. To better understand how menstrual cycle-related hormone fluctuations might influence depressive disorders, it is necessary to investigate whether these disorders and symptoms systematically differ between cycle phases. This question has been addressed in two longitudinal studies investigating participants with depression and in several studies investigating individual symptom fluctuations across the cycle in participants with depression, PMDD, or without mental disorders. An overview of these studies is presented in the following as a summary of the state of research on menstrual cycle-related changes in depressive symptoms.

To date, two prospective studies have investigated PME in depressive disorders from a longitudinal perspective (Fakhari et al., 2011; Hartlage et al., 2004), and suggested an overall perimenstrual worsening of individual symptoms (Hartlage et al., 2004) or a deterioration of

sum scores for depressive symptoms (Fakhari et al., 2011) in the majority of, but not all, participants. However, these studies did not investigate other cyclical fluctuations aside from perimenstrual exacerbation. Nevertheless, as demonstrated by Kiesner et al. (2011, 2016), a mid-cycle worsening of mental disorder symptoms around the time of ovulation may also be common, but has not yet been specifically investigated with regard to depression.

Besides the studies examining participants with depression, some studies have focused on individual symptoms that can occur in depressive disorders regarding their symptom fluctuation across the menstrual cycle in various different samples:

Mood changes across the cycle have been thoroughly investigated and systematically reviewed in non-help-seeking samples (Romans et al., 2012) and in participants with affective disorders (Farage et al., 2008). Most but not all of the reviewed studies reported associations between mood and cycle phases across participants (61.7%; Romans et al., 2012): In addition to premenstrual worsening of negative mood, associations with other phases have been highlighted (e.g. 8.5% non-premenstrual phase, 38.3% premenstrual and other phases; Romans et al., 2012). These heterogeneous results were found in populations with and without affective disorders and emphasize a high probability of individual differences in cyclicity (Farage et al., 2008; Romans et al., 2012).

Diminished interest, specifically anhedonia (i.e., reduced pleasure in normally pleasurable activities) has been investigated in a smaller number of studies. Using the Sweet Taste Test as a paradigm for initial response to reward, a cross-sectional study by Bedwell et al. (2019) revealed lower anhedonia in the luteal phase. It should be noted that the study compared women in the luteal phase to women in all other cycle phases and men combined, which might limit the generalizability of the findings. Rueda et al. (2019) investigated college students in two cross-sectional studies, and found no association between menstrual cycle

phase and overall anhedonia, but did find increased social anhedonia in the second half of the menstrual cycle.

Sleep across the menstrual cycle has been investigated in terms of both subjective sleep quality and objective measures of sleep (e.g. percentage of REM sleep). For subjective sleep, lower quality and increased sleep problems have been reported either in the perimenstrual phase (Driver et al., 2008; Owens and Eisenlohr-Moul, 2018) or at midcycle in some individuals (Van Reen and Kiesner, 2016). However, the timing and composition of sleep seem to remain relatively stable (Baker and Driver, 2007), with the exception of a slightly decreased percentage of REM sleep, a higher percentage of stage 2 sleep, and higher sleep spindle frequency (Baker and Driver, 2007; Driver et al., 2008) in the luteal compared to the follicular phase. This might be influenced by the increased basal body temperature in the luteal phase (Driver et al., 2008; Parry et al., 2006). Furthermore, women with irregular cycles (Baker and Driver, 2007; Nam et al., 2017), women with depression (Parry et al., 2006), and women with severe PMS (Baker and Lee, 2018) have reported more sleep problems during the luteal phase, possibly due to a decreased melatonin amplitude in the luteal phase in these individuals (Parry et al., 2006).

Changes in appetite and food intake have also been investigated with respect to ovarian hormone fluctuations in menstrual cycle phases (Roney and Simmons, 2017). One such study identified negative correlations between estradiol and food intake and positive correlations between progesterone and food intake, resulting in a periovulatory drop in eating (Roney and Simmons, 2017). This was the opposite pattern to shifts in sexual desire in the same sample, which the authors discussed as indicating a shift in motivational priorities from food intake to reproduction in the fertile window of the cycle. While Kammoun et al. (2017) revealed higher caloric intake not only in the luteal but also in the periovulatory phase compared to the follicular phase, a large majority of the studies reviewed by Asarian and Geary (2006) as well as studies

published since that review (Klump et al., 2013; Roney and Simmons, 2017) point at a periovulatory decrease and a luteal phase increase in appetite and food intake. The same pattern was found in animal studies (Fessler 2003; Klingerman et al. 2010; Schneider et al. 2013).

Low energy or fatigue has rarely been studied across the menstrual cycle. Carmichael et al. (2021) investigated five athletes and found lower energy and stronger fatigue in the luteal phase compared to the follicular phase. Li et al. (2020) reported a similar finding of increased mental fatigue in the mid-luteal compared to the early-follicular phase in participants without PMDD or anxiety. However, this was only investigated in small samples and not in a sample with depressive disorders.

Various studies have investigated concentration difficulties across the menstrual cycle in the scope of neuropsychological performance and cognitive functioning. The results were systematically reviewed by Souza et al. (2012) and Le et al. (2020), both indicating mild to no cyclical effects on average concentration abilities in healthy samples, with a tendency towards lower attention and concentration in the luteal phase. However, this effect was more pronounced in samples with severe PMS or PMDD, who showed a lower ability to concentrate in the luteal phase (Le et al., 2020; Souza et al., 2012). It has not yet been examined whether such increased cyclical fluctuations are likewise found in individuals with depressive disorders.

Feelings of worthlessness or guilt have not yet been investigated as a single symptom across the menstrual cycle. However, self-esteem, which can be associated with feelings of low self-worth, has been examined in different cycle phases, Thereby, some studies demonstrated lower self-esteem in the premenstrual phase compared to mid-cycle or the early follicular phase in women with (Fontana and Pontari, 1994) and without PMDD (Brock et al., 2016; Gonda et al., 2008). Moreover, one study reported higher self-esteem in the late luteal phase compared to the ovulatory phase (Hill and Durante, 2009), while others indicated only small (Bloch et

al., 1997) or no associations (Edmonds et al., 1995) between self-esteem and menstrual cycle phases.

Reduced movement or restlessness have likewise not been specifically examined across the menstrual cycle. As a closely related concept, however, exercise performance has been extensively investigated and reviewed, and was found to show small decreases in the early follicular phase compared to all other phases (McNulty et al., 2020). An investigation of reduced movement/restlessness as occurring in depressive disorder is still pending.

Suicidality, including suicidal ideation, suicide attempts, and completed suicide, has been a major focus with regard to cycle-related disorders and is also strongly associated with PMDD (Osborn et al., 2021; Owens and Eisenlohr-Moul, 2018). Multiple systematic reviews and meta-analyses have indicated an association between suicidality and menstrual cycle phases, suggesting an increase in suicidality in the menstrual phase (Jang and Elfenbein, 2019; Osborn et al., 2021; Owens and Eisenlohr-Moul, 2018; Saunders and Hawton, 2006). Suicidal ideation is increased in women during the reproductive years between menarche and menopause, similarly to depressive disorders (Owens et al., 2020). The risk of suicidal ideation has been shown to be increased in the menstrual and premenstrual phase (Owens and Eisenlohr-Moul, 2018; Saunders and Hawton, 2006), possibly due to interactions between lower estradiol concentrations and the serotonergic system. The risk of suicide attempts is two to three times higher in women than in men (Vijayakumar, 2015), and the menstrual phase was associated with a significantly increased risk of non-fatal suicide attempts in 15 out of 23 reviewed studies (Jang and Elfenbein, 2019; Saunders and Hawton, 2006). A similar pattern has been reported for completed suicide, with a 26% increased risk in the menstrual phase (Jang and Elfenbein, 2019). However, completed suicide is much harder to study due to methodological issues concerning cycle phase determination postmortem and the critical

timing of autopsies (Dogra et al., 2007; Saunders and Hawton, 2006). Overall, there are strong indications of cycle-related fluctuations in suicidality.

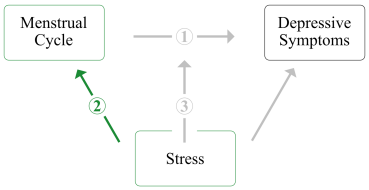
In summary, two longitudinal studies investigating overall depression, and multiple studies investigating individual depressive symptoms, have demonstrated heterogenous menstrual cycle effects. This heterogeneity highlights the need to use a symptom-specific approach when investigating menstrual cycle effects rather than merely drawing on sum scores of depression scales. Previous studies either reported aggregated scores for depression, focused on single symptoms, or only investigated the dichotomous occurrence of premenstrual exacerbation while failing to include mid-cycle deterioration. The next course of action is therefore to examine both perimenstrual and mid-cycle exacerbation of the full spectrum of depressive symptoms, with a focus on analyzing and comparing single symptoms individually.

Besides a potential influence of the menstrual cycle on depression, a converse effect of depression on menstrual cycle characteristics is possible. Studies have investigated the impact of current psychological symptoms and disorders on the reproductive hormone system, specifically the HPG axis (Grambsch et al., 2004; Meller et al., 2001). One way to assess HPG axis functioning is to measure the pulsatile rhythm of LH secretion, as it is regulated by GnRH release from the hypothalamus - the initiator of HPG axis functioning. In this regard, slower and dysrhythmic LH pulsatile release was observed in women with major depression compared to healthy controls (Meller et al., 2001). These changes suggest dysregulations in the hypothalamus during depressive episodes. However, while assessing LH pulsatility is the most accurate method to evaluate HPG axis regulation, it is also an expensive and time-consuming process. Alternatively, HPG axis dysregulation can be measured through outcomes such as menstrual cycle regularity (Mumford et al., 2012; Sasaki et al., 2016). Nillni et al. (2018) compared the prevalence of irregular menstrual cycles in 3,346 women with and without severe depressive symptoms, and found a 63% greater prevalence of irregular menstrual cycles among

those with depressive symptoms. Similarly, Yu et al. (2017) observed that adolescents who reported depressive symptoms in the preceding twelve months had a higher likelihood of experiencing irregular menstrual cycles. Importantly, neither of these latter two studies assessed both cycle regularity and depressive symptoms prospectively.

In addition to examining the variation of cycle length as a marker of menstrual cycle regularity, studies have also investigated ovulation status with regard to depression. Increased depressive mood was observed in ovulatory cycles compared to anovulatory cycles (meaning cycles in which no ovulation, and therefore no related hormone patterns, occurs; Harvey et al., 2009). To date, no other depressive symptoms have been investigated in relation to ovulation status.

2.4.2 Path 2: Menstrual cycle-related hormone fluctuations and stress



As a second interaction in the heuristic model, the state of research on the relationship of perceived and biological stress with the menstrual cycle is examined (see figure 2) and open questions investigated in this dissertation are presented.

Studies investigating perceived stress in terms of its potential association with the menstrual cycle have revealed changes in the perception of stressors in cycle phases in some, but not all, women. More specifically, women with PMDD perceived stressors as more severe and unpleasant in the premenstrual compared to the postmenstrual phase (Beddig et al., 2019; Brown and Lewis, 1993; Fontana and Badawy, 1997; Hoyer et al., 2013). This cyclicity was not found in healthy participants (Beddig et al., 2019; Brown and Lewis, 1993; Montero-López et al., 2018). However, when investigating specific ovarian hormone concentrations, healthy participants reported less subjective stress following a stress task in the ovulatory phase, when estradiol is high, compared to the early follicular phase, when estradiol is low (Albert et al.,

2015). Furthermore, psychosocial stress has been associated with changes in menstrual cycle regularity (Acevedo-Rodriguez et al., 2018; Ayrout et al., 2019; Kreisman et al., 2020; Ralph et al., 2016; Wagenmaker and Moenter, 2017) and even with the absence of ovulation, caused by an inhibitory effect of stress and stress hormones on the ovulation-inducing LH peak (Breen et al., 2005; Schliep et al., 2015; Wagenmaker and Moenter, 2017). In summary, the results of studies investigating perceived stress and the menstrual cycle reveal a bidirectional effect. Stress seems to show a potential cyclical fluctuation, especially in women with PMDD, indicating a role of hormone fluctuations in stress perception in some individuals. On the other hand, stress seems to have a potential influence on cycle characteristics, suggesting an interaction of ovarian hormone fluctuation and physiological stress responses.

The physiological response to internal and external stressors is mainly regulated by the hypothalamic–pituitary–adrenal (HPA) axis (Kudielka and Kirschbaum, 2005). In the face of stressors, the paraventricular nucleus (PVN) of the hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH). In turn, ACTH triggers the release of glucocorticoids by the adrenal glands. All of these hormones involved in HPA axis functioning play a crucial role in negative feedback inhibition of their own secretion, leading to a decrease in adrenal glucocorticoid production once the body reaches homeostasis. Cortisol is the main glucocorticoid in humans and the primary biomarker of HPA axis functioning and consequently the biological stress response (Esposito, 2012; Kudielka et al., 2012; Sapolsky et al., 2000). Given that almost all human cells can be stimulated by cortisol, it has a wide range of physiological effects in the organism (Kudielka and Kirschbaum, 2005), such as increasing cardiovascular activity and cognitive processes while inhibiting digestive, immune, and reproductive functions (Roy and Roy, 2017; Sapolsky et al., 2000). Due to its effects on behavior and cognition, cortisol release is closely linked to both physiological and psychological well-being (Bangasser and Valentino,

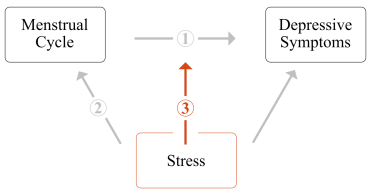
2014; Esposito, 2012). Cortisol can be measured in blood serum, saliva, urine, hair, and even fingernails (Fischer et al., 2020). Cortisol concentration can be assessed in both basal, unstimulated concentrations (which serve as an indicator of HPA axis activity) and in response to acute stressors (which serve as an indicator of HPA axis reactivity).

Research has revealed sex differences in HPA axis functioning (Liu et al., 2017), and more specifically associations with sex hormones and the HPG axis, with animal studies showing bidirectional interactions between the HPA and HPG axes at all levels of HPA axis functioning (Handa and Weiser, 2014; Heck and Handa, 2019; Oyola and Handa, 2017). For instance, research examining the HPA axis response to stressors over the four-day equivalent of the menstrual cycle in female rodents - the estrous cycle - has demonstrated a critical role of estradiol in regulating the neuroendocrine response to stress. The findings indicate that HPA axis reactivity is enhanced during estrous cycle phases, characterized by high estradiol concentrations (proestrus and estrus), and reduced during phases marked by low estradiol concentrations (diestrus) (Heck and Handa, 2019; Viau and Meaney, 1991) . Furthermore, progesterone mitigates the impact of estradiol on HPA output (Handa and Weiser, 2014; Heck and Handa, 2019; Herman et al., 2016; Viau and Meaney, 1991). In humans, research investigating the interaction between the HPA and HPG axes is sparse and has yielded heterogeneous findings (Heck and Handa, 2019; Kudielka et al., 2012; Kudielka and Kirschbaum, 2005). Therefore, it remains to be determined whether the interactions observed in animal studies can be generalized to humans. HPA axis activity and reactivity across the menstrual cycle has been investigated in some primary studies with rather small sample sizes. To allow for meaningful statements, a systematic review and meta-analytic comparison of HPA axis functioning across the menstrual cycle is necessary.

2.4.3 Stress and depressive symptoms

The substantial role of stress in the development, maintenance, and treatment of depressive disorders has been widely studied. In particular, a strong influence of physiological stress, perceived (chronic) stress, and stressful life events including traumatic experiences on depressive disorders has been demonstrated (Paykel, 2003; Quax et al., 2013; Rothe et al., 2020). In a systematic review of 190 studies, Rothe et al. (2020) examined physiological stress, in particular HPA axis functioning, in relation to major depression and burnout. The results revealed hypercortisolism and glucocorticoid resistance in the form of higher basal cortisol concentrations and a blunted cortisol reactivity to acute stressors in individuals with major depressive disorder (Rothe et al., 2020). Chronic perceived stress has also been associated with a higher risk of depression, possibly due to chronic HPA axis activation and consequent dysregulations of homeostatic cortisol concentrations (Quax et al., 2013). Furthermore, stressful life events increase the risk for the development of depressive disorders (Paykel, 2003). This also applies to experiences that are accompanied by tremendous stress, such as childhood trauma. For instance, childhood emotional, physical, and sexual abuse, violence, and neglect have been associated with a higher risk of developing depressive disorders in adulthood (Mandelli et al., 2015).

2.4.4. Path 3: Stress as a moderator of the hormone-depressive symptom relationship



Many stress-related disorders, including depression, show sex differences in terms of prevalence, symptoms, and trajectories (Bangasser and Valentino, 2014), indicating a possible interaction of all three discussed entities – depressive symptoms, perceived and biological stress, and menstrual cycle-related hormone fluctuations. This includes a possible moderating role of stress in the association between hormone fluctuations and depressive symptoms. The rationale for examining this moderating effect stems from observations that stressful life events

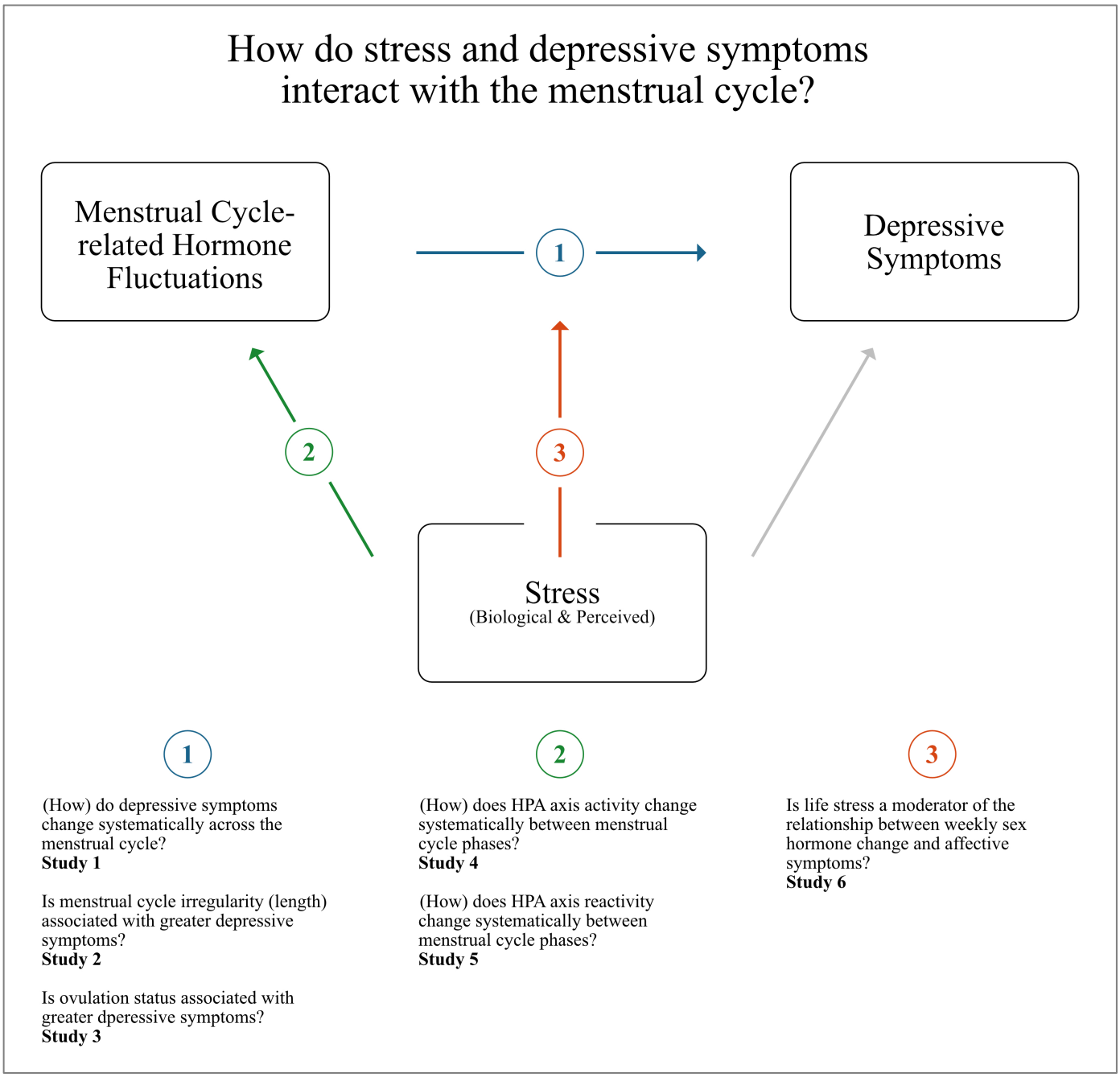
increase the emergence of reproductive mood disorders (Bromberger et al., 2011). For instance, in a study in perimenopausal women, the number of stressful life events was found to moderate the association between increased estradiol fluctuations and onset of depressive disorders (Gordon et al., 2016). However, it remains unclear whether and how stress moderates the association between menstrual cycle-related hormone fluctuations and depressive symptoms.

2.5 Scientific questions and overview of studies

The overarching research question of this dissertation is: **How do stress and depressive symptoms interact with the menstrual cycle?** By combining the evidence of respective associations between menstrual cycle-related hormone fluctuations, depressive symptoms, and stress (see sections 1.4.1 to 1.4.4), a triangular model emerges, which connects the three entities together (see figure 3). This dissertation aims to address previously discussed research gaps for each of the connections between these three entities. Additionally, it seeks to investigate the moderating role of stress in the interaction between hormone fluctuations and depressive symptoms. Each arm and research question of this model is investigated through the included studies, as depicted in figure 3.

Figure 3

Heuristic model for stress as moderating factor for hormone-mood sensitivity with scientific questions of this dissertation



Two of the studies (studies 1 and 2) are based on empirical data investigating depressive symptoms over the course of the menstrual cycle using an ambulatory assessment design. Two studies (studies 3 and 4) are based on empirical data, including hormone measurements from two studies investigating hormone and depressive symptom changes in peripubertal females. Two further studies (studies 5 and 6) meta-analytically investigated HPA axis functioning, specifically basal cortisol and cortisol reactivity in response to stressors, over the course of the menstrual cycle. The following scientific questions addressed by the papers are relevant for this dissertation:

Study 1 (presented in chapter 3) investigated the relationship between ovarian hormone fluctuations over the course of the menstrual cycle and depressive symptoms in a sample of 77 naturally cycling participants with and without depressive disorder. Using an ambulatory design, the study examined participants with regard to depressive symptoms across one menstrual cycle.

Q1: (How) do depressive symptoms change systematically across the menstrual cycle?

Study 2 (presented in chapter 4) investigated the association of menstrual cycle irregularity in terms of cycle length with depressive symptoms. For this purpose, two sub-studies were conducted – one cross-sectional study (n = 394) comparing self-reported cycle regularity with self-reported depressive symptoms and one longitudinal study (subsample from the cross-sectional study, n = 77) comparing depressive symptoms in the follicular phase of the cycle with the deviation from the expected next onset of menses.

Q2: Is menstrual cycle irregularity (length) associated with greater depressive symptoms?

Study 3 (presented in chapter 5) investigated the association of menstrual cycle irregularity in terms of ovulation status with depressive symptoms. A sample of 32 peripubertal females provided daily urine samples for the assessment of ovarian hormones, from which ovulation

status was determined, as well as daily ratings of depressive symptoms. As a prerequisite, a method review and systematic comparison was conducted to determine the most precise method to determine LH peaks and PdG rises for each participant, with the aim of accurately determining ovulation status in samples with a high prevalence of irregular and anovulatory cycles.

Q3: Is ovulation status associated with greater depressive symptoms?

Study 4 (presented in chapter 6) compared basal cortisol concentrations, as a marker of HPA axis activity, between broader (follicular vs. luteal) and more precise (menstrual, mid-to-late follicular, ovulatory, early-to-mid luteal, premenstrual) cycle phases. This comparison was undertaken through a systematic review and meta-analysis comparing k = 121 studies that assessed basal cortisol concentrations longitudinally in at least two menstrual cycle phases.

Q4: (How) does HPA axis activity change systematically between menstrual cycle phases?

Study 5 (presented in chapter 7) compared cortisol concentrations in response to acute physiological and psychosocial stressors, as a marker of HPA axis reactivity, between broader (follicular vs. luteal) and more precise (menstrual, mid-to-late follicular, ovulatory, early-to-mid luteal, premenstrual) cycle phases. This systematic review and meta-analysis included k = 12 studies that longitudinally investigated cortisol responses to stressors in at least two cycle phases.

Q5: (How) does HPA axis reactivity in response to acute psychological and physiological stressors change systematically between menstrual cycle phases?

Study 6 (presented in chapter 8) investigated the potential moderating role of stressful life events in the relationship between hormone fluctuations and depressive symptoms. This was examined in n = 35 pre- and post-menarchal females, who provided weekly urine samples for

the measurement of ovarian steroids (estrogens, progesterone, and DHEA), as well as weekly ratings of depressive symptoms and recent stressful life events (occurrence within the last year).

Q6: Is life stress a moderator of the relationship between weekly sex hormone changes and affective symptoms?

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CHAPTER 3

Study 1:

Menstrual cycle related depressive symptoms and their diurnal fluctuations – an ambulatory assessment study

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Menstrual cycle related depressive symptoms and their diurnal fluctuations – an ambulatory assessment study

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Abstract study 1

Background: Reproductive mood disorders indicate that within-person variation in depressive symptoms across the menstrual cycle can be related to ovarian hormone changes. Until now, such cycle-related symptom changes have been measured once daily, even though depression research indicates systematic diurnal changes in symptoms. Further, previous research often focused on aggregated depressive scores. This study examined whether three daily assessments of depressive symptoms follow similar trajectories across the menstrual cycle and investigated within-person cyclical fluctuation of all single symptoms and the aggregated score.

Methods: 77 naturally-cycling participants (35 with and 42 without depressive disorder) provided three daily ratings of depressive symptoms across one menstrual cycle.

Results: Reliability estimates (w) of the three diurnal measurements ranged from 0.56 to 0.78. Cyclicity showed significant interindividual differences for all symptoms, and single symptoms differed significantly from each other in their magnitude of cyclicity.

Limitations: Only one menstrual cycle was assessed to reduce participant burden. Further, ovulation testing dates were based on self-reported cycle lengths, and only LH peaks were tested without subsequent progesterone rises.

Conclusions: The results highlight the need for a symptom-specific approach to assess individual variance in cyclicity of depressive symptoms. Reliability for one daily assessment can be improved by using the afternoon value, a sum score for depressiveness, or multiple items per symptom. Furthermore, this study emphasizes, that depressive symptoms can systematically change across the menstrual cycle, and it is, therefore, important to include it in depression research. Exploring female-specific risk factors of depression will enable the development of person-tailored treatments.

3.1. Introduction

The risk for depression in women is twice as high as in men (Van de Velde et al., 2010). Studies have shown that this risk discrepancy for women begins with menarche and declines after menopause, suggesting that the menstrual cycle might play a crucial role in the etiology of depression (Newhouse and Albert, 2015).

The menstrual cycle in healthy females lasts 28 days on average and can broadly be divided into the follicular phase (onset of menses until the end of ovulation) and the luteal phase (ovulation until subsequent menses) or, more precisely, into the mid-luteal, perimenstrual, mid-follicular and periovulatory phase (Schmalenberger et al., 2021). Across these phases, ovarian hormone concentrations (e.g., estradiol, progesterone, etc.), regulated by the hypothalamic-pituitary-gonadal (HPG) axis, fluctuate significantly. Hormone fluctuations have been shown to influence physical and psychological states in some, hormone-sensitive people (Bloch, 2000; Sander et al., 2021; Schiller et al., 2022; Schmidt et al., 1998). Hence, ovarian hormone fluctuations across the menstrual cycle can cause cyclical change of depressive symptoms or *cyclicity of symptoms* (Eisenlohr-Moul, 2019). Most studies focus on a premenstrual exacerbation of symptoms, but a mid-cycle exacerbation of various physical and affective symptoms has also been shown to occur in a subgroup of individuals (Kiesner, 2011; Kiesner et al., 2016). Cyclicity has been especially investigated in premenstrual dysphoric disorder (PMDD), but can also occur in other mental disorders (Eisenlohr-Moul, 2019). For depressive disorders, Hartlage et al. (2004), showed that 58% of participants experienced premenstrual exacerbation of one or more depressive symptoms. Fakhari et al. (2011) reported a mid-cycle decrease in overall depressive symptoms calculated across all participants. These available longitudinal studies investigating cyclicity of depressive symptoms measure symptoms once a day (Hartlage et al., 2004) or once in three cycle phases (Fakhari et al., 2011) across one or two menstrual cycles. Daily measures are in line with the standards of menstrual cycle research,

which requires daily ratings of symptoms, ideally across multiple cycles, and thereby requires comparatively high effort from participants. However, previous studies with this intensive assessment approach did not specify the time of day for each measurement, nor did they schedule multiple assessments per day. This is understandable because multiple assessments per day increase participants' burden even further.

In addition to menstrual cyclic fluctuations, depressive symptoms can also show diurnal fluctuations, meaning systematic changes of symptoms within a day (Mendoza, 2019; Morris et al., 2009; Wirz-Justice, 2008) such as a morning- or evening-low (Murray, 2008). Given such diurnal patterns of depressive symptoms, the question arises whether a single daily symptom assessment can measure systematic change across the menstrual cycle well. Accordingly, systematic variance through time-of-day effects might imply the necessity for multiple measurement occasions to reliably investigate menstrual cycle effects, or to restrict daily samplings to the same timing.

Aside from this necessity to disentangle the interplay of cycle and diurnal fluctuations, a systematic investigation of single depressive symptoms is yet missing. A focus on single symptoms opposes to the analysis of an aggregate of depressive symptoms (e.g. subsuming loss of interest and decreased concentration, etc.) as defined by DSM-5 (American Psychiatric Association (2013)). The latter has drawn the general criticism that single symptoms can differ in their causes and underlying biology (Fried and Nesse, 2015). In accordance with this view, evidence from reproductive mood disorders such as PMDD, perimenopausal or postpartum depression indicates that hormonal influences can reflect differently on specific psychological symptoms (Eisenlohr-Moul et al., 2016; Gordon et al., 2019). For example, depressed mood (Angst et al., 2001; Bowen et al., 2011), non-fatal suicidality (Saunders and Hawton, 2006), and anger/irritability (Ko et al., 2013; Pearlstein et al., 2005) have shown specifically pronounced perimenstrual exacerbation in women with PMDD in contrast to, for example,

concentration problems (Le et al., 2020; Souza et al., 2012). Crucially in view of the present study, single symptoms were also shown to have different patterns and expressions of cyclicity (perimentstrual or mid-cycle increases of symptoms; Kiesner et al. (2011, 2016)). Interpreting solely a sum-score of all depressive symptoms across the menstrual cycle might thus mask single symptoms' variability. Therefore, a differential, symptom-specific approach is necessary to investigate menstrual cycle effects on depression. Previous studies that inform our knowledge of menstrual cyclicity in depression reported either aggregated scores for depression, focused on single symptoms such as sleep (Baker and Driver, 2007; Driver et al., 2008; Parry et al., 2006) or suicidality (Owens and Eisenlohr-Moul, 2018; Saunders and Hawton, 2006) or investigated the dichotomous occurrence of premenstrual exacerbation (Fakhari et al., 2011; Hartlage et al., 2004). The next step is to investigate how the full spectrum of depressive symptoms varies across the menstrual cycle while analyzing each symptom separately.

In summary, with this study, we aim to deepen the understanding of menstrual cycle patterns of depressive symptoms, using a symptom-based approach while also considering diurnal fluctuations. Further, we investigate not only premenstrual exacerbation of symptoms but also potential mid-cycle exacerbations, as introduced by Kiesner (2011). Specifically, we examined whether three daily assessments (morning, afternoon, evening) follow similar trajectories (aim I).

We further aimed to investigate within-person cyclicity of overall and single depressive symptoms (aim II). Here, we hypothesized that there are significant differences between participants regarding the pattern (perimenstrual vs. mid-cycle exacerbation) and individual magnitude of cyclicity of depressive symptoms (hypothesis 1). Participants with and without depressive disorder were then compared regarding their cyclicity. Further, against the background of the frequent use of the aggregated sum-scores and the associated risk for loss of

information, we investigated whether single symptoms show different trajectories across the menstrual cycle and hypothesized, that there are significant differences in cyclicity between single depressive symptoms (hypothesis 2).

3.2. Materials and methods

Our aims were tested with a daily ambulatory assessment study that investigated depressive symptoms of participants with and without depressive disorders (current major depressive episode and/or persistent depressive disorder) across one menstrual cycle. The study was preregistered at ClinicalTrials.gov (NCT04086316) and approved by the local ethics committee at Freie Universität Berlin (ID: 003.2019). All scripts for data preparation and analysis can be derived from the openly accessible R Script at https://osf.io/9x6uc/?view_only=75c0aefe34b941a1be992eab4e97ba23.

3.2.1 Participants

Participants were recruited on social media platforms, online marketplaces, online forums specialized in depression, and through the university's official website and outpatient clinic.

Inclusion criteria were age between 18 and 45 years, female biological sex (self-report), regular (+/- 2 days) menstrual cycle, and cycle length between 26 and 30 days. Exclusion criteria were hormonal contraception or psychotropic drug intake within the last six months, late-night shifts, more than one-hour time zone shift within the last month, menopause or the menopause transition, pregnancy, breastfeeding, being less than one-year post-partum, diagnoses of bipolar disorders, substance use disorder, eating disorders or schizophrenia and medications or chronic diseases that might influence hormone regulation (for a detailed list, see Appendix A). We also excluded participants with active suicidal ideation that would interfere with participation. In

this case, we informed them about treatment options and offered a follow-up phone call with a licensed psychotherapist.

3.2.2 Procedure

3.2.2.1 Screening

Interested participants completed an online screening questionnaire (Unipark, Questback GmbH, 2020) to assess eligibility. Firstly, a consent form was filled out. Aside from inclusion and exclusion criteria, age, biological sex, education, occupation, and health-related information, such as chronic diseases, medication, and psychological disorders were assessed. Furthermore, we assessed reproductive characteristics such as previous pregnancies, average cycle length, and the date of the previous menses onset.

Within seven days before the start of the daily ambulatory assessment, participants completed selected modules from the Structured Clinical Interview for DSM-5 Disorders (SCID-5-CV – German version (Beesdo-Baum et al., 2019)) by telephone to diagnose depressive disorders and to confirm inclusion criteria.

Data were collected between January 2020 and May 2021.

3.2.2.2 Daily ambulatory assessment across one menstrual cycle

Data collection was carried out on a study phone (Nokia 2.1 or Nokia 3.1) using the software "mobileQ" (Meers et al., 2020). During the study period, the corresponding application sent a prompting signal at three fixed time points (9.45 am, 2.45 pm, and 7.45 pm) every day across one menstrual cycle. Responses and response times were automatically time-stamped by the program. Participants had a 30-minute time window after the prompt to participate. Assessment started three days before the expected onset of menses and ended with

the onset of the next menstrual cycle. The days before menses onset were regarded as a familiarization period and were excluded from the analyses.

3.2.2.4 Menstrual cycle assessment

Menstrual cycle assessment and phase determination followed the recommendations by Schmalenberger et al. (2021). Ovulation was determined through LH ovulation tests (One + step, sensitivity: 20 mlU/ml). Tests were applied consistently at 5 pm for five consecutive days around the expected ovulation (based on menses onset and cycle length). Test results were immediately communicated to the study team and pictures of the test were taken for later confirmation by trained researchers. Menstrual bleeding and menstruation pain were assessed daily.

3.2.3 Measures

3.2.3.1 SCID-5-CV (selected sections)

The *Structural Clinical Interview for DSM-5 Disorders* (SCID-5-CV) is a semi-structured interview for the diagnosis of the major DSM-5 disorders (Cronbach's $\alpha > .7$; Beesdo-Baum et al., (2019)). We used the sections concerning affective episodes (Module A), psychotic and associated symptoms (Module B), substance-related disorders (Module E), and the screening questions for eating disorders (from Module I). Symptoms were rated as present or absent by a trained interviewer and diagnoses were made according to the DSM-5 diagnostic criteria.

3.2.3.2 PHQ-9 (adapted for ecological momentary assessment)

The severity of depressive symptoms across the menstrual cycle was measured with a version of the Patient Health Questionnaire (PHQ-9; Kroenke et al., 2001), adapted for ambulatory assessment purposes. Instead of assessing symptom severity retrospectively for the last two weeks, we asked participants to rate the current moment (*How much do you feel*

affected by the following symptoms right now?). The answering format was changed from rating symptom frequency across the last two weeks to rating the intensity in the current moment on a 6-point Likert scale from 0 - *not at all* to 5 - *very strongly*. The fully adapted version and the German original can be retrieved from Appendix B. If suicidality ratings >1 were reported, a clinical psychologist called the participants within one business day to assess the risk and refer them to support systems such as psychotherapists.

3.2.4 Analysis plan

All analyses were conducted with R version 4.2.0 (R Core Team, 2021), using packages lavaan (Rosseel et al., 2023), multilevelTools (Wiley, 2020), lmerTest (Kuznetsova et al., 2020) and ggplot2 (Wickham et al., 2023) for the main analyses and figures. All scripts and packages for the analyses can be retrieved from https://osf.io/9x6uc/?view_only=75c0aefe34b941a1be992eab4e97ba23.

3.2.4.1 Cycle phase standardization

To compare menstrual cycle days and phases between participants despite varying cycle and phase lengths, we standardized cycle days (days c0-c26). These standardized cycle days reflected the same phase in the menstrual cycle for each participant. The detailed procedure for standardizing the cycle day and example cycles and their recoding can be derived from Appendix C and the openly accessible R script (https://osf.io/9x6uc/?view_only=75c0aefe34b941a1be992eab4e97ba23). The standardization procedure was founded on recommendations by Schmalenberger et al. (2021). For days c0 – c9, we selected the first ten days of the assessment cycle to represent the menstrual and mid-follicular hormone pattern (low estrogen and progesterone). For days c10 – c16, we selected the seven days surrounding a positive LH test to represent the

periovulatory hormone pattern (strong rise and fall of oestradiol and LH). If no ovulation test result was available, we estimated the periovulatory hormone pattern by using days -17 to -11 counting back from the onset of next menses. For days c17 – c26, we used the last 10 days of the assessment cycle before onset of next menses, representing the mid-luteal and premenstrual hormone pattern (strong rise and fall of progesterone, slight rise and fall of estrogen).

3.2.4.2 Analysis to investigate diurnal variance in relation to cyclical fluctuations (Aim I)

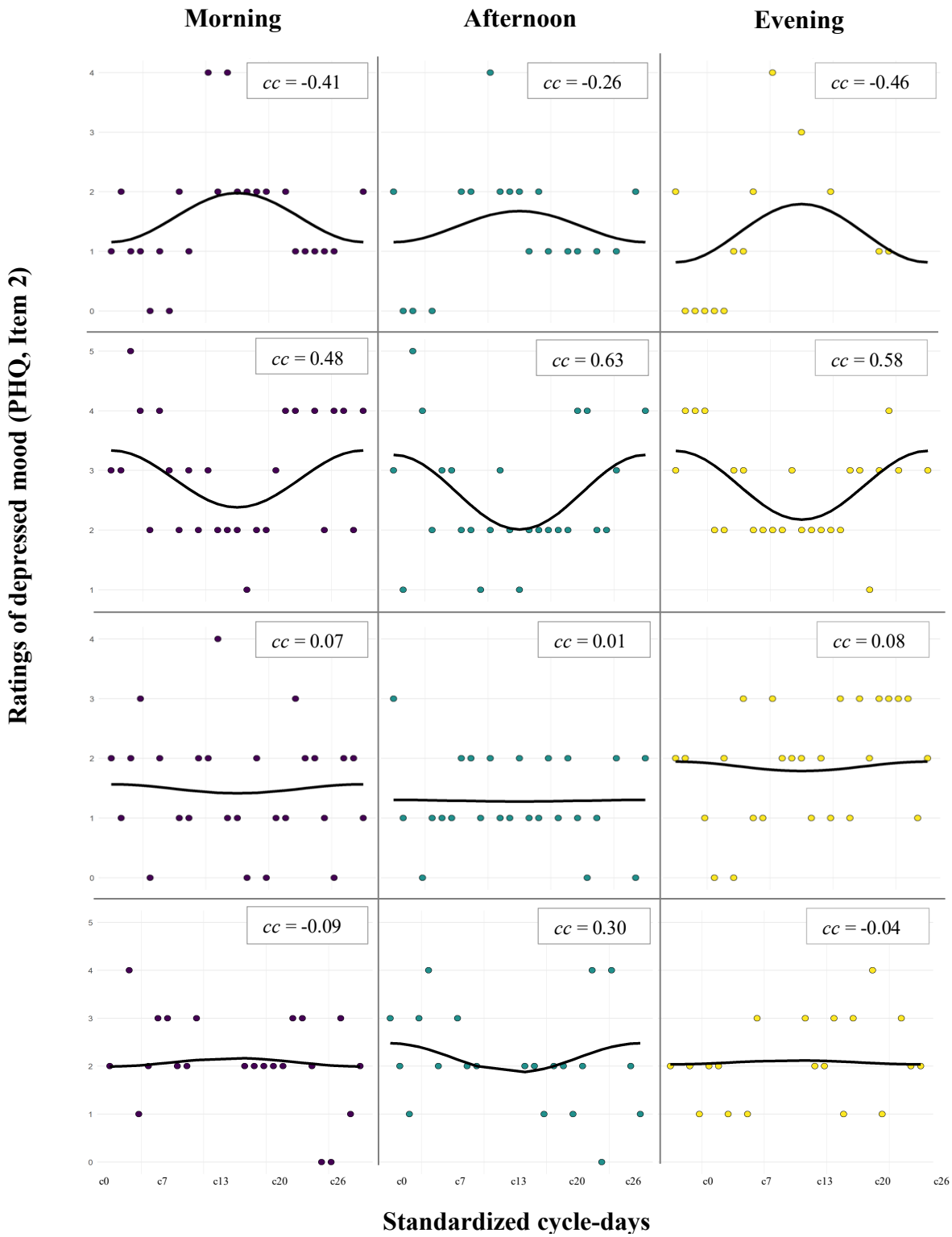
To examine whether within-day assessments of depressive symptoms follow similar trajectories across the cycle, irrespective of the sampling timings, we compared the cyclical fluctuations of the three daily measurements and examined the assessments' internal consistency (aim I). A high internal consistency would translate to similarly fluctuating symptoms of the morning, afternoon, and evening assessments across the menstrual cycle. A low internal consistency would translate to differently changing symptom trajectories across the cycle. Figure 3.1 illustrates the underlying concept of this analysis.

As a measure of within-person internal consistency, we computed the reliability index w, using multilevel confirmatory factor analyses (Geldhof et al., 2014). In our analysis, the three daily assessments, measured repeatedly across the cycle, were nested within individuals. Higher reliability (w-within) indicates that the three daily assessments fluctuate more similarly across the cycle days. We calculated the reliability for each symptom and the sum score of the PHQ items[3]. Reliability estimates range between 0 and 1 and can be interpreted as no reliability (0.0 – 0.1), slight (0.11 – 0.4), fair (0.41 – 0.6), moderate (0.61 – 0.8) and substantial (0.81 – 1.0) reliability (Nezlek, 2017; Shrout, 1998).

[3] Item 3 (on sleep quality) was excluded from the sum-score since it was only asked in the morning survey, after participants had slept.

Figure 3. 1

Example trajectories of depressed mood across the menstrual cycle in four participants.



Note. X-axis is scaled for standardized cycle days with c0 = onset of menses, c13 = ovulation, c26 = last day of the cycle; y-axis is absolute scale of Item 2 of the modified PHQ (symptom intensity from 0 = *not at all* to 5 = *very strongly*. cc = Cosine coefficients as measure for cyclicity for individual participant and time of day. Notes on internal consistency (aim I): According to the observed scores and the smoothed lines, the symptoms of the Participant 1-3 (from top to bottom) seem to follow the same trajectories on the different

*(Note continued).*daily measurement occasions. In Participant 4, instead, the trajectories do not follow the same path. Notes on within-person cyclicity (aim II): Participant 1 shows a mid-cycle exacerbation of symptoms (indicated by negative cosine coefficient), participant 2 shows a a perimenstrual exacerbation of symptoms (indicated by positive cosine coefficient), participant 3 shows no exacerbation (indicated by cosine coefficient very close to zero).

3.2.4.3 Analysis to investigate and describe cyclicity effects

The extent and pattern of cyclicity is heterogeneous and investigating average cyclical patterns across individuals can produce underestimations or false null findings (Eisenlohr-Moul, 2019; Kiesner et al., 2016; Schmalenberger et al., 2021). To address this, adapted a method from Kiesner et al. (2016), that incorporates cosine regressions in multilevel models to describe individual differences in cyclicity. Thereby, cosine regression is used to analyze non-linear time-series data, providing estimates of the amplitude (strength of cyclicity, described by the modulus of the regression weight) and pattern (perimenstrual or mid-cycle rise of symptoms, described by the sign before the regression weight) for each individual and symptom (Kiesner et al., 2016). To address aim II, we provide descriptive statistics to summarize and compare these estimates as a marker for cyclicity between participants, groups, and single depressive symptoms.

In more detail, a random intercept and random effects model of the cosine function of time (time = cycle day/26 standardized cycle days) was estimated to extract the random effect of the cosine function (for examples of cosine functions see Figure 3.1): $Y_{i,j} = \beta_{0,j} + \beta_{1.j} \cdot \text{Cosine}\left[2\pi \cdot \text{time}_{i,j}\right] + e_{i,j}$, with i = standardized cycle day and j = participant. If the random effect was significantly different from 0 (significance calculated through 95% confidence interval of the variance), it indicated that there were significant between-person differences in cyclicity measured by the cosine function (hypothesis 1). For all symptoms that showed such significant between-person differences, the cosine amplitude was extracted for each participant. These cosine coefficients were used as a marker for individual cyclicity. A positive cosine coefficient reflects a U-shaped cosine regression characterizing a perimenstrual

symptom increase and a mid-cycle symptom decrease. A negative cosine coefficient reflects an ∩-shaped (inverted U) cosine regression characterizing a mid-cycle symptom increase and perimenstrual symptom decrease. The higher the modulus of the cosine coefficient, the larger is cyclicity effect in that participant (see Figure 3.1 for examples).

The absolute cosine coefficients were compared between participants with and without depressive disorder using a t-test. They were further analyzed descriptively, and their distributions were compared visually with a raincloud plot - a combination of box-plot, violin plot, and jitter to visualize the distribution of all cosine coefficients of the sample.

Further, we calculated differences in cosine coefficients between symptoms to examine whether the intensity of their cyclicity differs (hypothesis 2). For this calculation, we applied pairwise t-tests (Bonferroni corrected for multiple testing) comparing the absolute value of cyclicity coefficients (the amplitudes' modulus) of each person for each symptom.

3.3 Results

3.3.1 Sample characteristics

The flowchart including the number of screened participants and reasons for exclusion can be found in Appendix D. Eighty participants met the inclusion criteria and started the study. One participant dropped out during data collection. Two participants were excluded because their menstrual cycle did not start within seven weeks after their previous menses. The remaining $n = 77$ participants were included in the statistical analyses. For demographic details, see Table 3.1.

Thirty-five participants met the diagnostic criteria for a current depressive disorder as assessed with the SCID-5 CV (Beesdo-Baum et al., 2019). Of those, n=29 met the criteria for a

current major depressive episode, n=12 for a persistent depressive disorder, and of those, n=6 fulfilled both criteria ("double depression").

Compliance of participants was high. On average, participants responded to 89.4% of prompts in the investigated cycle (min: 67.7%; max: 100%). After selecting standardized cycle days as described in section 3.2.4.1, 85.8% of all theoretically possible data points and items were available for the analyses.

3.3.2. Depressive symptom trajectories across the cycle: Reliability of daily assessments (aim I)

The reliability estimates indicating whether depressive symptoms follow similar trajectories across the cycle when measured on three different daily occasions can be derived from Table 3.2. The sum score of PHQ items showed the highest reliability (ω = 0.78 [0.76 – 0.80]), indicating that its trajectories of the three diurnal assessments fluctuated relatively similarly across the menstrual cycle. At the item level, PHQ Items 2 (depressed mood), 5 (changed appetite), 6 (feeling worthless), 7 (concentration problems), and 9 (suicidal ideation) showed reliabilities between ω = 0.61 and ω = 0.70, which falls within the range previously discussed as moderate (Nezlek, 2017; Shrout, 1998). Items 1 (diminished interest), 4 (low energy), and 8 (reduced movement/restlessness) showed fair reliabilities (ω = 0.56 - 0.60). Figure 3.1 shows exemplary trajectories of depressed mood (Item 2) across the menstrual cycle for all three daily measurements in four participants.

The multilevel factor analyses used for deriving reliability estimates also provide level-specific factor loadings (i.e., loadings of the three daily ratings on the time-varying latent variable [e.g., depressive mood across the cycle] and loadings of the three daily ratings on the person-level latent variable [e.g., depressive mood varying across persons]).

Table 3. 1

Demographic characteristics

	Non-depressed (N = 42)	Depressed (N = 35)	Total (N = 77)
Age			
Mean (SD)	27.6 (5.68)	29.4 (6.41)	28.4 (6.05)
Median [Min, Max]	27.4 [18.1, 44.1]	29.1 [19.3, 42.9]	27.5 [18.1, 44.1]
Age groups			
18 - 24 years	14 (33.3%)	12 (34.3%)	26 (33.8%)
25 - 29 years	12 (28.6%)	7 (20%)	19 (24.7%)
30 - 34 years	14 (33.3%)	8 (22.9%)	22 (28.6%)
35 - 39 years	0 (0%)	6 (17.1%)	6 (7.8%)
40 - 45 years	2 (4.8%)	2 (5.7%)	4 (5.2%)
Children			
No children	28 (90.5%)	31 (88.6%)	69 (89.6%)
Children	4 (9.5%)	4 (11.4%)	8 (10.4%)
Relationship status			
In a relationship	21 (50.0%)	18 (51.4%)	39 (50.6%)
Married	2 (4.8%)	3 (8.6%)	5 (6.5%)
Single	19 (45.2%)	13 (37.1%)	32 (41.6%)
Other	0 (0%)	1 (2.9%)	1 (1.3%)
Education			
College/University Degree	21 (50.0%)	14 (40.0%)	35 (45.5%)
Vocational Training	0 (0%)	2 (5.7%)	2 (2.6%)
High school	20 (47.6%)	17 (48.6%)	37 (48.1%)
Middle school	0 (0%)	2 (5.7%)	2 (2.6%)
Job			
Other	9 (21.4%)	17 (48.6%)	26 (33.8%)
Student	33 (78.6%)	18 (51.4%)	51 (66.2%)
Cycle length (of observed cycle)			
Mean (*SD*)	28.0 (3.09)	28.4 (2.95)	28.2 (3.01)
Median [Min, Max]	28.0 [24.0, 43.0]	28.0 [23.0, 38.0]	28.0 [23.0, 43.0]

	Non-depressed (N = 42)	Depressed (N = 35)	Total (N = 77)
Ovulation detected with LH test			
No	19 (45.2%)	12 (34.3%)	31 (40.3%)
Yes	23 (54.8%)	23 (65.7%)	46 (59.7%)
Depressive symptoms (Mean + SD)			
PHQ-9 score[a]	6.21 (3.94)	15.3 (4.07)	10.3 (6.10)
SCID score[b]	1 (1.41)	5.54 (1.65)	3.08 (2.72)

Note: [a]PHQ-9 sum-score at screening, significantly higher in depressed participants compared to non-depressed participants ($t(76) = -10.34$, $p < .001$). [b]Reported number of symptoms of major depressive episode in the SCID-5 CV interview (out of 9).

Table 3. 2

Reliability and factor loadings of within-day assessments (based on ω)

Symptom	Overall estimate	Morning	Afternoon	Evening
Diminished interest	0.60 [0.57 - 0.64]	0.51	0.76	0.46
Depressed mood	0.69 [0.66 - 0.72]	0.57	0.79	0.59
Low energy	0.60 [0.57 - 0.64]	0.42	0.81	0.49
Changed appetite	0.70 [0.67 - 0.73]	0.64	0.79	0.54
Feeling worthless	0.69 [0.66 - 0.72]	0.54	0.80	0.60
Concentration problems	0.65 [0.62 - 0.68]	0.61	0.67	0.57
Reduced movement/ Restlessness	0.56 [0.52 - 0.61]	0.58	0.55	0.53
Suicidal ideation	0.61 [0.58 - 0.65]	0.55	0.68	0.54
Sum score	0.78 [0.76 - 0.80]	0.68	0.86	0.67

The afternoon assessments showed the highest within-person factor loadings for all items except for Item 8 (reduced movement/restlessness). Therefore, the afternoon assessments best represent the cyclical fluctuation of depressive symptoms and were used in all following analyses. Because Item 3 was only assessed in the morning, it was discarded from the following analyses.

3.3.3 Investigation of within-person cyclicity of depressive symptoms (aim II)

To address aim II, we analyzed cyclical fluctuations of depressive symptoms and compared them between participants and symptoms.

Cyclicity was found to have significant between-person differences in all assessed depressive symptoms when modeled by the cosine function including random effects. This indicates that averaging a cosine function across all participants does not adequately describe individual change; instead considering between-person differences in cyclicity is necessary. The parameter estimates from the multilevel models can be derived from Table 3.3. Because all symptoms showed significant between-person differences in cyclicity, all symptoms were further analyzed and their patterns were described on an individual level.

The comparison of cyclicity coefficients yielded no significant difference between the depressed and non-depressed groups, except for symptoms of diminished interest (p = .042) with higher mean absolute cosine coefficients in the depressed group (mean = 0.15 ± 0.07) compared to the non-depressed group (mean = 0.11 ± 0.08), and suicidality (p = .409) with slightly higher mean cosine coefficients in the non-depressed group (mean = 0.02 ± 0.03) than in the depressed group (mean = 0.01 ± 0.01).

Table 3. 3

Parameter estimates from multilevel models estimating cyclicity across all participants

Symptom	Fixed effect cosine function	CI fixed effects	SD random effect cosine function	CI for SD of random effect
Diminished interest	0.13	[0.06 - 0.19]*	0.15	[0.02 - 0.23]*
Depressed mood	0.16	[0.07 - 0.24]*	0.29	[0.21- 0.37]*
Sleep problems	0.05	[-0.08 - 0.17]	0.33	[0.21- 0.46]*
Low energy	0.19	[0.10 - 0.27]*	0.22	[0.10 - 0.32]*
Changed appetite	0.10	[0.00 - 0.20]*	0.33	[0.24 - 0.42]*
Feeling worthless	0.07	[0.01 - 0.14]*	0.18	[0.11 - 0.25]*
Concentration problems	0.07	[-0.01 - 0.14]	0.23	[0.16 - 0.31]*
Reduced movement/Restlessness	0.07	[0.01 - 0.12]*	0.19	[0.14 - 0.24]*
Suicidal ideation	0.01	[-0.00 - 0.03]	0.04	[0.01 - 0.06]*
Sum score	0.84	[0.42 – 1.21]*	1.24	[0.85 - 1.64]*

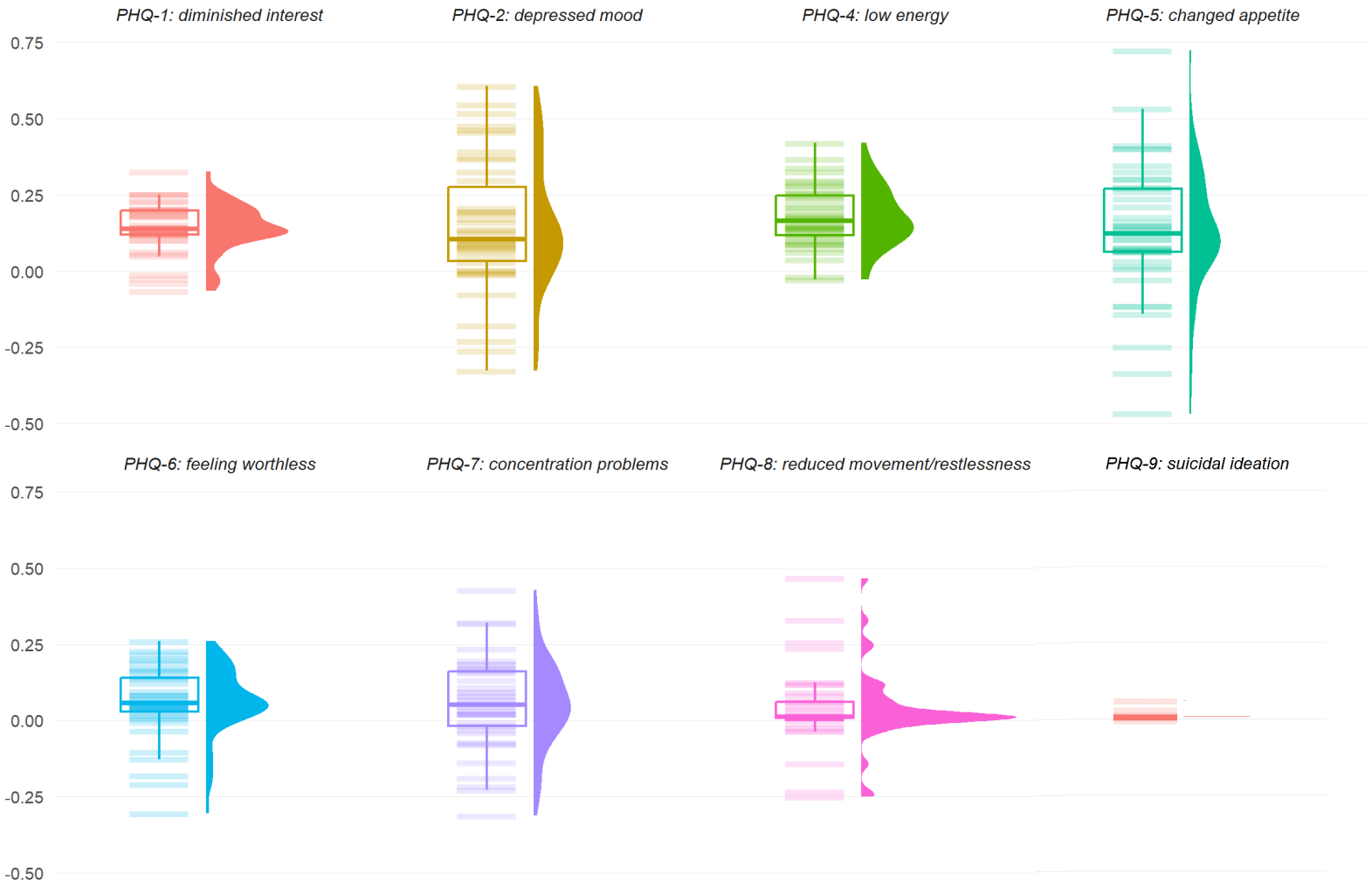
Note: CI = 95% confidence interval, SD = Standard deviation, *confidence intervals, that do not include zero are marked as significant.

The two cyclical patterns (perimenstrual or mid-cycle exacerbation of symptoms) proposed by Kiesner et al. (2016) could also be identified in this sample's participants. Figure 3.1 provides such exemplary cycle trajectories. The majority of participants showed a positive cosine coefficient, indicating a perimenstrual worsening of symptoms (sum score: 85.7 %, diminished interest: 92.21%; depressed mood: 77.92%; changed appetite: 75.32%; feeling worthless: 79.22%, concentration problems: 68.83%; reduced movement/restlessness: 81.82%; suicidal ideation: 94.81%; sleep problems: 59.74%). Across all symptoms, between 0% (suicidality) and 31.2% (depressed mood) of participants showed a cosine coefficient larger than 0.25.

Figure 3.2 shows a raincloud plot representing the mean and distribution of participants' cyclicity coefficients, separately for all symptoms. The absolute values of cosine coefficients (as measures for cyclicity strength and not pattern) of single symptoms significantly differed from each other in 19 out of 28 possible comparisons. Unsurprisingly, comparisons of suicidality (which showed low variance and low expression of cyclicity coefficients) yielded especially high effect sizes with strong significance when compared to symptoms with rather high mean cyclicity coefficients such as diminished interest (see Figure 3.2). The detailed results of pairwise t-tests and a visualization of their effect sizes can be derived from Appendix E.

Figure 3. 2

Raincloud plot of individual cosine coefficients as a marker for cyclicity for different symptoms of depression.



Note. Coefficients are derived from the afternoon measurement. PHQ = Patient Health Questionnaire.

3.4. Discussion

3.4.1 Summary and interpretation of results

This study investigated menstrual cyclicity of depressive symptoms in a sample of 77 participants with and without current depressive disorder. The results generate knowledge on menstrual cycle-related biological underpinnings regarding the etiology and development of depression and other disorders that incorporate depressive symptoms.

Using an ambulatory assessment design with three measurements per day, we investigated the internal consistencies of depressive symptom trajectories when measured in the morning, afternoon and evening, across one menstrual cycle. The reliability analyses of the three diurnal assessments revealed reliability estimates ranging from 0.56 to 0.78. Except for Item 8 (reduced movement/restlessness), the three daily assessments seem to follow rather similar trajectories across the cycle.

When investigating how depressive symptoms fluctuate across the menstrual cycle (i.e., examining cyclic patterns), multi-level-models revealed significant individual differences in cyclicity, confirming hypothesis 1. Cyclicity coefficients did not differ significantly between participants with and without depressive disorder, except for diminished interest and suicidality, with higher absolute cosine coefficients as a measure for cyclicity in depressed participants. Further, the intensity of cyclicity varied between single depressive symptoms, both descriptively (Figure 3.2) and when statistically comparing cyclicity coefficients between symptoms. This confirms hypothesis 2.

3.4.1.1 Aim I: Interpretation of results - Reliability of daily assessments

The reliability estimates of the three diurnal measurements varied across symptoms with estimates ranging from 0.56 to 0.78. On the one hand, these estimates indicate, that the single measures were not perfectly consistent ($\omega = 1$) and three daily measures improve the

cyclicity measurement. On the other hand, the reliability estimates of the three daily measures were mostly moderate, indicating that the trajectories across the cycle represent the same underlying latent variable causing cyclicity. Considering practical constraints and participants' burden, one could argue for one daily measurement when examining menstrual cycle-related fluctuation of symptoms across the menstrual cycle.

The aggregated PHQ score showed the highest reliability estimate, which is expected because an aggregated measure is less sensitive to measurement errors (Shavelson et al., 1989; Wittmann, 1988). Accordingly, when considering single symptoms measured with single items, reliability was lower. An inspection of the factor loadings from these analyses revealed that the afternoon assessments had the highest loadings, indicating that these assessments are the best indicator of the latent variable driving symptom variability across the cycle.

The practical implications of these reliability analyses are diverse and depend on future studies' constraints. Ideally, studies interested in day-to-day variations of single symptoms across the menstrual cycle would measure symptoms multiple times a day. Subsequent analyses could then work with the resulting latent variable that corrects for unreliability due to within-day variation. One could even improve such study designs by including multiple items per assessment occasion. Considering participant burden and potential other constraints, such ideal designs might not be realistic. Choosing the best indicator of the latent variable, the afternoon assessment might be the next best alternative. In case practical reasons speak against planning assessments on afternoons due to more variable daily routines at this time of the day, it seems especially relevant to increase reliability through the inclusion of multiple items per symptom on morning- or evening assessments. In contrast to the PHQ-9, the Daily Rating of Severity of Problem (DRSP; Endicott et al., (2006)) assesses some symptoms with multiple items (e.g. increased appetite/food cravings), and might thus be considered as an alternative for these

symptoms. Future cycle research could benefit greatly from the development of even more precise item batteries that assess single depressive symptoms.

In summary, these results show that if one is interested in investigating cycle-related variation of depressive symptoms, multiple items for each symptom and/or multiple daily assessments can be recommended to gain more precise results. At the same time, reducing assessments and the number of items is especially relevant in menstrual cycle research where daily measures are necessary, ideally across multiple cycles, and the burden for participants is high. Using one measurement timepoint – either with an aggregated measure, measuring in the afternoon, and/or using multiple items per symptom - can optimize feasibility and should yield reliable measurements.

3.4.1.2 Aim II: Interpretation of results - Within-person cyclicity of depressive symptoms

As revealed by multilevel models, depressive symptoms show significant individual differences in cyclicity of depressive symptoms, confirming hypothesis 1. This is in line with previous research, in which individual differences in symptom sensitivity to hormone fluctuation were revealed repeatedly (Eisenlohr-Moul, 2019; Schmidt et al., 1998). This concept of hormone sensitivity stating that some individuals suffer from abnormal symptom sensitivity to normal hormone change has been investigated and strengthened in various reproductive mood disorders (i.e. premenstrual dysphoric disorder, perinatal depression, or perimenopausal-onset depression) for the last three decades (Bloch, 2000; Schiller et al., 2022; Schmidt et al., 2017, 1998). Even though the exact mechanisms for increased sensitivity to hormone change remain poorly understood, individual differences have been observed across disorders and studies. Our results confirm the presence of individual differences in hormone sensitivity for depressive symptoms in participants with and without a current depressive disorder. The results emphasize that an overall measure of cyclicity, summarizing all participants, is highly problematic and can result in underestimations of individual cycle

effects. This is especially relevant when taking different cyclicity patterns into account (perimenstrual vs. mid-cycle worsening of symptoms), which can cancel each other out if averaged (Kiesner et al., 2016).

Further, our results indicate differences in cyclicity patterns between single depressive symptoms, confirming hypothesis 2 This is in line with previous research showing that single (affective) symptoms had especially high hormone sensitive properties (Andersen et al., 2023; Eisenlohr-Moul et al., 2016; Kiesner et al., 2016). Furthermore, this emphasizes that summarizing depressive symptoms into one score can lead to a loss of information on cyclicity; symptoms with low cyclicity might mask cyclicity effects of other symptoms.

In summary, our results indicate that cyclicity is person-specific and item-specific. Future studies should use a symptom-specific approach and allow for between-person variance in cyclicity to reduce the risk of information loss or dampened effects due to averaging symptoms or participants.

3.4.2. Limitations

The first limitation of the study is that only one menstrual cycle was investigated per subject. Measuring at least two cycles would have been preferable, but the burden of multiple daily questionnaires for one cycle was already high. The implications of this paper contribute to the future possibility of using one daily measure while still achieving reliable results (e.g., through focusing on the afternoon assessments while working with sum scores or multiple items per symptom).

Secondly, the group comparison is limited by a solely dichotomous group assignment. Even though the SCID-CV is the gold standard in assessing depressive disorders (Shabani et al., 2021), a number of participants reported subclinical depressive symptoms. Therefore, a more

dimensional approach would be preferred in future studies with larger power to determine the precise associations between depressiveness and cyclicity.

Thirdly, limitations need to be considered regarding two items from the PHQ-9: reduced movement/restlessness (Item 8) and suicidality (Item 9). Participants reported confusion about Item 8 because it incorporates two experiences in one – reduced movement *or* restlessness. This resulting ambiguity might have caused the rather low reliability estimate ($\omega = 0.56$). Therefore, the results of reduced movement/restlessness need to be considered with caution. Further, the item on suicidality showed very low variance across assessments (e.g. see Figure 3.2). This is most likely caused by active suicidal ideation being an exclusion criterion for this study. Therefore, it is not possible to provide generalizable statements on the cyclicity of suicidal ideation based on this sample. However, as reviewed by Owens and Eisenlohr-Moul (2018), there are indications for cyclical fluctuations of suicide risk and this should be further considered in future studies. Additionally, practical and ethical reasons to monitor suicidality in a clinical study on depressive symptoms might speak for including the suicidality item regardless of its poor reliability and low variance.

Lastly, the time frame for ovulation testing was based on self-reported cycle length and regularity. While self-reported cycle length has regularly been shown to be sufficiently reliable, previous research revealed higher irregularity in samples with depressive disorders (Klusmann et al., under review). Accordingly, it is possible that the time windows for ovulation testing were not accurately classified due to a higher chance of irregular cycles. Furthermore, to confirm ovulation, only LH rise was assessed and no additional ovarian steroid concentrations were measured. While this procedure complies with current guidelines on menstrual cycle assessment, daily measures of ovarian steroid concentrations should be added in future studies to confirm ovulation through a rise in progesterone.

3.4.4 Conclusion

On a larger scale, this study emphasized, that depressive symptoms can be systematically influenced by the menstrual cycle and therefore it is of utmost importance to include the menstrual cycle in research on depressive symptoms. It is highly imperative to increase our understanding of female-specific factors of depression, as this may reduce personal burden, and enhance both efficacy and cost-efficiency of mental health care for women. Research on the role of ovarian steroids and the menstrual cycle in depression etiology leaves many open questions, such as the exact biological underpinnings of symptom sensitivity to hormone fluctuations or person-tailored and more effective treatment options for cyclicity of depressive symptoms. As of now, this study, along with others, highlights that the menstrual cycle needs to be considered when aiming at understanding depressive symptoms thoroughly.

3.5 References for study 1

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Author Contributions for study 1

Hannah Klusmann, Sarah Schumacher and Sinha Engel designed the study. Hannah Klusmann conducted the investigation.

Sebastian Laufer assisted with data acquisition. Hannah Klusmann, Annette Brose, Lars Schulze and Elise Bücklein set up the analysis plan. Hannah Klusmann performed the data analysis. Elise Bücklein, Lars Schulze and Sebastian Laufer assisted with application of R for the data analysis.

Hannah Klusmann drafted the manuscript. All authors critically revised the manuscript.

Sarah Schumacher and Christine Knaevelsrud supervised the study.

CHAPTER 4

Study 2:

Higher depressive symptoms in irregular menstrual cycles - converging evidence from cross-sectional and prospective assessments

Klusmann, H., Kapp, C.., Engel, S., Laufer, S., Schumacher, T., Bücklein, E., Knaevelsrud, C., & Schumacher, S. (under review). Higher depressive symptoms in irregular menstrual cycles - converging evidence from cross-sectional and prospective assessments. *Psychopathology.*

Higher depressive symptoms in irregular menstrual cycles - converging evidence from cross-sectional and prospective assessments

Short title: Depression and menstrual cycle irregularity

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Abstract study 2

Background: Menstrual cycle regularity is an important marker of reproductive health and associated with physiological and psychological illnesses, as well as experiencing stress. We hypothesized that individuals with irregular menstrual cycles report higher depressive symptom severity, after controlling for stress occurrence.

Methods: The hypothesis was examined through two measurement approaches: a cross-sectional and a prospective, longitudinal study. In the cross-sectional study, participants (n = 394) reported depressive symptoms and their overall menstrual cycle regularity. In the longitudinal study, participants (n = 77; 37 with depressive disorder) completed questionnaires on depressive symptoms and stress during the mid-follicular and periovulatory phase of one menstrual cycle. Depressive symptoms were compared between participants with regular and irregular cycles through a Welch t-test and an ANCOVA.

Results: Participants with irregular menstrual cycles reported more depressive symptoms in the cross-sectional analysis. Similarly, in the longitudinal analysis, the group with a current irregular menstrual cycle reported more depressive symptoms after controlling for stress occurrence. When including only complete data sets without multiple imputation (n = 52), the direction of the effects remained, but did not reach statistical significance.

Limitations: Although we investigated the menstrual cycle prospectively, it would have been more precise to include two or more cycles and daily sex hormone measurements. Further limitations were the suboptimal statistical power and the data collection during the COVID pandemic.

Conclusions: The results indicate an association between depressive symptoms and menstrual cycle irregularity. We give recommendations on how to incorporate this in future study designs on women's mental health.

4.1 Introduction

Menstrual cycle characteristics are highly important markers of female reproductive health (Mumford et al., 2012; Sasaki et al., 2016). Typical menstrual cycles approximately last 25-35 days with substantial variation in length between women[4] (Diedrich et al., 2013; Fraser et al., 2011). Deviation in cycle length stems from differences in the follicular phase (onset of menses to ovulation). The luteal phase (ovulation to onset of next menses), has a more consistent length, determined by the life span of the corpus luteum (Fehring et al., 2006). Above interindividual differences in average length, intraindividual regularity of length has been associated with a higher risk for some somatic and mental illnesses (Bleil et al., 2013; Solomon et al., 2002, 2001; Toffol et al., 2014). Influencing factors of cycle regularity are age (Creinin et al., 2004; Harlow et al., 2000), night work (Lawson et al., 2011), jet lag (Baker and Driver, 2007), excessive exercise (Torstveit and Sundgot-Borgen, 2005), and stress (Acevedo-Rodriguez et al., 2018; Ayrout et al., 2019; Wagenmaker and Moenter, 2017). Psychological symptoms related to menstrual cycle irregularity have been investigated by Nillni et al. (2018), who estimated a 63% greater prevalence of irregular menstrual cycles for pregnancy-planners reporting severe depressive symptoms. Similarly, Yu et al. (2017) found an increased chance of irregular menstrual cycles in adolescents who reported depressive symptoms within the previous twelve months. Importantly, neither study assessed severity of depressive symptoms continuously. Moreover, Yu et al. (2017) investigated exclusively adolescent participants, which show unique menstrual cycle characteristics (e.g. high rate of anovulatory and/or cycles, lower progesterone concentrations) and can therefore differ compared to an adult population (Carlson and Shaw, 2019; Gunn et al., 2018).

This study aims to investigate the association between menstrual cycle irregularity and

[4] The term “women” is often incorrectly restricted to individuals with the female biological sex instead of gender. In the following text we will use a more precise description of the designated group, wherever possible.

depressive symptoms by incorporating a cross-sectional and longitudinal design in adult female humans. Thereby, we either controlled for or eliminated other influencing factors of menstrual cycle regularity, specifically stress occurrence, age, jet lag and night work. We hypothesized that women with an irregular menstrual cycle report higher depressive symptom severity than participants with a regular menstrual cycle. Further, this study contributes to the foresighted planning of future studies by discussing how to address menstrual cycle irregularity when assessing depressive symptoms.

4.2 Methods

The hypothesis was investigated with two approaches: A cross-sectional and a longitudinal study. In both, depressive symptoms were compared between participants with and without irregular menstrual cycles. Depressive symptoms were defined by the criteria for major depressive episode (MDE) in the Diagnostic and statistical manual of mental disorders (DSM V) (American Psychiatric Association, 2013). Data for both studies was collected between January 2020 and May 2021.

This is a secondary analysis of a study on depression and stress in the menstrual cycle. It is preregistered on ClinicalTrials.gov (NCT04086316) and approved by the ethics committee at Freie Universität Berlin (ID:003.2019). Written consent was given twice, once for the cross-sectional and once for the longitudinal study.

4.2.1 Procedure

The cross-sectional study was carried out through an online questionnaire (Questback GmbH, 2020), in which participants reported demographics, cycle characteristics, and depressive symptoms, as described below.

For the longitudinal study, a subset of participants from the cross-sectional study, that

fulfilled additional inclusion criteria, was selected. Across one menstrual cycle, they reported daily if menstrual bleeding occurred and additional ovulation tests were carried out to determine menstrual cycle phase. Furthermore, participants completed a set of questionnaires on current depressive symptoms and stress occurrence at two time points: once in the mid-follicular phase (T1) and once in the periovulatory phase (T2). For more details, see 4.2.3.

4.2.2 Participants

4.2.2.1 Cross-sectional study

Participants for the cross-sectional study were recruited on social media platforms, via the university's official website, and its outpatient clinic. Exclusion criteria were male sex, shift work, age <18 or >45 years, menopause, hormonal contraception use, pregnancy in the last year, current breastfeeding, psychotropic medication in the past 6 months, lifetime diagnosis of bipolar disorders, psychotic disorders, substance-related disorders, or eating disorders, and current diagnosis of a chronic disease (for a full list of excluded chronic diseases see appendix A).

4.2.2.2 Longitudinal study

Participants for the longitudinal study consisted of a subgroup of the cross-sectional study. Due to the necessity to plan assessments in specific cycle phases, a self-reported regular menstrual cycle between 26 and 30 days was an additional inclusion criterion. Participants who planned to travel to a destination with more than one-hour time difference were excluded to account for potential jet lag. Furthermore, individuals that reported moderate to severe suicidal ideation were excluded and referred to a psychotherapist.

4.2.3 Menstrual cycle assessment

4.2.3.1 Cross-sectional study

General menstrual cycle characteristics were assessed by self-report for both studies in the cross-sectional questionnaire, including average menstrual cycle length, dates of previous and next expected menses and mean cycle length.

There is no uniform definition of the range of days in variability that is considered regular and irregular (Ludwig, 2012). Standard deviations of mean menstrual cycle length vary greatly, depending on age range and included cycle lengths, but are typically estimated between 2 and 4 days (Creinin et al., 2004; Fehring et al., 2006; Siekmann, 2016). We chose a rather strict approach and defined a cycle range of more than 5 days, i.e., variations of more than +/- 2 days around the expected onset of menstruation, as irregular.

Survey participants self-reported their menstrual cycle as either regular or not, whereas the definition of regular as +/- 2 days was provided.

4.2.3.2 Longitudinal study

In the longitudinal study, ovulation and menstrual cycle phase were assessed to ensure that the questionnaires would fall into the mid-follicular (T1) or periovulatory phase (T2). Following the guidelines of Schmalenberger et al. (2021) the mid-follicular phase was defined as days +4 to +7 after the onset of menstruation. The periovulatory phase was defined as days -2 to +1 around a positive ovulation test or, if no valid test was available, days -15 to -12 before onset of the next menstruation.

On five consecutive days, scheduled based on self-reported cycle length, participants were asked to perform a commercially available LH-test to determine ovulation (One+ step® ovulation test). They were prompted to send a photo of the test strip 10 minutes after taking the test. Ovulation was confirmed, as soon as a positive test result was followed by a negative test

result. A daily question provided via smartphone app (mobileQ; Meers et al., 2020) was used to assess current menstrual bleeding. Thereby, the exact length of the investigated menstrual cycle was determined from the first indicated menstrual bleeding up to the second time when menstrual onset was confirmed.

Participants were assigned to the irregular cycle group if the length of the longitudinally investigated menstrual cycle fell into a range of 5 days, i.e., variations of +/- 2 days, around their self-reported cycle length. All other participants were assigned to the regular menstrual cycle group.

4.2.4 Measures

4.2.4.1 Sociodemographic and health-related information

Sociodemographic for sample description (biological sex, age, relationship status, occupation) and health-related information for determining inclusion (chronic diseases, medication, hormonal contraception, menopausal status, psychological disorders, specifically psychotic, bipolar, substance-related, and eating disorders) were assessed by self-developed items in the cross-sectional questionnaire.

4.2.4.2 Cross-sectional study: assessment of depressive symptoms

Patient Health Questionnaire-8 (PHQ-8). To assess symptoms of depressive disorders, the eight-item version of the PHQ-9 (PHQ-8) was used (Gräfe et al., 2004; Spitzer et al., 1999). This version excludes the item for suicidal ideation (Kroenke et al., 2009; Kroenke and Spitzer, 2002). Each item assesses one symptom of a major depressive episode (MDE) on a 4-point Likert-Scale (0 = not at all, 3 = nearly every day). As a marker for overall depressive symptoms a sum score ranging from 0 to 24 is calculated.

4.2.4.3 Longitudinal study: assessment of depressive symptoms and stress

Structured Clinical Interview for DSM-5 disorders (SCID-5 CV, German version). The SCID-CV (Beesdo-Baum et al., 2019) was used to determine current affective disorders and to confirm absence of exclusion criteria. The following modules were administered by professionally trained, certified researchers: affective episodes (Module A), psychotic and associated symptoms (Module B), substance-related disorders (Module E), and screening questions for eating disorders (from Module I). Reliability of the SCID-5 CV is ≥.70 but may vary depending on study design, interviewer training and sample population (Beesdo-Baum et al., 2019).

IDAS-Based Symptom Measure of Depression (IDAS-b; modified version). This scale aims to represent individual symptoms of depressive disorders as latent variables. Items were in part translated and adapted from the Inventory of Depression and Anxiety Symptoms (Watson & O'Hara, 2017; Watson et al., 2008, 2012) and complemented by additional items. Participants rated how often they experienced described emotions or behaviors on that day, using a rating scale from not at all (0) to extreme (4). Depressive symptom severity was estimated by the mean of all responses, ranging from 0 to 4. Validation of the scale is in progress (Heinrich et al., in prep).

Weekly Hassles Scale (WHS). The WHS (Weckesser et al., 2019) consists of 30 items that list stressors. They are rated once regarding their occurrence in the last week (yes/no) and, if they occurred, rated for severity of perceived stress on a five-point Likert scale. Sum scores are calculated for frequency of occurred stressors (WHS_{occur}) and experienced intensity of the occurred stressors (WHS_{intens}).

4.2.5 Statistical analysis

Statistical analyses were conducted with R (v.4.0.2).

4.2.5.1 Cross-sectional study

For the cross-sectional sample, a Welch t-test for independent groups was conducted with overall menstrual cycle regularity as group variable and PHQ-8 score as dependent variable. Only complete datasets were included. To account for group differences in age and differing sample sizes, a subsequent covariate matching using optimal matching with one matching partner in each group was applied with the MatchIt package (Ho et al., 2011).

4.2.5.2 Longitudinal study

For the longitudinal study, a covariance analysis (ANCOVA) was conducted with the IDAS-b sum score as the dependent variable and WHS_{occur} and menstrual cycle regularity as predictors. Depression and stress scores were averaged over T1 and T2 to be represented in a single score.

Missing data in the longitudinal analysis were estimated with the mice package (van Buuren and Groothuis-Oudshoorn, 2011) which applies an algorithm for multiple imputation. For further analyses, the estimated parameters and confidence intervals were pooled (Heymans and Eekout, 2019). 27 of 77 records were incomplete (35.06%). For T1, eight surveys were unavailable because timepoints were missed (5 surveys) or retrospectively excluded because they did not fall in the mid-follicular phase (3 surveys). For T2, 19 surveys were unavailable because timepoints were missed (8 surveys) or retrospectively excluded because they did not fall in the periovulatory phase (11 surveys).

4.3. Results

4.3.1 Sample characteristics

4.3.1.1 Cross-sectional study

394 participants were included into the cross-sectional analysis. Of those, 83 (21.07%) reported an irregular menstrual cycle and were assigned to the irregular cycle group. 311

participants were assigned to the regular cycle group. The two groups were mostly comparable, but differed significantly in age and menstrual cycle length (age: $t(140.05)=2.11$, $p=.04$; cycle length: $t(140.61)=-3.09$, $p=.003$). Table 4.1 summarizes further group characteristics.

4.3.1.2 Longitudinal study

77 participants were included into the longitudinal analysis. 80 participants started the study, but two dropped out, because their menstrual cycle did not start within seven weeks after their previous menses and one dropped out due to unknown reasons. Of the remaining 77 participants, 18 (23.38 %) had an irregular menstrual cycle and 59 (76.62 %) a regular menstrual cycle. The two groups did not differ significantly in age nor any other examined sociodemographic characteristic, as measured by a t-test (for continuous numeric variables) or χ^2-test (for categorical variables). The mean PHQ score at enrollment was significantly higher in the irregular group ($p = .018$) They Table 4.1 summarizes further sample characteristics.

4.3.2 Results of the cross-sectional study

In the irregular cycle group, the mean depression score (PHQ-8) ($M=11.81$, $SD=5.31$) was significantly higher ($t(130.33)=-2.57$, $p=0.006$) than in the regular cycle group ($M=10.23$, $SD=5.32$). The effect size estimate was small ($d=-0.32$, 95% CI [-∞; -0.11]). Because the groups differed significantly in age, a matching procedure was applied, which showed similar results ($t(164.0) = -1.91$, $p = .029$, $d = -0.30$, 95% CI [-∞; -0.05]). The comparison is visualized in figure 4.1.

Table 4. 1

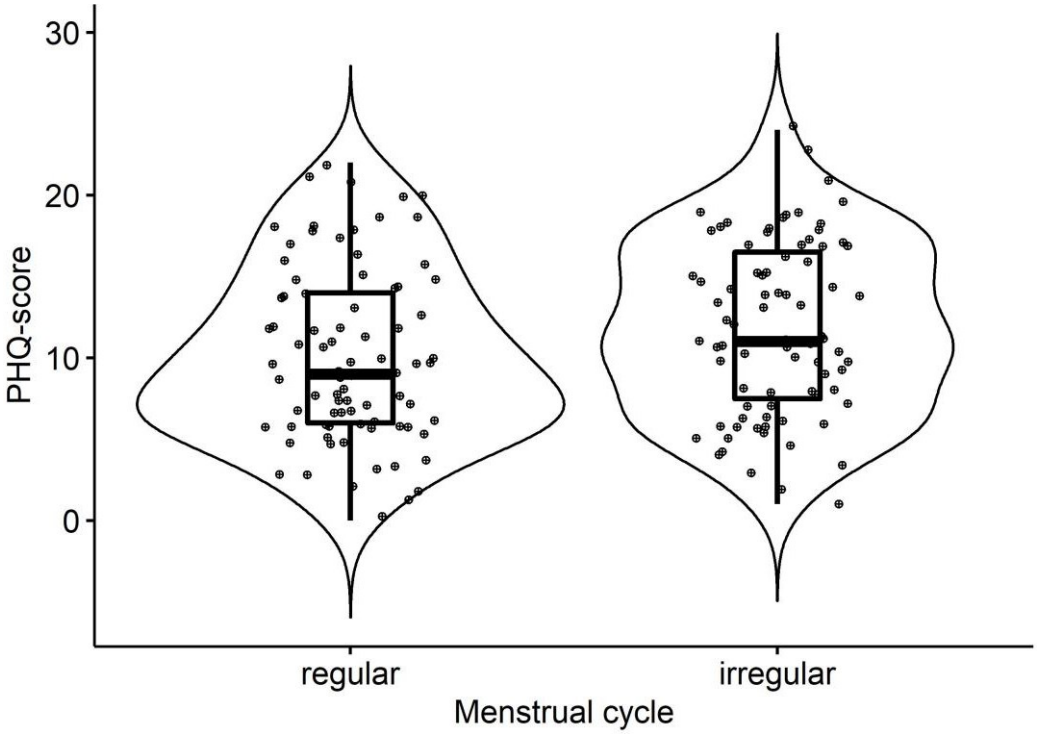
Demographic characteristics by group for both studies

	Cross-sectional study						Longitudinal study					
	Regular menstrual cycle (N = 311)			Irregular menstrual cycle (N = 83)			Regular menstrual cycle (N = 59)			Irregular menstrual cycle (N = 18)		
Variable	M	SD	%	M	SD	%	M	SD	%	M	SD	%
Age	28.02	6.32		26.50	5.73		27.92	6.91		28.55	7.04	
Menstrual cycle length	28.49	1.59		29.27	2.12		28.31	1.22		28.44	0.92	
PHQ score	10.23	5.32		11.81	5.31		9.39	5.96		13.3	5.73	
Permanent relationship[ab]			54.66			56.63			54.24			72.22
Children[ac]									10.2			22.1
Student[a]			58.71			62.65			67.80			61.11
Chronic disease[a]			2.57			1.20			6.78			5.56

Note. [a]The percentage indicates the proportion of participants to whom the status applies. [b]Dichotomous variable summarizing any current permanent partnership irrespective of legal status (e.g., marriage).[c]Percentage of participants with one or more children.

Figure 4. 1

Violin plots of depression scores by group



Note. Violin plots of depression scores (PHQ-8) by group (regular menstrual cycle versus irregular menstrual cycle) in age-matched samples (N = 166). The plot depicts a standard box-plot, the observed data and the kernel probability density of the data at different values (Kassambara, 2020).

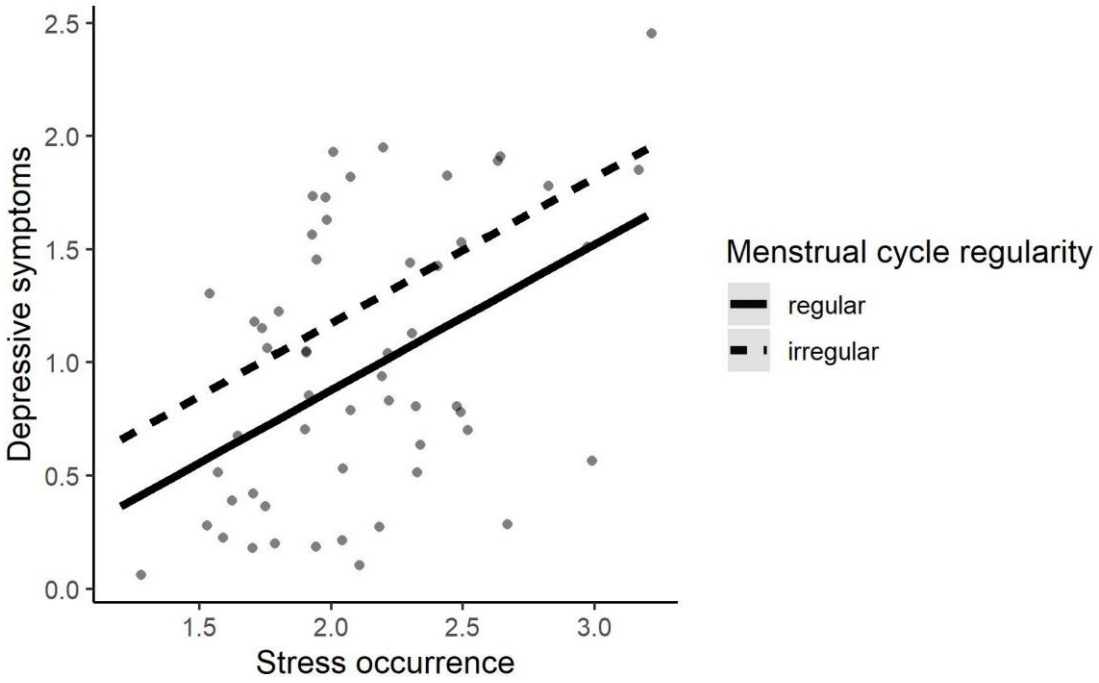
4.3.3 Results of the longitudinal study

An ANCOVA was fitted as a linear model with menstrual cycle irregularity as categorical predictor and stress occurrence as continuous predictor. In the imputed-data analysis (n=77), the pooled model estimate for the unstandardized regression weight of menstrual cycle irregularity was significantly different from zero (b=0.30, t(65.04)=2.04, p=.045), indicating that, irrespective of stress occurrence, depressive symptoms were higher in women with menstrual cycle irregularities. In the complete-case analysis (n=52), the unstandardized regression weight for menstrual cycle irregularity was estimated at b=0.38 (t(49)=1.83, p=.07, η^2_p=0.06). The direction of effect was equivalent to the estimation based

on the imputed data set but did not reach statistical significance, presumably due to the smaller sample and the loss of statistical power. Figure 4.2 visualizes the values of the model for complete cases.

Figure 4. 2

Group difference of depressive symptoms



Note. Group difference of depressive symptoms (IDAS-b) between participants with a current regular or irregular menstrual cycle after adjusting for stress occurrence (Weekly Hassles Scale). X-axis is number of reported stressful events, y-axis is mean of all items of the IDAS-b, ranging from 0 ("not at all") to 4 ("extreme").

4.4 Discussion

4.4.1 Summary of results

Evidence from a cross-sectional and longitudinal study consistently showed that women with an irregular menstrual cycle reported significantly higher depressive symptom severity than regularly cycling women. We further showed that the association between cycle irregularities and depressive symptoms was also present when controlling for relevant

influencing factors, specifically stress occurrence (by including it as covariate), age (through matching), shift work and jet lag (by excluding affected participants).

4.4.2 Interpretation of results

The observed higher depressive symptom severity in irregular cycles complements findings from Nillni et al. (2018) and Yu et al. (2017), who predicted menstrual cycle regularity from depressive symptom severity. In previous studies on depression-related conditions, such as premenstrual dysphoric disorder (PMDD), hormone fluctuation has been shown to influence affective symptoms in some hormone-sensitive individuals (Schmidt et al., 1998). Our results suggest that, beyond the regular fluctuation of sex hormones, the deviation from regular, predictable cycles could be a relevant factor in the development of depressive symptoms.

Furthermore, our study results give more insights into the accuracy of self-reported cycle lengths. Previous studies have investigated the accuracy of self-reported length and irregularity with mixed results (Creinin et al., 2004; Small et al., 2007; Weller and Weller, 1998). In the current sample, a substantial number of participants (22.5%) had irregular cycles by our definition, after self-reporting to have a regular one. Our results show that depressive symptoms are associated with cycle regularity and therefore might be a factor influencing differences between self-report and actually observed menstrual cycles. This is highly relevant when planning studies with cycling participants.

4.4.3 Strengths and limitations

The major strength of these studies is the interlocking of two methodological approaches that ideally complement each other: The results from a large, cross-sectional study that relied on self-reported cycle irregularities were validated in a longitudinal study with a smaller sample, but objective methodology for cycle assessment. We applied prospective assessments to associate cycle irregularities with depressive symptoms, and further considered

stress, which has been identified as an important correlate of both, cycle irregularities and depressive symptoms (Acevedo-Rodriguez et al., 2018; Ayrout et al., 2019; Wagenmaker and Moenter, 2017). Further, as depressive symptoms were assessed across the follicular and ovulatory phase, potential cycle phase effects on depressive symptom severity were controlled for.

A limitation of our longitudinal study is the suboptimal statistical power (25.33% for the estimated effect of $f = 0.21$), which is due to the fact that our a-priori power analysis was not based on this secondary analysis. Further, to investigate menstrual cycle irregularities even more precisely, two or more consecutive cycles as well as daily hormone measurements would be an excellent addition for future studies on the topic.

It has to be noted that both study periods were affected by the COVID-19 pandemic. Preliminary evidence indicates that the pandemic impacted menstrual cycle characteristics (Bruinvels et al., 2021; Phelan et al., 2021). Although we were not able to eliminate this problem, we tackled it by asking women to report any pandemic-related stressors they experienced. Including pandemic-related stressors did not change the results of the analyses (see appendix B).

Acknowledging these limitations and also the strengths of our two studies, their results provide evidence for an association between cycle irregularities and depressive symptoms and lay a foundation for future studies on the topic.

4.4.4 Implications for future research

Notably, our results demonstrated that 22.5% of women who reported to have a regular menstrual cycle did not fulfill this criterion in the longitudinal study. This is highly relevant for future studies that depend on predictable lengths of hormonal patterns within the menstrual cycle. Oftentimes, psychoneuroendocrinological studies investigate depressive symptoms related to the menstrual cycle, for example within premenstrual dysphoric disorder (PMDD).

Thereby, it is important to account for menstrual cycle irregularities, which can be more common with higher depressive symptoms, as our results show. To enable a reliable study design and assessment planning, we suggest taking the following practical measures when conducting a study related to the menstrual cycle: the precise definition of menstrual cycle regularity, including a defined cycle range, is essential when aiming to include regularly cycling participants, as otherwise the term “regular” may be defined differently by different people. We suggest including a cycle range of at least 7 days to account for variability more common in participants with higher depressive symptoms. A further step to determine regularity is to assess the start days of the last three menses (as suggested by Schmalenberger et al. (2021)) and to inquire how often an irregular cycle appeared within the last year. All these measures aim to identify people with regular menstrual cycles. However, it is essential to be prepared for possible irregularities, e.g., through incorporating additional assessment days before and after the expected onset of menses when using daily measures.

In general, the aim should not be to exclude irregularly cycling participants from research, but instead to further investigate psychological and physiological influencing factors. The exact biological mechanisms of these interactions and a future investigation of the causational direction of the association between depression and menstrual cycle regularity deserve attention in further studies. This is not only relevant to conduct precise and predictable studies, but also for cycling individuals with depressive symptoms, interested in natural contraception, family planning, or individualized treatment options for their symptoms.

4.4.5 Conclusion

Female reproductive health is important for all women and thus, half of the world population. The menstrual cycle is an important indicator of female reproductive health. As our studies showed, its impact goes beyond physical health, but might influence women's mental health, too. Given that cycle irregularities have been associated with depressive

symptom severity, irregular cycling women might have an elevated long-term risk to develop a depressive disorder. This needs to be elaborated in further studies. Further sex-sensitive mental health research holds the potential to transform women's mental and physical health care by developing and researching appropriate and specifically tailored prevention and treatment options.

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Author Contributions for study 2

Hannah Klusmann, Sarah Schumacher, Claudia Kapp and Sinha Engel designed the study. Hannah Klusmann conducted the investigation. Claudia Kapp assisted with data collection and data preparation.

Hannah Klusmann and Claudia Kapp set up the analysis plan. Claudia Kapp, Elise Bücklein and Hannah Klusmann performed the data analysis with R software. Hannah Klusmann drafted the manuscript. All authors critically revised the manuscript.

Sarah Schumacher and Christine Knaevelsrud supervised the study.

CHAPTER 5

Study 3:

Analyzing the Atypical – Methods for studying irregular menstrual cycles in adolescents and adults

Klusmann, H., Eisenlohr-Moul, T., Baresich, K., Schmalenberger, K.M., Girdler, S. & Andersen, E. Analyzing the Atypical – Methods for studying irregular menstrual cycles in adolescents and adults.

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Analyzing the Atypical – Methods for studying irregular menstrual cycles in adolescents and adults

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Abstract study 3

Background. The female pubertal transition is characterized by a rapidly changing hormone milieu, which is heavily influenced by the first menstrual cycle – menarche. The first year following menarche is associated with menstrual cycles that are irregular and anovulatory. Peripuberty also marks the beginning of a female-biased risk for suicidality and depression, suggesting some influence by the menstrual cycle and ovarian hormone fluctuations. However, there are limited methods and guidelines for studying the menstrual cycle and related affective symptoms in this developmental window. Thus, this study's objective was to identify the most accurate methods for detecting ovulation in irregular cycles (Part 1) and develop guidelines based on these methods for determining menstrual cycle phases. These methods were applied to investigate hormones and affective symptoms based on cycle phase and ovulation status in a sample of peripubertal females (Part 2).

Methods. Thirty-two peripubertal females (ages 11-14) provided daily urine samples of estrogen (E1G) and progesterone (PdG) metabolites and luteinizing hormone (LH), and ratings of affective symptoms for one menstrual cycle. Ten literature-derived methods for determining the presence of an LH-peak or PdG rise were compared, focusing on their feasibility for psychological research.

Results. Methods by Sun et al. (2019) and Park et al. (2007) most accurately detected PdG rises and LH peaks in this sample, identifying 40.6% of cycles as ovulatory. As expected, ovulatory participants showed greater LH in the periovulatory phase (p=.001), greater PdG in the mid-luteal phase (p<.0001), and greater E1G in the periovulatory phase (p=.001) compared with anovulatory participants. Participants with anovulatory cycles had greater affective symptoms of loneliness (p=.032) and difficulty concentrating (p=.025).

Conclusions. Recommendations and guidelines for studying the menstrual cycle in irregular cycling adolescents and adults are offered. Novel methods for ovulation detection identified phase-specific hormonal and affective symptom patterns in irregular adolescent cycles.

5.1 Introduction

The pubertal transition (peripuberty) is characterized by extensive physical maturation and a rapidly changing reproductive hormone milieu. Aside from these biological changes, peripuberty marks the beginning of a sex disparity in risk for psychopathology that continues throughout the female reproductive lifespan. Rising ovarian hormone levels and their increased fluctuation across the menstrual cycle may contribute to this abrupt rise in vulnerability to psychopathology (i.e., suicidal attempts, depression) in peripubertal females (Angold and Costello, 2006; Owens et al., 2020; Schiller et al., 2016; Thapar et al., 2012). Given the challenges of assessing the often anovulatory and irregular cycles of peripubertal participants, psychological symptoms accompanying hormone changes are rarely studied. The present study sought to fill this critical research gap by providing novel recommendations for studying the menstrual cycle in peripubertal participants and samples in which irregular cycles are common (e.g., perimenopause).

5.1.1 The peripubertal menstrual cycle

The onset of menstrual cycling, or menarche, is a biological, and for some, also a psychosocial milestone in female adolescent development that typically occurs between the ages of 12 and 13 (American Academy of Pediatrics et al., 2006). Across the menstrual cycle, hormones, including estrogens, progesterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) finely coordinate the growth of follicles and the release of an oocyte into the uterus (ovulation) for fertilization (Bale and Epperson, 2017). Absent a pregnancy, the endometrium is excreted through menstrual bleeding and a new cycle starts. These hormones fluctuate systematically throughout the cycle and thereby characterize distinct phases of the cycle (mid-follicular, periovulatory, mid-luteal, and perimenstrual phases).

The first year post-menarche, also referred to as the first gynecological year, is associated with irregular cycle lengths and frequent anovulatory cycles (Gunn et al., 2018; WHO, 1986). The mean cycle length in the first gynecological year ranges from 32 to 61 days with high variability (Gunn 2018). This contrasts with adult (>18 years) menstrual cycles that have a more stable mean length of approximately 28 days, with normal variations between 22 and 36 days (Fehring et al., 2006). Gunn et al. (2018) also reported that prevalence of ovulatory cycles in the first gynecological year ranges from 0 to 45%. Furthermore, shorter luteal phases and/or lower progesterone concentrations compared to adults are common (Carlson and Shaw, 2019). In general, female adolescents show lower mean output of estradiol and progesterone concentrations than adults throughout the cycle, yet equivalent concentrations of LH across the cycle (Carlson and Shaw, 2019; Sun et al., 2019a). Cycle irregularity and anovulation often continue after the first gynecological year. It can take six years until normal cycle length is established (American Academy of Pediatrics et al., 2006). Anovulatory cycles show different hormonal patterns from ovulatory cycles; specifically, lower overall concentrations and smaller amplitudes of estradiol, and LH and almost no progesterone output (Hambridge et al., 2013).

Characterizing the menstrual cycle and distinguishing its phases is critical to investigating underlying hormonal patterns and related risk for psychological symptom changes during peripuberty (see 1.2). Recently, guidelines on how to study the menstrual cycle in adults have been developed (Schmalenberger et al., 2021), and thresholds for biological markers to determine cycle phases have been proposed for various specimens (i.e., blood, serum, and urine) (Arslan et al., 2022; Barbieri, 2014; Fiers et al., 2017) using different analysis methods (Tivis et al., 2005). Yet, these recommendations are unsuitable for peripubertal cycles due to their irregularities in ovulation, cycle length, and hormone concentrations discussed previously (Yu et al., 2017). Frequent transvaginal ultrasounds are considered the gold standard for determining ovulation (Ecochard et al., 2001; Lynch et al.,

2014); however, this approach is not feasible for most research studies. Alternative methods for determining ovulation in the early years post-menarche are scarce and require additional study guidelines.

5.1.2 Impact of the menstrual cycle characteristics on psychological symptoms

Across the last quarter century, a series of experimental studies have demonstrated that affective symptoms tied to reproductive events are caused by an aberrant "hormone sensitivity" to normal hormone changes, although the underlying neurobiology of this sensitivity remains poorly understood. Hormone sensitivity has been experimentally demonstrated in patients with premenstrual dysphoric disorder (PMDD; luteal phase confinement of affective symptoms; Schmidt et al. (2017)), perinatal depression (Bloch, 2000; Schiller et al., 2022), and perimenopausal-onset depression (Schmidt et al., 2015). In vulnerable females, hormone-induced affective symptoms can be triggered by periovulatory surges in progesterone metabolites (Martinez et al., 2016; Schiller et al., 2014; Schmidt et al., 1991) as well as by perimenstrual estradiol withdrawal (Barone et al., 2022; Eisenlohr-Moul et al., 2022). In addition to rising hormone concentrations and variability that accompany puberty, the cyclical sources of hormone-sensitive affective symptoms described above are expected to emerge and increase risk of psychopathology at peripuberty.

We recently demonstrated that many peripubertal females (pre- and post-menarche) show affective sensitivity to weekly changes in estrone and testosterone. However, this investigation was restricted to weekly salivary hormone and affective symptom assessments and did not consider menstrual cycle characteristics (Andersen et al., 2022). Few studies have examined affective symptoms accompanying hormone change in peripubertal females, possibly due to challenges in detecting ovulation and cycle irregularity in the first-year post-menarche. While multiple ovulation detection methods have been used in adult research, the results vary substantially and have not been tested in an adolescent population (Lynch et al., 2014).

5.1.3 Research objectives of the present study

The absence of guidelines for investigating the menstrual cycle in adolescents (including ovulation detection) inhibits the ability to elucidate the effects of the menstrual cycle and associated hormonal fluctuation in the emergence of adolescent psychopathology. The objectives of the present study were to 1) review methods for ovulation detection and empirically determine the most reliable ovulation detection method to develop guidelines and recommendations for determining ovulation and to 2) adapt guidelines for determining menstrual cycle phases for adolescents or adults with irregular cycles. As an exploratory example, we applied these recommendations to a sample of peripubertal participants and investigated effects of ovulatory status on affective symptoms. Daily dried urine samples of ovulatory hormones and affective symptom assessments in a sample of peripubertal girls (≤ 1-year post menarche) were analyzed to identify the most accurate method for detecting ovulation status (Part 1) and to characterize menstrual cycle phases (Part 2), and to exemplary compare affective symptom patterns between ovulatory and anovulatory participants.

5.2 Methods

5.2.1 Study design and procedure

Following a screening questionnaire and enrollment session, eligible participants completed daily affective symptom ratings and hormone measurements for 28 days or one menstrual cycle (up to 48 days). Forty-nine participants answered daily questionnaires on current affective symptoms for 28 days or one menstrual cycle on a study iPad. After completion and applying additional exclusion criteria (see section 2.2.), 32 participants provided sufficient cycle data to be included in this study. The first 24 participants (n = 15 of final sample) provided data for 28 days; however, because several participants showed substantially longer cycle lengths (more than 41 days), this assessment time frame was

modified to assess a full menstrual cycle of the individual up to 48 days. The protocol was approved by the Institutional Review Board at University of North Carolina at Chapel Hill and was conducted in accordance with the Declaration of Helsinki.

5.2.2 Participant recruitment and inclusion

Peripubertal participants between the ages of 11 and 14 were recruited through flyers posted in schools and the local community, mass emails to university members, postings on middle school parent websites, and word-of-mouth. Participants were assigned female at birth, physically healthy, and undergoing a natural pubertal transition. Participants were mid-puberty, determined by pubic hair growth and breast development underway but not complete according to the pubertal development scale (Petersen et al., 1988) and Tanner staging with line drawings, which was self and parent-reported. We excluded participants who were over 15 months post-menarche (based on parent's report) at the enrollment session, non-English speaking, pregnant, taking hormonal contraception, taking pharmaceutical regimens that directly alter cardiovascular or neurological function, a reported personal history of any chronic medical condition, psychotic or bipolar disorders or active suicidality (assessed using an abbreviated Structured Clinical Interview for DSM-V *(SCID-CV)* (First et al., 2017) and Columbia-Suicide Severity rating scale (Posner et al., 2008)). Parents provided written consent and adolescent participants provided written assent to participate in the study. Participants were compensated with a $180.00 gift card for full compliance.

5.2.3 Biological measures

To examine ovarian hormone concentrations and determine ovulation status for cycle phasing, urinary metabolites of estradiol (estrone-3-glucuronide, E1G) and progesterone (pregnanediol glucuronide, PdG), luteinizing hormone (LH) and creatinine were measured daily. Urine samples were collected on filter paper immediately upon awakening to capture the

peak hormone response and dried completely (at least 24 hours) before being stored in the participant's home freezer. Pregnanediol (PdG), the primary urinary metabolite of progesterone, is particularly sensitive for detecting ovulation (Carlson and Shaw, 2019). Further, dried urine sampling offers a noninvasive, non-burdensome method with superior feasibility for daily collections. Precision is comparable to serum concentrations (Newman et al., 2019) and liquid urine samples (Appendix D), while offering superior stability. At the end of the collection period, samples were retrieved and transferred to a -80°C laboratory freezer before being sent to ZRT laboratory (Beaverton, OR) for analysis.

A 6-mm hole punch, 96 well-fritted filter block and a buffered extraction solution were used to prepare the samples for analysis. Enzyme-linked immunosorbent (ELISA) assays were used for E1G, PdG and LH and a colorimetric assay for creatinine was used. Intra-assay and inter-assay coefficients of variation for EIG, PdG, and LH were between 0.6-11.6% and 4.9-18.7%, respectively. Limits of detection were 1.07 mIU/mL for LH, 4.02 ng/mL for E1G, 57.58 ng/mL for PdG, and 0.026 mg/mL for creatinine. Hormone measurements were corrected for creatinine using the following equation: analyte divided by creatinine after matching volume units.

5.2.4 Assessment of affective symptoms

Daily Record of Severity of Problems (DRSP): 11 items modified from the DRSP were used to assess affective symptoms (Endicott et al., 2006). The selected 11 items included a variety of symptoms typically associated with premenstrual dysphoric disorder, including depression, loneliness, hopelessness, anxiety, mood lability, anger, low interest, sensitivity to rejection, interpersonal conflict, feeling overwhelmed, and difficulty concentrating. Participants rated symptoms using a 6-point scale ranging from 1 (not at all) to 6 (extreme). Test-retest reliability for the DRSP is above 0.70, internal consistency scores are high, and

correlations with measures such as the Hamilton Depression Rating Scale or the Quality of Life Enjoyment and Satisfaction Questionnaire are moderate to high (Endicott et al., 2006).

5.2.5 Part 1: Determining ovulation status

5.2.5.1 Protocol for identifying ovulation

Ovulation status can be determined from ovulatory hormone concentrations if the following occur: (1) an LH peak that is (2) closely followed by a rise in progesterone. Available methods for detecting ovulation status are either based on adult hormone level thresholds or yield no consistent procedure (Lynch et al., 2014). Thus, we identified and systematically compared ten[1] determination methods in the literature to isolate the most accurate methods for identifying an LH peak and PdG rise (Apter et al., 1978; Brown, 1977; Hoff et al., 1983; Johansson et al., 1971; Park et al., 2007; Sun et al., 2019b; Testart et al., 1981; Zhang et al., 2008). Descriptions of the determination methods and their comparison can be found in Table 5.1.

(1) **LH peak detection protocol:** For normally cycling adults, LH concentrations range from 18-25 mIU/mL and triple at peak to approximately 60 mUI/mL (Barbieri, 2014). Because LH levels are likely more variable in adolescents (unpublished data), a relational criterion dependent on individual mean LH concentrations is necessary, rather than a threshold based on absolute concentrations. We applied seven relational methods used in previous publications to identify the most accurate method for the present sample[5].(2) **PdG rise in luteal phase:** Similar to LH, PdG concentrations are lower in peripuberty compared to adulthood. Therefore, absolute threshold measures are not reliable, and a relational method is necessary for determining the rise of PdG during the luteal phase.

[5] Hoff et al. (1983) and Sun et al. (2019) used the same approach, therefore six methods were derived from the seven studies

Criteria for evaluating these methods were based on 1) whether the prevalence of detected LH peaks is plausible for this age group (less than 80% of participants showing a peak), 2) a distinct LH peak (meaning only peak across the cycle) was isolated without detecting multiple minor rises, and 3) the majority of the PdG rise occurred during the luteal phase. After discarding methods that did not fulfill these criteria, a total of 6 methods were systematically tested against each other (marked in table 5.1). Two experts (HK and KB) independently applied each method to each participant of our sample and used the following rating system to assess how precisely each method detected an LH peak and PdG rise (refer to Figure 5.1 for an example). Two points were given when the method accurately detected an LH peak (or PdG rise), one point was given if the method identified a visible peak/rise but not exclusively, and no points were issued if the method failed to detect or incorrectly detected a peak/rise. A third researcher (EA) resolved rating disagreements. The methods with the highest score for accurately detecting an LH peak and PdG rise were used for all further analyses in this paper.

(3) **Combined ovulation status:** The determination of ovulation status for each participant was based on the detection of both (1) an LH peak, (2) and PdG rise, and (3) whether the LH peak preceded the PdG rise by no more than seven days. If all criteria were met, the cycle was considered ovulatory. If not all of the criteria were met, the cycle was deemed non-ovulatory/abnormal.

The classification was carried out with R Studio (R Core Team, 2021). All scripts for data preparation, determination of ovulation status and further analyses are openly accessible at https://osf.io/cmg3r/?view_only=1f824d13a9134483940a480f5f2bf7e6.

Table 5. 1

Overview of literature-derived methods to determine LH peak and PDG rise

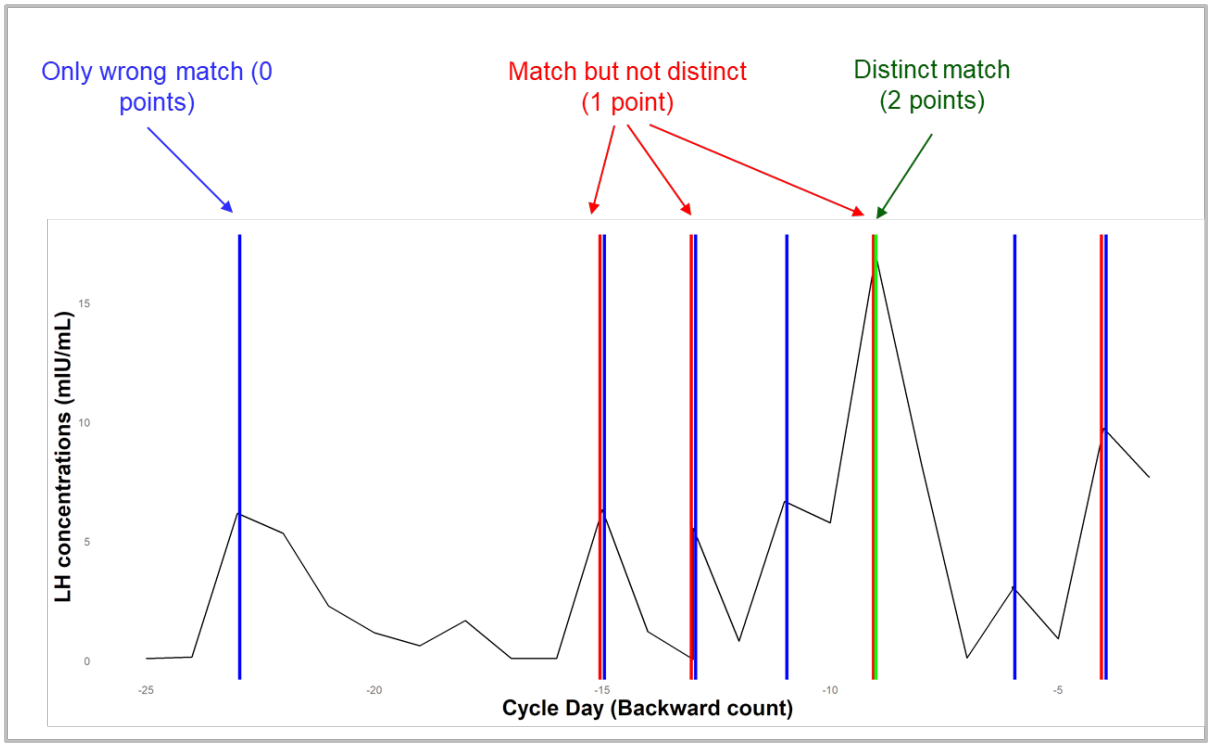
General information on method			Application to adolescent sample		
Reference	**Detection algorithm**	**Adolescent specifics**	**% of participants with detected peak**	**Mean number of LH peaks**	**Points from rating**
	LH peak detection				
Park et al. (2007)	LH-surge defined as 2.5 increase in LH from that of mean of five preceding days	No	84%	1.97	42
Brown (1977) (as cited by Lynch)	LH surge defined as first LH value exceeding +3 SD above averages of both the immediately preceding and following five days that is also of ≤ 2 days duration	No	41%	0.5	34
Johansson et al. (1971)	LH-surge defined as 4-fold increase in LH from the day before	No	62%	1.38	24
Apter et al. (1978)	Highest value	Yes	100%	1	-
Hoff et al. (1983)	First LH value exceeding the mean + 2 sd of the six preceding values	No	94%	3.19	-
Testart et al. (1981) (as cited by Lynch)	LH surge defined as LH level > 180% above the average of the preceding four LH values	No	94%	3.53	-
Zhang et al (2008)	≥ mean + 3 SD of the five preceding values	Yes	94%	3.2	-

Progesterone rise detection					
			% of participants with detected rise	**% of PdG rises, that fall in last 14 days of cycle**	**Points from rating**
Sun et al. (2019)	Threefold increase in urine Pd above mean follicular phase (FP) levels (modified Kassam algorithm)	Yes	50%	90%	31
Zhang et al (2008)	Lowest 5-day average as baseline; rise is threefold increase for at least three days, and only for PdG: ≥ 1.0 mg/g Cr	Yes	34%	85%	20
Kassam et al. (1996)	Threefold increase for three or more days compared of average of day 6-10	No	25%	100%	15

Note. * methods were applied to the dataset and compared with the rating system described in the manuscript. Methods are selected from comparison paper of ovulation detection algorithms (Lynch et al., 2014), review on peripubertal transition and hormones (Carlson and Shaw, 2019) and snowball search for further methods.

Figure 5. 1

Rating system for determining method accuracy.



Note. LH concentration curve (mIU/mL, creatinine corrected) of an example participant with peaks detected by different methods in different colors. The x-axis reflects the backward-count cycle days leading up to the subsequent onset of menses (day 0). Refer to the text for description of the rating system.

5.2.5.2 Exploratory analysis: affective symptoms by ovulation status

As an exemplary analysis to demonstrate how ovulation status might be used in studying affective symptoms, we compared individual DRSP items and the DRSP sum score between participants with and without ovulatory cycles in this sample of peripubertal females. As a fluctuation-sensitive measure, the area under the curve (with respect to ground (AUCg)) was calculated for affective symptom ratings across the menstrual cycle (Hambridge et al., 2013; Pruessner et al., 2003). The AUCg was compared between ovulatory and non-ovulatory participants using a t-test. To account for cycle length variability, and therefore number of assessments, AUCg measurements were divided by the number of assessments to compare

between participants. This analysis was conducted with R version 4.2.0 (R Core Team, 2021) and the script is accessible at https://osf.io/cmg3r/?view_only=1f824d13a9134483940a480f5f2bf7e6.

5.2.6 Part 2: Cycle phase determination

Based on the results of Part 1 and the recommendations proposed by Schmalenberger et al. (2021) for cycling adults, we propose the following recommendations for determining precise cycle phases for individuals with highly irregular cycles and short luteal phases, characteristic of the first-gynecologic year. These determination methods aim to catch specific hormonal events (levels and/or acute changes) associated with the cycle phase.

Using the dates of menses onset (before and after the assessment period) and the date of the LH peak as anchor points, as proposed by Schmalenberger et al. (2021), the mid-follicular phase (very low progesterone, rising estrogen) consists of days -7 to -3 before the LH peak, and the periovulatory phase consists of day -2 to +1 surrounding the LH peak (strong rise and fall of estrogen, slight increase of progesterone). The perimenstrual phase (falling and low estrogen and progesterone) can be classified as the four days surrounding the onset of menses (day -2 to +2), including one fewer day premenstrual than the recommendations for adults, due to shorter luteal phases. To account for typically shorter luteal phases in adolescents (median in this sample: 8 days, compared to 13.3 days currently proposed for adults; see Fehring et al., 2006, for meta-review), we recommend defining the length of the mid-luteal phase as 35% of the individual luteal phase length (rounded to full days). This individualized approach avoids relatively long luteal phase lengths that might wrongly include the periovulatory or the premenstrual phase. We recommend determining the halfway point between the LH peak and the onset of menses and anchoring the predefined phase length around this mid-point. If no halfway point can be determined since the luteal phase has an even number of days, we

recommend using the days closer to menstruation to avoid including periovulatory hormone fluctuations. If an assessment day can be classified as falling into both the mid-luteal and perimenstrual (or periovulatory) phase, we recommend assigning the day to the perimenstrual (or periovulatory) phase. A mixed model approach with phase as a repeated variable (mid-follicular, periovulatory, midluteal, perimenstrual) was used to examine phase differences in mean hormone levels between ovulatory and non-ovulatory/abnormal cycles. Thus, the measure for each cycle phase was calculated with the mean of all daily values in that phase.

The detailed procedure can be derived and replicated from the openly accessible R-script (https://osf.io/cmg3r/?view_only=1f824d13a9134483940a480f5f2bf7e6).

5.3. Results

5.3.1 Sample characteristics

Following screening and enrollment, 49 participants were enrolled in the study. Of those, 11 were excluded from the menstrual cycle analyses because they provided fewer than 75% of daily urine samples between day six and the end of the investigated cycle (7/11 participated in the shorter, unmodified protocol and 4/11 participated in the longer, modified protocol). Three participants dropped out of the study, either before starting the daily measures (n = 1), soon after starting the daily measures (n = 1) or in the middle of participation (n = 1). Further, 4 participants were excluded because the self-reported and parent-reported menarche dates differed by one or more years or the participant was more than 15 months post-menarche. One participant that fulfilled inclusion criteria at screening could not be scheduled for the enrollment session within 15 months after menarche. As such, 32 participants provided sufficient cycle data and were included in the present analysis.

The mean age of participants was 13.3 years old (SD = 0.78), the mean gynecological age (time since menarche at enrollment) was 9.66 months (SD = 4.20) (Table 5.2).

Demographic characteristics did not differ significantly between participants with ovulatory and non-ovulatory/abnormal cycles.

5.3.2 Part 1: Determining ovulation status

5.3.2.1 Ovulation Status

LH peak detection: After applying the seven methods to our dataset, four methods were immediately excluded because they detected LH peaks in 90 – 100% of participants, which is unlikely one year post-menarche (Apter et al., 1978; Hoff et al., 1983; Testart et al., 1981; Zhang et al., 2008). These methods also detected multiple LH peaks per cycle (on average three peaks for Hoff et al. and Testart et al.). The remaining three methods (Brown, 1977; Johansson et al., 1971; Park et al., 2007) were compared using the rating system described in the previous section (Figure 5.1). The interrater reliability for the doubled rating yielded moderate to almost perfect unweighted Cohen's Kappa values ($k_{Brown} = 0.94$, $k_{Johansson} = 0.89$, $k_{Brown} = 0.73$), based on the interpretation of kappa from McHugh (McHugh, 2012). The method proposed by Park et al. 2007 yielded the highest score, detecting an LH peak if the LH concentration exceeds a 2.5-fold increase of the rolling average of the last five days. The Park et al. 2007 method detected an LH peak in 84.38% of participants.

PdG rise: After applying three relational methods for detecting PdG rises to our dataset (Kassam et al., 1996; Sun et al., 2019b; Zhang et al., 2008), we confirmed that the majority of detected PdG rises were in the luteal phase and that no more than 80% of cycles showed a PdG rise, which would be implausible in this age group. The methods were compared using the described rating system. The interrater reliability for the doubled rating yielded moderate unweighted Cohen's Kappa values for the methods proposed by Kassam et al. (1996) and Sun et al. (2019b) ($k_{kassam} = 0.62$, $k_{Sun} = 0.78$) and weak interrater reliability for the method proposed by Zhang et al. (2008) ($k_{kassam} = 0.5$). The method proposed by Sun et al. (2019b) yielded the

most points. The method proposed by Sun et al., (2019b) detects a PdG rise if the PdG concentration exceeds a threefold increase of the mean PdG concentration in the follicular phase and accurately identified a PdG rise in 50% of participants.

Ovulation status: Out of the participants that showed both an LH peak and a PdG rise, three did not meet the time criterion to be considered ovulatory, with two having a PdG rise before the LH peak and one having a PdG rise 30 days after the LH peak. Five participants had neither an LH peak nor a PdG rise. Except for the three participants that missed the time criterion, all other participants with no ovulation detected had only an LH peak but no PdG rise (n = 11). This resulted in n = 13 (40.62%) participants with an ovulatory cycle and n = 19 (59.38%) with no ovulation detected.

Table 5. 2

Demographic characteristics

	Total (N=32)	Ovulatory (N=13)	Non-ovulatory (N=19)
	Mean ± SD [Min, Max] or *n* (%)		
Age	13.3 ± 0.78 [11.9, 14.8]	13.3 ± 0.81 [12.3, 14.7]	13.3 ± 0.81 [11.9, 14.8]
Gynecological age (in months)	9.66 ± 4.20 [0.77, 15.7]	10.4 ± 3.51 [3.13, 15.2]	9.10 ± 4.65 [0.77, 15.7]
Age at menarche	12.0 ± 0.84 [11.0, 14.0]	11.8 ± 0.99 [11.0, 14.0]	12.1 ± 0.73 [11.0, 13.0]
BMI	19.9 ± 3.57 [14.6, 30.2]	20.4 ± 4.04 [14.6, 30.2]	19.5 ± 3.26 [15.0, 27.3]
Member of LGTBQ community			
No	27 (84.4%)	12 (92.3%)	15 (78.9%)
Yes	3 (9.4%)	0 (0%)	3 (15.8%)
Race			
Black or African-American	5 (15.6%)	3 (23.1%)	2 (10.5%)
Asian	1 (3.1%)	1 (7.7%)	0 (0%)
White/Caucasian	21 (65.6%)	7 (53.8%)	14 (73.7%)
More than 1 race or other	5 (15.6%)	2 (15.4%)	3 (15.8%)
Ethnicity			
Hispanic and/or Latina	2 (6.3%)	1 (7.7%)	1 (5.3%)
Not Hispanic or Latina	29 (90.6%)	12 (92.3%)	17 (89.5%)
Highest education of parent			
Associate's degree	3 (9.4%)	0 (0%)	3 (15.8%)
Bachelor's degree	10 (31.3%)	5 (38.5%)	5 (26.3%)
Completed some postgraduate	4 (12.5%)	1 (7.7%)	3 (15.8%)
Law, or medical degree	2 (6.3%)	1 (7.7%)	1 (5.3%)
Master's degree	5 (15.6%)	3 (23.1%)	2 (10.5%)
Other advanced degree beyond a Master's degree	7 (21.9%)	3 (23.1%)	4 (21.1%)
Estimated annual income of parent			
Less than $20,000	3 (9.4%)	3 (15.8%)	0 (0%)
$20,000 to $49,999	2 (6.3%)	2 (10.5%)	0 (0%)
$50,000 to $99,999	15 (46.9%)	8 (42.1%)	7 (53.8%)

	Total (N=32)	Ovulatory (N=13)	Non-ovulatory (N=19)
		Mean ± SD [Min, Max] or *n* (%)	
Over $100,000	11 (34.4%)	5 (26.3%)	6 (46.2%)

Note. [1]Based on menarche date reported by parent; [2]reported by parent at enrollment. Income and education of other parent were missing for the majority of participants and therefore not included in this table.

5.3.2.2 Menstrual cycle characteristics

Menstrual cycle characteristics of included participants are reported in Table 5.3. Cycle length varied between 20 and 43 days. Of note, cycles were longer (mean = 45.27 days, SD = 11.10; min: 21 days; max: 93 days; n = 11) for the participants excluded due to insufficient data.

For the 13 participants classified as ovulatory, the LH peak was determined on average 9.53 days before the onset of next menses (arithmetic mean of backward count -9.54; sd = 2.22 days; min: -13; max: -6 days). Applying the classification of Sun et al. (Sun et al., 2019b), 9 participants had short luteal phases (5 – 9 days starting from the day after LH peak until the onset of menses). The other four participants had a luteal phase length between 10 and 14 days.

Visual inspection of hormone profiles revealed notable irregularities, including three participants with an elevation in PdG early in the cycle (within three days after menses onset), which was the sole peak or accompanied by a later PdG rise. Moreover, one participant showed an early (seven days after menses onset), and two participants exhibited a late LH peak (within two days before next menses onset). Two additional participants showed unusually early elevations of LH and PdG, however; upon closer inspection, the creatinine on the respective day was marked as low, therefore the creatinine-corrected concentration was overestimated, and the non-corrected hormone concentrations did not differ from those of the surrounding days.

Table 5. 3

Cycle characteristics and affective symptoms by ovulation status

	Ovulatory (N=13)	Non-ovulatory (N=19)	p-value
Biological and cycle characteristics			
Cycle Length			
Mean ± SD	27.8 (5.44)	27.8 (6.85)	0.979
Median [Min, Max]	27.0 [22.0, 41.0]	27.0 [20.0, 43.0]	
Follicular phase length			
Mean ± SD)	19.3 (4.21)		
Median [Min, Max]	18.0 [15.0, 29.0]		
Luteal phase length			
Mean ± SD	8.54 (2.22)		
Median [Min, Max]	8.00 [5.00, 12.0]		
LH AUCg (Mean ± SD)	16.1 (5.50)	24.5 (37.7)	0.351
E1G AUCg (Mean ± SD)	31.1 (8.99)	30.5 (13.7)	0.896
PdG AUCg (Mean ± SD)	819 (342)	499 (297)	**0.0115***

Note. N = number, SD = standard deviation, LH = luteinizing hormone, PdG = pregnanediol glucuronide, E1G = estrone-3-glucuronide; AUCg = Area under the curve with respect to ground, measure for overall output across the cycle.

5.3.2.3 Exploratory analysis: affective symptoms by ovulation status

Non-ovulatory participants exhibited significantly greater symptoms of loneliness (AUCg) ($p = .032$) and difficulty concentrating (AUCg) ($p = .025$) compared with ovulatory participants. For all other symptoms, there was a non-significant trend towards greater symptom ratings for the non-ovulatory group relative to the ovulatory group (see Appendix A, additional figures for affective symptoms in cycle phases can be found in Appendix B).

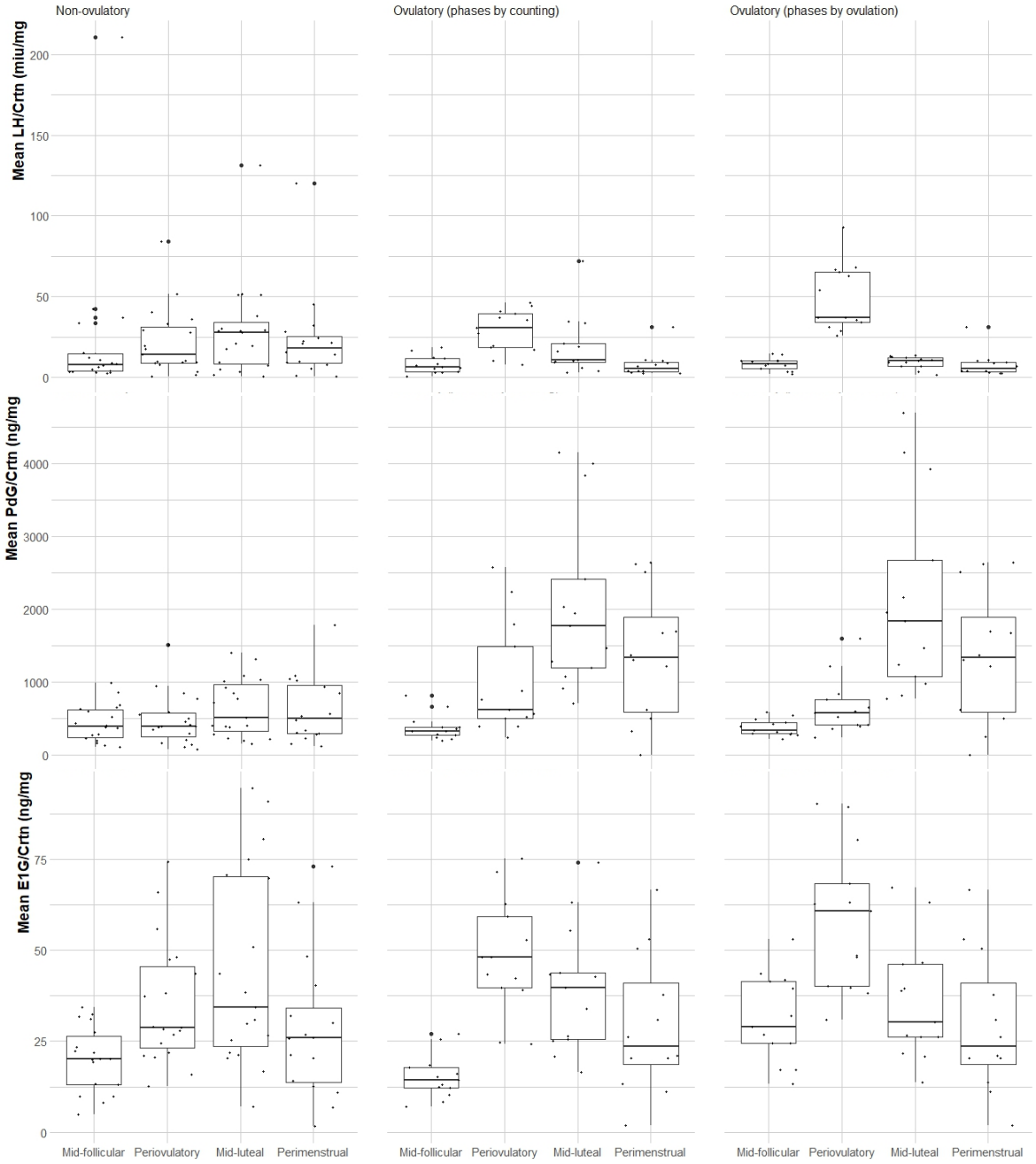
5.3.3 Part 2: Cycle phase determination

Cycle phases were determined according to the previously described procedure for all ovulatory participants. For comparison purposes, cycle phases were additionally determined

for participants with non-ovulatory/abnormal cycles using counting methods (Figure 5.3). E1g ($F(3,28)=15.05$, $p<.0001$), PdG ($F(3,20.5)=8.91$, $p=.0006$) and LH ($F(3,29.5)=9.19$, $p=.0002$) levels differed by phase, with PdG levels greater overall in ovulatory participants (Group: $F(1,29.8)=14.27$, $p=.0007$). A Group X Phase interaction was found for E1g ($F(3,28)=5.44$, $p=.005$), such that E1g was greater for ovulatory participants during mid-follicular ($F(1,25)=6.08$, $p=.021$, $\eta_p^2=.20$) and periovulatory ($F(1,30)=12.89$, $p=.001$, $\eta_p^2=.30$) phases. Additionally, PdG (Group X Phase: $F(3,20.5)=7.36$, $p=.002$) and LH (Group X Phase: $F(3,29.5)=17.01$, $p<.0001$) profiles differed by ovulatory group status (Figure 5.2). Specifically, planned pairwise comparisons demonstrated that ovulatory participants exhibited greater PdG during mid-follicular ($F(1,25)=36.50$, $p<.0001$, $\eta_p^2=.59$) and mid-luteal phases ($F(1,30)=21.10$, $p<.0001$, $\eta_p^2=.41$), and had greater LH levels during periovulatory ($F(1,30)=13.45$, $p=.001$, $\eta_p^2 = .31$) and mid-luteal ($F(1,30)=5.60$, $p=.025$, $\eta_p^2=.157$) phases compared with anovulatory participants.

Figure 5. 2

All participants' hormone concentrations by cycle phase and ovulation detection



Note. LH = luteinizing hormone, PdG = pregnanediol glucuronide, E1G = estrone-3-glucuronide. Boxplot shows the median, the 1st, and the 3rd quartile. The whisker extends from the hinge to the largest value no further than 1.5 * inter-quartile range from the hinge. For participants that were ovulatory, cycle phases were determined in relation to the day of ovulation. For non-ovulatory participants, cycle phases were determined according to counting methods as proposed by Schmalenberger et al. (2021).

5.4 Discussion

5.4.1 Summary of results

Using daily prospective ratings of affective symptoms and urinary metabolites of ovarian hormones, the present study identified the most effective methods for classifying peripubertal ovulation status based on the occurrence of a LH peak and PdG rise. After comparing ten relational methods (based on individual mean hormone concentrations and not absolute thresholds), those proposed by Park et al. (2007) for an LH peak and Sun et al. (2019b) for a PdG rise demonstrated the best fit. These methods were applied to determine menstrual cycle phases and to compare affective symptom patterns tied to hormone changes between ovulatory and non-ovulatory participants.

Using the methods proposed by Park et al. (2007) and Sun et al. (2019), 40.6 % of participants had an ovulatory cycle, which is consistent with prior reports of the proportion of ovulatory cycles within the first gynecological year (Gunn et al., 2018). By examining individual hormone trajectories, we found short luteal phases consistent with Sun et al. (2019b) and Carlson and Shaw (2019). Ovulatory participants exhibited greater concentrations of LH and E1G during the periovulatory phase and higher PdG during the mid-luteal phase, consistent with previous results (Hambridge et al., 2013); these hormone patterns were not observed in non-ovulatory participants. Further, patterns deviating from expected hormone trajectories across the cycle were found in 23% of participants, including early PdG elevations and late LH peaks. An early PdG rise and late LH peak (immediately preceding menses onset) may have resulted from misinterpreting mid-cycle spotting as menstruation (prevalent in 4.8% of regular cycling adults (Dasharathy et al., 2012)). To account for this possibility, we assessed days of bleeding to confirm menses length of at least three consecutive days.

Affective symptoms, particularly loneliness and difficulty concentrating, were greater for non-ovulatory participants. It is possible that heightened stress exposure influenced symptom expression in non-ovulatory participants, as stress has been shown to affect both menstrual cycle regularity (Acevedo-Rodriguez et al., 2018; Ayrout et al., 2019) and affective symptoms (Hammen, 2005). Future research is warranted to investigate the role of stress on hormone and affective symptom relationships and to examine biological underpinnings of potential symptom reducing effects of ovarian hormones.

5.4.2 Proposed guidelines and recommendations derived from the present study

Based on the results and study design challenges of the present study, the following guidelines and recommendations are proposed to facilitate further investigation of the peripubertal menstrual cycle, detection of ovulation status, and identification of more precise cycle phases. These guidelines are not limited to studies examining the pubertal transition, but can also be applied when investigating irregular or anovulatory cycles in other populations. Thereby, they will need to be tested and possibly adapted to particularities of conditions, including perimenopause. These guidelines are meant to evolve and adapt with advancements in our understanding of the peripubertal menstrual cycle and psychological characteristics. A more extensive checklist of these recommendations for study planning purposes can be found in Appendix C.

5.4.2.1 General recommendations for studying the menstrual cycle in peripuberty

Due to the high variability in peripubertal cycle length, we recommend specifying an assessment time frame that allows for very long and short cycles. Cycles longer than 40 days are common (28.5% in our sample before excluding participants) and almost all reviewed studies by Gunn et al. report mean cycle lengths between 32 and 45 days. Therefore, we recommend allowing at least 45 days to assess a complete menstrual cycle. It is also essential

to differentiate between mid-cycle spotting and menstruation by determining the length (through asking daily "Did you experience menstrual bleeding or spotting today?" and intensity (i.e. using pictograms of pads as reviewed by Magnay et al. (2018)) of menses.

Further, due to varying cycle lengths, irregularity in ovulation occurrence (41% ovulatory in our sample, 0-45% ovulatory as reviewed by Gunn et al. (2018)), and a high percentage of luteal phases shorter than nine days (69% in our sample, 22% reported for a sample up to 3.5 years post-menarche by Sun et al. (2019)), frequent measures of ovulation status are necessary. We recommend daily measurements to avoid missing ovulation or to be confident that ovulation did not occur. Specific methods to determine ovulation status are discussed below. Daily symptom assessments are also recommended when comparing psychological measures between precise cycle phases. If feasible, the assessment of multiple cycles is recommended to reveal stable cycle characteristics (Schmalenberger et al., 2021) and examine the effects of irregular or anovulatory cycles on subsequent cycles (Hambridge et al., 2013). This is particularly important for studies focused on cyclical affect changes, since retrospective (single timepoint) reports of cyclical affective changes have proven vulnerable to false positive reports of premenstrual affective changes (reviewed in Eisenlohr-Moul et al.(2021)).

5.4.2.2 Determine ovulation status and cycle phases

This study highlighted the variety of ovulation detection methods that produced profound inconsistencies in the detection of ovulatory cycles in peripuberty, consistent with previous research in adults (Carlson and Shaw, 2019; Lynch et al., 2014). As such, recommended methods for adults by Schmalenberger et al. (2021) are not applicable for this gynecological stage. While the gold standard for precisely assessing ovulation is a vaginal ultrasound, this method is not easily accessible or feasible for most longitudinal psychological research studies.

With this study we aimed to explore other available methods to determine ovulation status (measurement of ovarian hormones, LH test, counting methods) to provide recommendations for their accuracy. An overview of these recommendations can be derived from figure 5.3. Of note, these recommendations are meant to evolve as research progresses.

When possible, we strongly recommend measuring ovarian hormones (preferably daily) to assess ovulation status. Therefore, it is advised to use relational methods instead of absolute thresholds to determine the occurrence of LH peaks and PdG (or progesterone) rises in relation to each individual's mean hormone concentrations. Different methods derived from the literature can be found in table 5.1 and their application is presented in the open-source R script (https://osf.io/cmg3r/?view_only=1f824d13a9134483940a480f5f2bf7e6). We developed a rating system to find the best fitting method to a sample, which is described in section 2.3.1. This can be applied and adapted to other data sets and studies. In the present study, the methods by Park et al. (2007) and Sun et al. (2019b) yielded the most accurate determinations of PdG rise and LH peak (described in 2.2.3). That said, alternative methods may be superior for other age groups, assessment specimen, or hormone analysis methods. In the present study, dried urine samples provided a feasible and precise method for assessing hormone patterns and determining ovulation status, consistent with Carlson and Shaw (2019). We recommend using creatinine corrected hormone concentrations to account for hydration status, which can alter urinary biomarker concentrations. Evaluating samples with low creatinine concentrations (below 0.1mg/mL) is essential because they may skew corrected hormone levels. If frequent hormone collections are available, visually inspecting hormone curves provides a valuable method for detecting and addressing unusual patterns, which are common in peripuberty. The open-source R script provides a subscript to plot and save individual hormone curves along with detected LH peaks and PdG rises.

Figure 5. 3

Overview of ovulation detection and phasing procedure

	Progesterone + LH assessment	**LH testing only**	**Cycle day counting only**	**Estimations for planning phases in advance**
Ovulation determination	Determining LH peak & Progesterone rise (e.g. with proposed method)	Determining LH peak (yes/no)	No ovulation determination	
Cycle phase determination	**Ovulatory**[a]	**Ovulatory**[a]		
Mid-follicular:	-7 to -3 before LH peak	-7 to -3 before LH peak	+4 to +7 after menses onset	+4 to +7 after menses onset
Periovulatory:	-2 to +1 surrounding LH peak	-2 to +1 surrounding LH peak	Not recommended	-8 to -11 before next expected menses onset
Mid-luteal:	Individual phase length (35% of luteal phase) around midway point between LH peak and onset of menses	Individual phase length (35% of luteal phase) around midway point between LH peak and onset of menses	-6 to -3 before onset of menses (to be used with caution)	6 to -3 before next expected menses onset
Perimenstrual:	-2 to +2 surrounding menses	-2 to +2 surrounding menses	-2 to +2 surrounding menses	-2 to +2 surrounding menses
Remarks	High frequency of hormone measurement necessary (preferably daily)	Frequent LH testing necessary (preferably throughout the whole cycle)	***Note of Caution:*** *results may be biased, and cycle effects may be blunted when ovulation status is not evaluated.*	*These days can also be selected for comparisons of anovulatory cycles (with ovulatory cycles, as done in this study)*

Note. LH = luteinizing hormone. [a]in non-ovulatory cycles, no ovulation occurs. Therefore, the typical hormonal fluctuations across the cycle do not occur and determining "phases" is only useful when phases of ovulatory cycles need to be contrasted with days in anovulatory cycles, that are similar in length and time-point of assessment (as done in this study)

LH testing to determine ovulation status is advised when hormone assessment is impossible; however, this is less reliable as LH testing alone cannot indicate if a PdG rise is present. In our sample, the sole measurement of LH peaks (using the Park et al. 2007 method) would have resulted in falsely classifying n = 11 (34%) participants. Specifically, those with an LH peak but no PdG rise (assuming that all LH peaks detected through daily hormone measured would have been detected by an LH test). To assess the validity of LH testing, we compared LH tests and ovarian hormone measurements in another sample of older adolescents. Twelve participants used ovulation detection kits (Clearblue, sensitivity 40mIU/ml) for 10-18 days starting on days 3-5 (depending on age) and assessed dried urine every second day across one or two cycles (13 cycles available in total). Comparing ovulation status based on LH tests and the determination method based on Sun et al and Park et al., 54% of cycles (7/13) showed consistent results for the two methods (ovulatory vs. non-ovulatory/abnormal). One participant showed an LH peak with both methods but did not show a PdG rise and was therefore classified inconsistently. Three participants (25%) had a positive ovulation test, yet did not show a LH peak with dried urine testing. Two participants did not have a positive LH test but showed a LH peak and PdG rise in dried urine testing. In summary, urine LH tests with a threshold of 40 mIU/ml correctly classify ovulation in more than half of participants and can be considered superior to simple counting methods (as long as the LH testing covers the majority of the cycle); however, high frequency LH and PdG sampling remain superior for detecting ovulation in peripubertal females. Still, for most studies, ovulation detection with LH tests is more feasible for most research studies than daily hormone assessment, and thus, establishing the validity and the optimal sensitivity of LH tests for adolescent participants are important areas for future research.

When LH testing and frequent hormone samples are not available, counting methods can be used to estimate ovulation date and cycle phases. However, it is not advised to use

counting methods to identify ovulation dates, mainly because high rates of anovulatory cycles are expected. Therefore, the periovulatory phase cannot be reliably determined when only counting methods are available. Further, luteal phases can be relatively short in peripuberty (mean = 8.54 when counting the day of ovulation as day 0) compared with adults. Therefore, counting back 14 days from the onset of menses would lead to missed detection of ovulation in a large proportion of participants. In our sample, no participant showed ovulation exactly 14 days before the onset of menses. Therefore, assessment of mid-luteal phase based on only counting methods can be imprecise and might include unrecognized periovulatory days of short luteal phases. As such, we emphasize that identifying the mid-luteal phase based only on counting methods is not recommended. If conducted, it should include days very close to menses and must be interpreted with utmost caution. The perimenstrual and mid-follicular phases can be estimated using the start date of menses (before and after assessment) as anchor points (Figure 5.3).

5.4.2.3 Study design and feasibility with an adolescent sample

Overall participants were compliant, especially when parents were involved and informed about the study tasks. We aimed to make participation exciting and manageable by providing a checklist with study tasks, binder clips with the study logo, and allowed participants to use their personal smartphone device or a study-supplied tablet to complete daily affective symptom ratings. We maintained active communication with participants using the survey app's encrypted messenger and logged pictures of the sample cards' times and dates to ensure accurate timing of hormone sampling.

It is recommended and necessary to have parents fill out the screening questionnaire, and both parents and participants complete the enrollment forms to identify discrepancies (e.g., date of menarche). In the present study, the majority (61%) of participants reported a menarche date differing from their parent's response, some by over a year. This deviation may have been

caused by a falsely reported date or miscommunication between parents and the participant. To prevent these discrepancies in future studies, it is advised to ask both the parent and the participant for important information such as menarche date, and provide an opportunity for discussion so that a consensus can be reached and the date can be corrected. Further, it is recommended to systematically assess whether participants have told their parents about the onset of menstrual cycles at the time of menarche. Lastly, we recommend assessing contextual information about the time point of menarche (e.g., age and season it occurred, grade level, on a memorable family trip etc.).

5.4.3 Limitations

Despite the dense collection frequency of hormones and affective symptoms during peripuberty in the present study, results should be interpreted in light of several limitations. The focus of this investigation was to evaluate and improve methods for studying the peripubertal menstrual cycle. The protocol was refined as the study progressed to account for challenges that emerged, including extending the collection period from 28 to 48 days to capture the majority of menstrual cycle days.

While the sample size did not allow for including predictors and risk factors or focusing on individual patterns of hormone-sensitivity in more complex analyses, it was sufficient to conduct exploratory analyses as an example of the proposed recommendations. The results of these analyses need to be further investigated with larger sample sizes in future studies. Despite cycle irregularity and long cycle lengths in peripubertal participants, it is recommended to collect data for more than one menstrual cycle when investigating cycle effects. This would be important for examining individual cycle regularity and hormone trajectories of surrounding cycles.

Lastly, we did not validate the proposed ovulation detection methods with ultrasound measurements, which has been done previously (Sun et al. 2019). Further research is needed

to compare ultrasound to the different LH peak/PdG rise detection methods, as highlighted by Lynch et al. (2014).

5.4.4 Future research

Developing ovulation detection methods (algorithms to detect peaks and optimal LH test kits) and validating methods with ultrasounds on large samples of peripubertal participants is one important step for future research. Additionally, the results derived from this study indicate that ovulation status is relevant to assessing hormone-affect relationships, suggesting future research possibly including the impact of stress is warranted. Further, previous research suggests that anovulatory cycles might be affected by the hormone milieu of previous cycles and influence following cycles (Hambridge et al., 2013). Thus, it would be important to investigate longer periods of cycles to examine the interaction of these varying hormone patterns. The recommendations provided herein can be used to investigate biological underpinnings of the interaction of hormones and affective symptoms across the menstrual cycle to determine which symptoms are especially susceptible to hormone change.

5.4.5 Conclusion

Given the challenges of assessing the adolescent menstrual cycle with often anovulatory and irregular cycles, a critical research gap on reproductive mental health in adolescents has developed. The results and the derived recommendations of this study sought to inform future research on investigating menstrual cycle characteristics in samples in which irregular cycles are expected, including peripuberty and perimenopause. Results demonstrate that it is possible to investigate the menstrual cycle despite its irregularity in peripuberty and shows that menstrual cycle characteristics can be associated with affective symptom change. Further, understanding the impact of the menstrual cycle and associated hormone patterns on affective

symptoms will assist in identifying early risk factors for hormone related psychopathology during peripuberty, a critical developmental window for prevention and intervention efforts.

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Elizabeth Andersen and Susan Girdler supervised the study.

CHAPTER 6

Study 4:

HPA axis activity across the menstrual cycle - a systematic review and meta-analysis of longitudinal studies

Due to copyright reasons the article is excluded from the online version of this dissertation.

Klusmann, H., Schulze, L., Engel, S., Bücklein, E., Daehn, D., Lozza-Fiacco, S., Geiling, A., Meyer, C., Andersen, E., Knaevelsrud, C. & Schumacher, S. (2022). HPA axis activity across the menstrual cycle - a systematic review and meta-analysis of longitudinal studies. *Frontiers in Neuroendocrinology*. 66, 100998. https://doi.org/10.1016/j.yfrne.2022.100998

HPA axis activity across the menstrual cycle - a systematic review and meta-analysis of longitudinal studies

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Daria Daehn, Serena Lozza-Fiacco, Angelika Geiling, Elise Bücklein, Caroline Meyer and Elisabeth Conrad (master student) performed the data extraction and quality rating, under Sarah Schumacher's and Hannah Klusmann's supervision.

Hannah Klusmann contacted the authors of all primary studies for which data for the meta-analysis were not reported in the publication.

Hannah Klusmann and Lars Schulze set up the analysis plan and performed the statistical analyses.

Hannah Klusmann drafted the manuscript. All authors critically revised the manuscript for important intellectual content.

Sarah Schumacher, Christine Knaevelsrud and Elisabeth Andersen supervised the study.

CHAPTER 7

Study 5:

Menstrual cycle-related changes in HPA axis reactivity to acute psychosocial and physiological stressors - a systematic review and meta-analysis of longitudinal studies

Due to copyright reasons the article is excluded from the online version of this dissertation.

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Menstrual cycle-related changes in HPA axis reactivity to acute psychosocial and physiological stressors - a systematic review and meta-analysis of longitudinal studies

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Hannah Klusmann and Noemi Luecking contacted the authors of all primary studies for which data for the meta-analysis were not reported in the publication.

Hannah Klusmann and Noemi Luecking set up the analysis plan. Hannah Klusmann performed the statistical analyses. Hannah Klusmann drafted the manuscript. Meike Katharina Blecker assisted with tables and figures. All authors critically revised the manuscript for important intellectual content.

Sarah Schumacher and Christine Knaevelsrud supervised the study.

CHAPTER 8

Study 6:

Life stress influences the relationship between sex hormone fluctuation and affective symptoms in peripubertal female adolescents.

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Life stress influences the relationship between sex hormone fluctuation and affective symptoms in peripubertal female adolescents

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Abstract study 6

Female adolescents have a greatly increased risk of depression starting at puberty, which continues throughout the reproductive lifespan. Sex hormone fluctuation has been highlighted as a key proximal precipitating factor in the development of mood disorders tied to reproductive events; however, hormone-induced affective state change is poorly understood in the pubertal transition. The present study investigated the impact of recent stressful life events on the relationship between sex hormone change and affective symptoms in peripubertal female participants. 35 peripubertal participants (ages 11-14, premenarchal, or within 1 year of menarche) completed an assessment of stressful life events and provided weekly salivary hormone collections (estrone, testosterone, dehydroepiandrosterone (DHEA)) and mood assessments for eight weeks. Linear mixed models tested whether stressful life events provided a context in which within-person changes in hormones predicted weekly affective symptoms. Results indicated that exposure to stressful life events proximal to the pubertal transition influenced the directional effects of hormone change on affective symptoms. Specifically, greater affective symptoms were associated with increases in hormones in a high stress context and decreases in hormones in a low stress context. These findings provide support for stress-related hormone sensitivity as a diathesis for precipitating affective symptoms in the presence of pronounced peripubertal hormone flux.

8.1 Introduction

The pubertal transition (peripuberty) is characterized by nearly a 3-fold greater risk of a depressive disorder in female adolescents (SAMHSA, 2020). Among other factors, this increased risk may be a result of simultaneous increases in sex hormone fluctuations and interpersonal stressors in female adolescents (Balzer et al. 2015). The sex disparity first emerges at mid-puberty (Tanner stage 3 or 4) and continues throughout the female reproductive lifespan, suggesting a role of ovarian hormone fluctuations in vulnerability to psychopathology (Angold, 1993; Angold et al., 1999; Schiller et al., 2016). An abnormal neurobiological sensitivity to normal reproductive hormone flux can trigger affective symptoms in hormone-sensitive individuals. Furthermore, stress exposure interferes with the maturation of neurodevelopmental trajectories (Walker et al., 2004) and can predict who is at risk for developing reproductive mood disorders (Bromberger et al., 2011; Namavar Jahromi et al., 2011). However, the biological mechanisms by which life stress exposure interacts with the peripubertal reproductive endocrine environment to promote depression risk are poorly understood. The present study examines how within-person deviations in sex hormones may predict depressive symptoms in peripubertal female adolescents, particularly in the context of recent stressful life events.

8.1.1 Mechanisms of depression risk during the pubertal transition

8.1.1.1 Reproductive hormone environment

The pubertal transition is accompanied by extensive changes in ovarian and adrenal hormones (Andersen et al. 2022; Apter 1980; Apter et al. 1989), heightened interpersonal stress exposure and a critical window in which neurodevelopmental trajectories are established (Blakemore, 2008). Adrenarche, increased activity of the hypothalamic-pituitary-adrenal (HPA) axis, initiates the developmental hormone cascade with the production of androgens, including

dehydroepiandrosterone (DHEA). This is followed by reactivation of the hypothalamic-pituitary-gonadal (HPG) axis (i.e., gonadarche) and stimulation of sex steroid production, for example estradiol (E2), estrone (E1), and testosterone (T). Moreover, not only absolute concentrations but also the degree of hormone fluctuation increases with the onset of the first menstrual cycle (menarche)(Andersen et al. 2022; Janfaza et al. 2006). The first gynecological year (first year after menarche) is characterized by frequent anovulatory (up to 66%) and irregular cycles that further increase exposure to hormone fluctuation (Carlson and Shaw, 2019a; Gunn et al., 2018). This dramatic reproductive endocrine instability may contribute to the abrupt emergence of affective illness during the pubertal transition in female adolescents (Angold, 1993; Patton et al., 2008).

Given the significant regulatory effect of sex hormones on mood and behavior, women are more likely to experience affective dysregulation during reproductive transition events characterized by significant hormonal flux, including premenstrual, postpartum, and perimenopausal periods (Balzer et al. 2015; Schiller et al. 2016). Hormone manipulation studies simulating reproductive transition events indicate that a vulnerability to normal changes in sex hormones, rather than the absolute level, precipitate the emergence of depressive symptoms in women with a history of reproductive hormone-related mood impairment (Bloch et al. 2000; Gordon et al. 2015, 2016; Schmidt et al. 1998, 2017). Accordingly, fluctuations in sex hormones such as estrogens (e.g., estradiol, estrone), progesterone and its derivatives, and testosterone have been shown to trigger the development of affective symptoms in a subset of hormone-sensitive individuals across the lifespan (premenstrual: Schmidt et al. 1998; perinatally: (Bloch et al., 2000); perimenopausal: (Sander et al., 2021; Schmidt et al., 2015); in males: (Schmidt et al., 2004). We recently demonstrated that a large proportion of peripubertal female adolescents showed mood sensitivity to sex hormone flux. Specifically, 57% of participants exhibited increased dysphoric mood with

weekly changes (i.e., increase, decrease or large change in either direction from the previous week) in estrone and 37% of participants were mood sensitive to weekly changes in testosterone (Andersen et al. 2022). Our previous findings suggest that peripubertal female adolescents' mood sensitivity to sex-hormone flux can be categorized into four different hormone sensitivity profiles: 1) insensitive (no increase in mood symptoms with weekly change in hormone); 2) withdrawal sensitive (increased mood symptom severity with *decreases* in hormone change from the previous week); 3) increase sensitive (increased mood severity with *increased* hormone change); and 4) change sensitive (increased mood severity with large changes in hormone, regardless of direction) (Andersen et al. 2022). The identification of these different hormone sensitivity profiles highlights the importance of assessing within-person hormone variability in determining mood and hormone relationships.

8.1.1.2 Life stress exposure and HPA axis dysfunction

Along with profound physical developmental changes, the pubertal transition is characterized by elevated life stress exposure (Spear, 2009). The impact of life stress is especially relevant during critical developmental windows characterized by dramatic neurodevelopment, including childhood and adolescence (Blakemore, 2008). Exposure to life stress during these early developmental periods has been shown to modify biological stress response systems (i.e., HPA axis), and is associated with sustained alterations in brain function, behavior and cognition (Lupien et al., 2009). As such, dysregulation of the HPA axis can have long-term consequences and increase the risk for psychological disorders (De Carvalho Tofoli et al., 2011). The deleterious effect of stress may be even more pronounced for adolescents who experience earlier pubertal maturation relative to their peers (i.e., early pubertal timing)(Hamlat et al., 2014). African American or Black and Latino/Latina adolescents, youth from lower socioeconomic contexts

(James-Todd et al., 2010), and those exposed to early life adversity (Colich and McLaughlin, 2022) are disproportionately more likely to experience early pubertal timing (Colich and McLaughlin 2022). Therefore, social context and diversity may converge with the unique reproductive endocrine features of puberty to promote greater risk of psychopathology during the pubertal transition (Mendle et al., 2019).

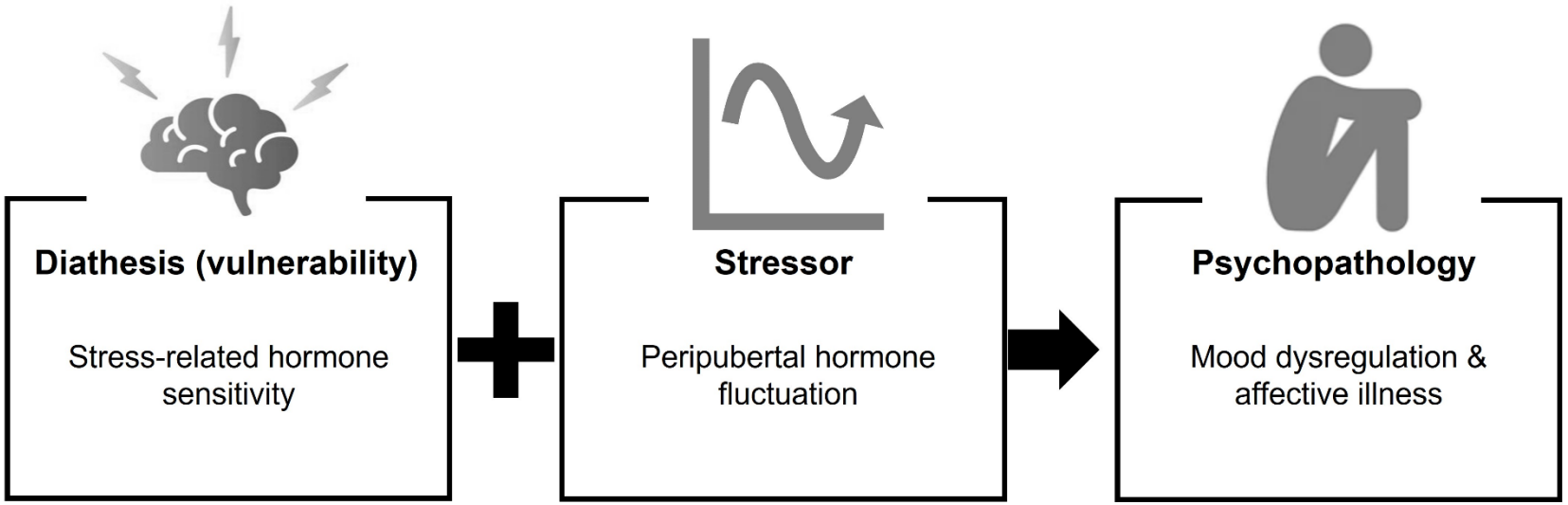
Although null findings exists (Meadows et al., 2006), a number of studies report increased stress exposure for female compared to male adolescents (Ge et al., 2001, 1994). This applies particularly to interpersonal stress (Hankin et al., 2007), possibly due to social gender roles (McLeod and Kessler, 1990; Turner et al., 1995). Female adolescents also experience increased rates of severe stressful life events, including physical and sexual abuse, which also contributes to long-term modifications in stress reactivity (LeMoult et al., 2020, 2015). Exposure to stressful life events is strongly associated with an increased risk of affective disorders, including depression (Hammen, 2005; Monroe and Reid, 2008; Paykel, 2003). The association of stress exposure and depression has been shown to be stronger in female adolescents compared to male adolescents (Ge et al., 1994; Shih et al., 2006). Further, stressful life events proximal to reproductive transition events have been shown to be strong predictors of the emergence of reproductive mood disorders (Bromberger et al., 2011). As such, in initially euthymic perimenopausal women, the number of recent stressful life events in combination with greater estradiol variability over the menopause transition predicted the emergence of depression (Gordon et al. 2016). However, this relationship between hormone fluctuation and affective state change is poorly understood during the pubertal transition, which shares many parallels with the menopause transition and is similarly characterized by substantial reproductive hormone flux and heightened life stress.

8.1.2 Aims of the current study

The present study employed a longitudinal, dimensional approach to examine within-person relationships between life stress, weekly changes in sex hormones (estrone, testosterone, DHEA) and affective state change in peripubertal female adolescents. The present study tests a novel diathesis-stress model of adolescent depression in which we propose that life stress modifies the brain's sensitivity to hormone fluctuation and precipitates vulnerability (i.e., a diathesis) to depressive symptoms in the presence of normal peripubertal hormone flux (i.e., an acute hormonal stressor). The rationale for examining stress-related hormone sensitivity as a diathesis for affective symptoms is twofold. First, sensitivity to hormonal flux during specific reproductive events has been shown to trigger affective symptoms in women who are more susceptible to stress and, second, sex hormones are powerful neuroregulators of frontal and limbic neural networks (Ottowitz et al., 2008) and the HPA stress axis, each of which is implicated in depression (Pandya et al., 2012). Specifically, the present analysis examines recent life stress as a moderator of the relationship between weekly hormone deviations and specific affective symptom constructs (e.g., interpersonal depressive symptoms, low positive affect, somatic symptoms) (Figure 8.1). The initial investigation of peripubertal hormone and mood relationships was restricted to general dysphoric mood and did not examine hormone-induced changes in distinct symptom domains (i.e., anxiety, irritability, etc.). This additional decomposition of affective symptoms is warranted given that previously reported hormone-related mood disturbances are symptom and hormone specific (Eisenlohr-Moul et al. 2016; Schiller et al. 2022; Schmidt et al. 1998). Consistent with hormone-mood relationships observed in other female reproductive transitions (i.e., premenstrual phase, perimenopause), weekly deviations in hormones (estrone, testosterone, DHEA) in the context of high life stress were expected to be associated with greater depressive symptoms and distress.

Figure 8. 1

Diathesis-stress model of hormone-related mood dysregulation and affective illness.



Note. Illustration of the proposed model in which life stress modifies the brain's sensitivity to hormone fluctuation and precipitates vulnerability (i.e., a diathesis) to depressive symptoms in the presence of normal peripubertal hormone flux (i.e., an acute hormonal stressor).

8.2 Methods

8.2.1 Participants

35 participants assigned female at birth, between the ages of 11 and 14, and who were undergoing a healthy pubertal transition were recruited from the local community using flyers, mass emails to university staff and students, word-of-mouth, and online posts on middle school parent sites. The protocol was approved by the local Institutional Review Board and was conducted in accordance with the Declaration of Helsinki. Parents provided written consent and adolescent participants provided written assent to be involved in the study. Participants received a $150.00 Visa gift card for full compliance.

8.2.2 Procedure

The study was conducted between 2018 and 2020, and consisted of three phases, including 1) screening, 2) enrollment, and 3) weekly hormone and mood measurements, described in more detail below and in Andersen et al. (2022). Participants included in the present analysis were a subsample of a larger study (n=52) who all had weekly (vs. daily) mood assessments and morning (vs. afternoon) salivary hormone collections. The present analysis with this subsample (n=35) permitted the investigation of life stress and its effect on hormone-related changes in distinct symptom constructs of depression with refined collection parameters.

8.2.2.1 Screening

Parents of interested participants completed an online screening form to assess eligibility. Participants were recruited based on parental and self-reported pubertal stage (Tanner Stage 3 or 4, breast development and pubic hair growth started, but not complete - as assessed by the Pubertal Development Scale (PDS) at screening and enrollment) and were premenarchal or within one-year

post-menarche. Non-English speakers and participants with psychotic or bipolar disorders, active suicidal ideation, or severe symptoms that would interfere with participation were excluded. To obtain a wide range and adequate variance of depressive symptoms, current self-reported clinical diagnoses of depression or anxiety were permitted if no suicidal ideation, and no symptoms of psychosis or mania were reported at enrollment. Participants did not use hormonal agents or contraceptives, or mood-altering medications (e.g., antidepressants). If eligible, the screening was followed by a phone call with the parent to discuss the specifics of the study procedure and confirm eligibility before scheduling the enrollment session.

8.2.2.2 Enrollment

At enrollment, participants and their parents completed questionnaires assessing personal and family medical history, including self-reported clinical diagnoses, demographic characteristics, mood, stressful life events, and pubertal stage. An abbreviated Structured Clinical Interview for DSM-V (SCID) was administered to screen for psychosis symptoms or bipolar disorder. Height and weight measurements were collected to calculate age-corrected BMI according to Centers for Disease Control and Prevention guidelines, as BMI consistently increases with pubertal maturation (Bini et al., 2000). Parental reports of highest educational attainment and total household income were collected and assessed as indicators of socioeconomic status. Participants and their parents were instructed on best practices for collecting saliva samples and were given clearly labeled collection vials.

8.2.2.3 Weekly hormone and mood assessments

Following the enrollment session, participants completed eight consecutive weeks of saliva and mood assessments. Using the unstimulated passive drool technique, participants provided 3mL of saliva into cryovials immediately upon awakening to capture the expected peak hormone levels

for 8 consecutive weeks (Hucklebridge et al., 2005; Janfaza et al., 2006; Kuzawa et al., 2016). To prevent contamination and dilution, participants were instructed not to visit the dentist within two days before the next collection, refrain from drinking (water included) and brushing their teeth thirty minutes prior to their collection, and refrain from eating one hour before the collection. Saliva samples were immediately placed in the participant's home freezer before being transferred to a -80°C laboratory freezer every 1-3 weeks. Weekly compliance checks were completed along with the surveys to determine if there were any changes in activity level, diet, sleep, or medication use prior to the saliva collection.

8.2.3 Measures

8.2.3.1 Enrollment assessments

Pubertal Development was self-reported using a combination of the Pubertal Development Scale *(PDS;* Petersen et al., 1988) and Tanner line staging, in which participants selected line drawings of breast development and pubic hair growth that best matched their own (Taylor et al., 2001). Line drawings corresponded to Tanner stage criteria, with 1 indicating no development ("breasts are flat"; "no hair") and 5 indicating complete development ("only the nipple sticks out beyond the breast"; "hair has spread over the thighs"). Participants rated menarche status, height or growth spurt, breast development, pubic hair growth and appearance of acne on the PDS using a 4-point scale ranging from 1) no development/no menses, 2) barely begun, 3) definitely underway, to 4) completed/menses. The PDS score was calculated as the mean of the five responses. The category PDS score corresponds to the sum of the items pertaining to pubic hair growth, breast development and menarche status, as described previously (Carskadon and Acebo, 1993). Category scores range from 3- prepubertal to 12 post-pubertal. PDS and tanner line staging scores were averaged for breast development and pubic hair growth. Self-reported PDS for female breast and pubic hair

development has shown excellent agreement with a physical examination (85% within 1 Tanner stage) (Schmitz et al., 2004) and good reliability (Cronbach's alpha for PDS in the present sample was 0.70).

Recent Stressful Life Events were assessed using a modified version of the Coddington Life Events Scale (CLES) (Coddington, 1972). Participants were asked to indicate whether or not they had experienced any of the 25 stressful experiences listed in the measure in the past year by checking 'yes' or 'no' for each item. Samples items include "death of a family member," "end of romantic relationship," "serious personal injury/illness," "change in acceptance by peers," "move or change in residence," and "parents' divorce." A total score was calculated from the aggregated number of stressful life events that the participant reported. The number of stressful life events (continuous) was standardized using a z-score.

Mood and Feelings Questionnaire *(MFQ;* Costello and Angold 1988) is a 33-item self-report inventory to assess adolescent depressive symptoms. Participants rate how they have been feeling or acting over the past 2 weeks by indicating whether each statement is not true (0), somewhat true (1) or mostly true (2) for them. The total score reflects the sum of the 33 items and ranges from 0 to 68, with larger scores reflecting greater depressive symptoms. Cronbach's alpha for MFQ in the present sample was 0.96.

8.2.3.2 *Weekly mood assessments*

The Center for Epidemiological Studies Depression Scale for Children (CES-DC) is a 20- item assessment for child and adolescent depressive symptoms (Roberts et al., 1990). Items are scored 0 (not at all) to 3 (a lot) with 4 items reverse coded for a range between 0-60. Cronbach's alpha for all 20 items in the present sample was 0.95. Previous studies on reproductive mood disorders

have reported that the psychological relevance of hormone change may be specific to certain symptom domains (Eisenlohr-Moul et al., 2016; Gordon et al., 2019). As such, the current study employed a dimensional approach to elucidate specific mood and hormone relationships. To refine the investigation of depressive symptom domains, four predefined subscales were computed (reviewed in Shafer, 2006), including interpersonal (2 items, α (present sample)=.862), low positive affect (4 items, α (present sample)= .852), depressed affect (7 items, α (present sample)=.932) and somatic symptoms (7 items, α (present sample) =.855).

The Perceived Stress Scale (for children) is a 10-item self-report assessment to evaluate perceived coping and distress associated with stressful situations (Cohen et al., 1983). Items are rated on a 5-point Likert scale from 0 (never) to 4 (very often). Summary scores were calculated after reversing 4 items. Higher scores represent higher subjective stress. Consistent with previous reports, two factors were confirmed in the current sample: a positive-worded factor associated with perceived coping and a negative-worded factor indexing perceived distress (Ezzati et al., 2014; Hewitt et al., 1992) both of which have been found to predict depression symptoms. Correlations among scale items ranged from r=.210 to r=.742, with Cronbach's α=.896 for all 10 items, α=.780 for the coping factor, and α=.889 for the distress factor.

Weekly Subjective Stress Rating. Each week, participants were asked to indicate whether they experienced "any problems" over the last week by checking a box next to relevant items or filling in their own response. The seven items included in the list were: 1) performed poorly on an exam or assignment, 2) received a bad report card, 3) felt overbooked with extracurricular activities, 4) got in a fight with a friend(s), 5) didn't get along with parents, 6) experienced bullying, 7) didn't get along with siblings, and 8) other. They were also asked to enter the most stressful event that

happened each week and how stressed it made them feel on a sliding bar from 1 (not very stressful) to 10 (extremely stressful).

8.2.4 Salivary hormone collection and analysis

Salivary samples were assayed at ZRT Laboratory (Beaverton, OR) using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to achieve the most sensitive and accurate quantification of salivary hormones. Average inter-assay precision was 7.27% for estrone, 9.77% for testosterone, and 7.03% for DHEA, with the average intra-assay coefficients of variance as follows: 9.47% for estrone, 4.20% for testosterone, and 7.27% for DHEA. Minimum detection limits were 0.4 pg/mL for estrone, 3.2 pg/mL for testosterone, and 17.1 pg/mL for DHEA. Measurements that fell below assay sensitivity were assigned a value that was one-half the limit of detection. Estrone was selected over estradiol (E2) for the present analyses because estrone rises before E2 during the pubertal transition (Biro et al., 2014) allowing for easier detection, and has been shown to be highly correlated with E2 in previous reports ($r=0.80$, $p<.0001$) (Andersen et al., 2022). Lower concentrations of E2 are expected in peripubertal females – especially premenarche (Janfaza et al., 2006).

8.2.5 Analytic Plan

8.2.5.1 Data coding and preparation

Analyses were performed in SAS On Demand for Academics. Hormone measurements were centered within-person (CWP) to extract within-person hormone variance (i.e., [hormone level at this sample] – [participant's average hormone level across all samples]). As such, greater values of hormone CWP reflect increased levels relative to a participant's individual mean (Eisenlohr-Moul et al., 2015).

8.2.5.2 Weekly hormone and mood relationships

Separate multilevel models were performed to predict each affective symptom outcome (CES-DC-depressed, CES-DC-interpersonal, CES-DC-low positive affect, CES-DC-somatic, PSS_coping, PSS_distress) from weekly deviations from an individual's average hormone level (DHEA, testosterone, estrone), number of stressful life events (SLE, sample standardized), and all associated interactions. "Week" was specified as a repeated statement to account for serial correlation of weekly hormone and mood assessments, and a random slope within-person was applied with a variance component structure. Null multilevel models (without predictors) were used to calculate the intraclass correlation coefficient (ICC) for each predictor and outcome variable (see Table 8.1). Given the small sample size, restricted maximum likelihood estimation (REML) was implemented to account for potential biases in random-effect variances and the Kenward-Roger correction was used to address biases in standard error estimation (McNeish and Matta, 2018). Age, race, parental education (as a proxy for socioeconomic status) and BMI did not significantly contribute to the models and were therefore excluded from final analyses.

The effect of stress was further probed by calculating the simple slopes of hormone (CWP) at various levels of stress for each combination of symptom and hormone pair. The simple slopes were calculated from three levels of SLE: mean SLE = 5.029, mean SLE + standard deviation (sd) = 7.578, and mean SLE – sd = 2.479.

8.2.5.3 Power Analysis

With 35 participants that completed CES-DC ratings and hormone collections, the current study had 86% power with α=0.05 to detect small effects (f=0.15) of within-person weekly hormone and mood associations (8 repeated measures with 0.50 correlation).

8.3 Results

8.3.1 Descriptive information

Demographic and sample characteristics are presented in Table 8.1. 35 participants (mean age =12.48, SD=1.01) provided weekly mood ratings and salivary hormone samples. The majority of participants identified as White (74.3%), with 11.4% identifying as multiracial, 11.4% as Hispanic/Latino or Spanish, and 2.9% Black. Participants' parents were highly educated (graduated or postgraduate) and were affluent middle to upper class, representative of the local community. Participants were self-reported mid-puberty (PDS category score: mean = 8.33, SD = 2.30), with 51.4% (18/35) of the sample pre-menarche and 48.6% (17/35) of participants post-menarche within one year. Pictorial rating of breast (mean = 3.23, SD = 0.69) and pubic hair development (mean = 2.94, SD = 0.87) subjectively confirmed peripubertal status. One participant identified as male. Pubertal status, BMI, MFQ, number of stressful life events and average estrone, testosterone and DHEA levels did not differ by race or ethnicity, nor were these variables associated with parental education (as a proxy for socioeconomic status). Pubertal status was correlated with BMI (r_s=.484, p=.004), and associated with greater estrone (r_s=.535, p=.001), testosterone (r_s=.477, p=.004) and DHEA (r_s=.398, p=.018) levels.

Table 8. 1

Demographic and sample characteristics

	N	Range (min - max)	Mean ± SD
Age (months)		132 – 176	149.77 ± 12.11
Race			
White	26 (74.3%)		
Black or African American	1 (2.9 %)		
Multiracial	4 (11.4%)		
Hispanic/Latino/Spanish	4 (11.4%)		
BMI (z-score)		-1.73 – 3.31	-0.021 ± 1.09
Female Gender Identity	34 (97.1%)		
Identify as LGBTQIA+			
No	29 (82.8%)		
Yes	3 (8.6%)		
No response	3 (8.6%)		
Pubertal Development Score		1.8 – 3.8	2.66 ± 0.63
PDS (avg) category score		5 – 12.5	8.33 ± 2.30
Menarche status	17 (48.6%)		
Breast (pictorial score)		2 – 5	3.23 ± 0.69
Pubic hair (pictorial score)		1 – 5	2.94 ± 0.87
Stressful Life Events		2 – 11	5.03 ± 2.55
Mood & Feelings		0 – 53	14.43 ± 13.32
Parental Education (%)		4.5 – 7	6.26 ± 0.68
High School Diploma	0		
>4 Years of College	5 (14.28%)		
4-Year College Degree	15 (42.86%)		
Professional Degree	15 (42.86%)		
Household Income			
<$30,000	1 (2.86%)		
$30,000 – $70,000	1 (2.86%)		
$70,000 - $100,000	6 (17.14%)		
$101,000 - $150,000	12 (34.29%)		
$150,000+	15 (42.86%)		

	ICC	Range (min-max)	Mean ± SD
Weekly Self Report			
CES-DC-interpersonal	0.704	0 – 6	1.28 ± 1.73
CES-DC-depressed	0.760	0 – 18	4.14 ± 4.68
CES-DC-somatic	0.774	0 – 24	6.00 ± 5.62
CES-DC-low positive affect	0.770	0 – 12	3.81 ± 3.06
PSS-coping	0.581	0 – 14	6.02 ± 2.86
PSS-distress	0.652	0 – 22	9.38 ± 5.34
Hormones			
Estrone	0.364	0.20 ± 4.20	1.10 ± 0.68
Testosterone	0.638	1.50 ± 30.00	6.89 ± 4.28
DHEA	0.537	8.50 ± 319.00	77.68 ± 47.12

Note. Means are presented ± standard deviation. BMI: body mass index, LGBTQIA+: lesbian, gay, bisexual, transgender, queer, intersex, asexual and more, PDS: Pubertal Development Scale average category score (averaged PDS and tanner line staging score for breast development and pubic hair growth), Primary parent completed education level, CES-DC: Center for Epidemiological Studies Depression Scale for Children, PSS: Perceived Stress Scale, DHEA: dehydroepiandrosterone.

The frequency of stressful life events ranged from 2 to 11 events, with an average of 5.03 (SD = 2.55). As expected, there was a wide range of depressive symptom severity reported on the mood and feelings questionnaire, ranging from 0 to 53 (mean=14.43, SD=13.31, a score of 29 or above is considered clinically significant (Burleson Daviss et al., 2006)), with increased symptom severity associated with a greater number of stressful life events (r_s=.385, p=.022). Further, more stressful life events were related to higher DHEA levels (r_s=.380, p=.024). The most frequently experienced weekly stress was associated with academic performance (26.84%), followed by conflicts with parents (16.67%) and "other" (16.67%). Descriptions of "other" included being excluded from a sleepover, feeling jealous, not getting enough sleep, getting braces, a sick pet, etc. There were no major life events reported during the 8-week collection period.

Study adherence was high, with only 13 weekly surveys missed (4.6%, 267/280) and 5 saliva samples missed (1.8%, 275/280) across all participants. Estrone and testosterone were below the limit of detection in 4% (E1:12/275; T:10/275) of the samples, and DHEA was undetectable in 2% (6/275) of the samples collected.

8.3.2 Intraclass correlations of repeated hormones and outcomes

Intraclass correlations indicated that the majority of the variance in symptoms could be attributed to stable between-person differences (trait-like; ICCs between 0.58 and 0.77). Variability in estrone was primarily at the within-person level (ICC=0.36), whereas 64% of testosterone and 54% of DHEA variance was trait-like (Table 8.1).

8.3.3 Interactive effects of stressful life events and hormone change on symptoms

Results of models examining the moderating role of stressful life events on the association between within-person deviations in hormones (CWP) and symptom expression for all outcomes are

reported in Table 8.2. Presented *p*-values are FDR corrected and Cohen's f^2 was calculated for mixed-effects models using recommendations proposed by Selya et al. 2012.

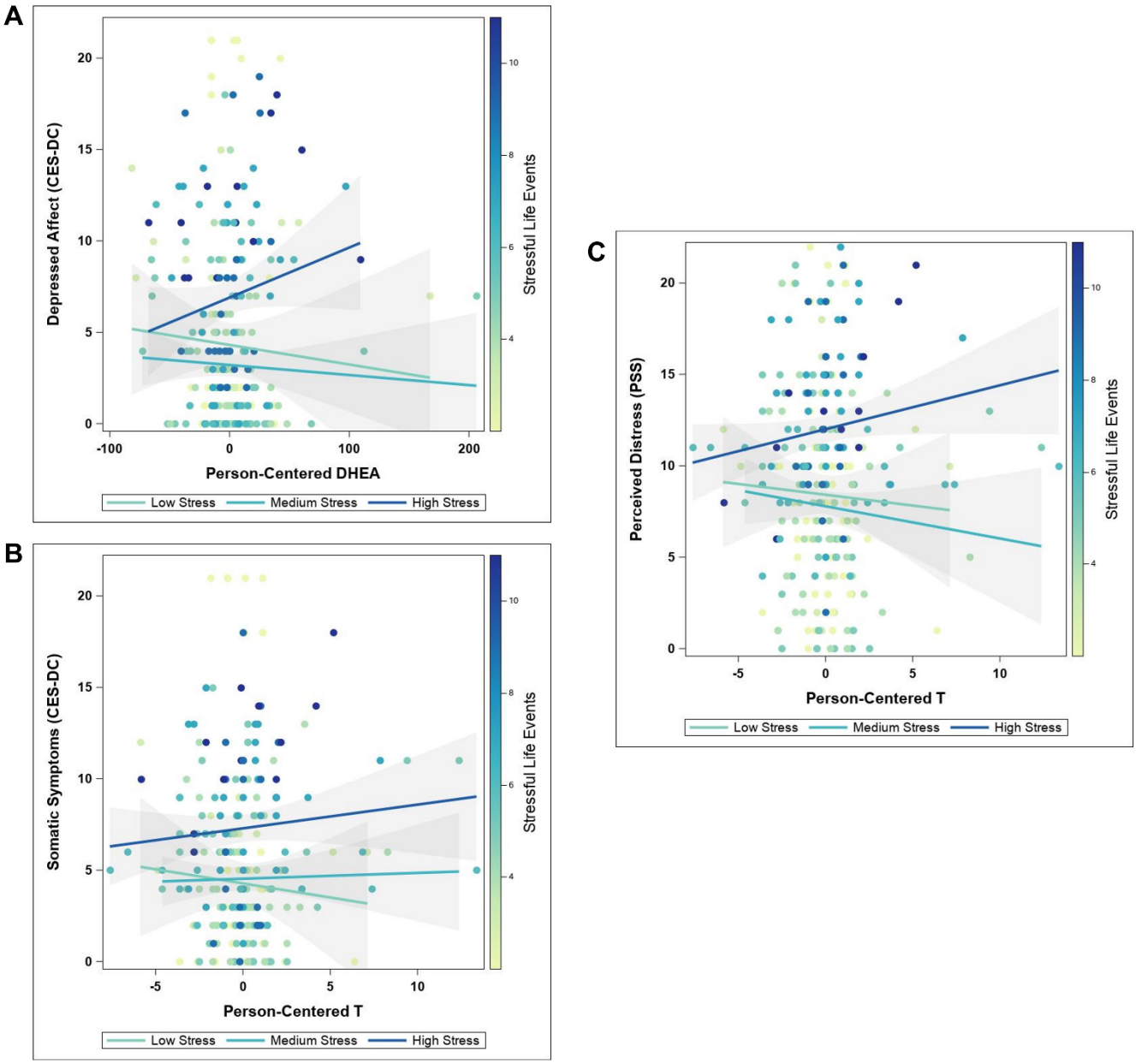
8.3.3.1 Stressful life events and within-person weekly hormone deviations

Estrone. Stressful life events interacted with estrone CWP to predict somatic (F(1,230)=6.32, *p*=.013), interpersonal (F(1,230)=6.78, *p*=.01), and depressed affect (F(1,230)=5.62, *p*=.019) symptoms. Specifically, within-person elevations of estrone predicted greater somatic (*t*(230.2) = 2.67, *p*=0.008) and interpersonal (*t*(230.1) = 2.31, *p*=0.022) symptoms in the context of high stress (SLE+sd), and lower-than-usual estrone predicted greater depressed affect (*t*(230.1) = -2.14, *p*=0.033) at low stress (SLE-sd).

Testosterone. Within-person deviations in testosterone interacted with stressful life events to predict somatic (F(1,230)=9.16, *p*=.003), depressed affect (F(1,230)=11.81, *p*=.001), and perceived distress (F(1,230)=15.74, *p*<.001) symptoms. Probing the impact of stress revealed differential effects of within-person deviations in testosterone on somatic symptoms and perceived distress, with higher-than-usual testosterone predicting greater symptoms at high stress (somatic: *t*(230.2) = 2.65, *p*=0.009; distress: *t*(230.2) = 3.04, *p*=0.003), and lower-than-usual testosterone predicting greater symptoms at low stress (somatic: *t*(230.1) = -2.11, *p*=0.036; distress: *t*(230.2) = -3.12, *p*=0.002) (Figure 8.2). Additionally, low (*t*(230.1) = -4.05, *p*<0.001) to average (*t*(230.1) = -2.72, *p*=0.007) stress was associated with greater depressed affect following lower-than-usual testosterone.

Figure 8. 2

Stress unmasks differential effects of within-person hormone deviations on affective symptoms.



Note. The stress context created a divergence in DHEA-related depressed affect (a) and testosterone-related somatic symptoms (b) and perceived distress (c). Stressful life events are presented as a gradient (with a low number of events indicated by yellow and a high number of events represented by dark blue colors). The regression lines represent tertile groups with 90% confidence intervals. DHEA: dehydroepiandrosterone, CES-DC: Center for Epidemiological Studies Depression Scale for Children, PSS: perceived stress scale.

Table 8. 2

Fixed effects of stressful life events, within-person hormone deviations, and their interactions on symptoms.

	Weekly CES-DC score															
Parameter	Somatic				Low positive affect				Depressed affect				Interpersonal			
Model: E1 × SLE	Est.	(SE)	*p*	f^2	Est.	(SE)	*p*	f^2	Est.	(SE)	*p*	f^2	Est.	(SE)	*p*	f^2
Intercept	5.454	0.686	<.0001		3.838	0.445	<.0001		4.783	0.777	<.0001		1.277	0.246	<.0001	
zSLE	1.725	0.701	0.019		1.033	0.454	0.030		1.816	0.794	0.029		0.498	0.251	0.055	
$E1_{CWP}$	0.396	0.290	0.172		0.190	0.179	0.289		-0.115	0.304	0.706		0.076	0.113	0.499	
$zSLE*E1_{CWP}$	0.848	0.337	0.028*	0.119	0.155	0.208	0.482*	0.091	0.838	0.354	0.036*	0.103	0.342	0.132	0.026*	0.069
Model: T × SLE																
Intercept	5.456	0.686	<.0001		3.839	0.445	<.0001		4.786	0.777	<.0001		1.277	0.246	<.0001	
zSLE	1.721	0.701	0.019		1.031	0.454	0.030		1.812	0.793	0.029		0.498	0.251	0.055	
T_{CWP}	0.005	0.060	0.932		0.005	0.037	0.888		-0.171	0.062	0.007		0.022	0.024	0.360	
$zSLE*T_{CWP}$	0.226	0.075	0.014*	0.122	0.086	0.046	0.091*	0.094	0.264	0.077	0.006*	0.111	0.054	0.029	0.091*	0.065
Model: DHEA × SLE																
Intercept	5.446	0.686	<.0001		3.835	0.445	<.0001		4.774	0.777	<.0001		1.274	0.245	<.0001	
zSLE	1.701	0.700	0.021		1.023	0.454	0.031		1.801	0.794	0.030		0.492	0.250	0.058	
$DHEA_{CWP}$	0.014	0.005	0.005		0.002	0.003	0.449		-0.001	0.005	0.864		0.003	0.002	0.193	
$zSLE* DHEA_{CWP}$	0.015	0.005	0.014*	0.121	0.007	0.003	0.042*	0.095	0.015	0.005	0.019*	0.106	0.005	0.002	0.026*	0.072

Table 8.2 continued

Parameter	Weekly PSS Score: Coping				Weekly PSS Score: Distress			
Model: E1 × SLE	Est.	(SE)	*p*	f^2	Est.	(SE)	*p*	f^2
Intercept	6.024	0.386	<.0001		9.453	0.707	<.0001	
zSLE	0.449	0.395	0.263		1.777	0.722	0.019	
$E1_{CWP}$	-0.010	0.223	0.966		0.690	0.375	0.067	
$zSLE*E1_{CWP}$	-0.253	0.259	0.395*	0.013	0.775	0.437	0.099*	0.110
Model: T × SLE								
Intercept	6.026	0.386	<.0001		9.458	0.707	<.0001	
zSLE	0.450	0.395	0.262		1.767	0.722	0.002	
T_{CWP}	-0.081	0.046	0.083		-0.040	0.077	0.609	
$zSLE*T_{CWP}$	-0.030	0.057	0.595*	0.013	0.377	0.095	.0002*	0.130
Model: DHEA × SLE								
Intercept	6.023	0.386	<.0001		9.448	0.708	<.0001	
zSLE	0.448	0.394	0.264		1.756	0.724	0.021	
$DHEA_{CWP}$	-0.006	0.004	0.109		0.008	0.006	0.215	
$zSLE*\ DHEA_{CWP}$	0.003	0.004	0.482*	0.014	0.016	0.007	0.030*	0.112

Note: Estimates are presented with standard error (SE) and p value. SLE: stressful life events, zSLE: standardized stressful life events score, CWP: Centered Within Person, E1: estrone, T: testosterone, DHEA: dehydroepiandrosterone, CES-DC: Center for Epidemiological Studies Depression Scale for Children, PSS: perceived *stress scale. *FDR adjusted p-values*

DHEA. DHEA CWP interacted with stressful life events to predict somatic (F(1,230)=8.88, *p*=.003), low positive affect (F(1,230)=5.05, *p*=.026), depressed affect (F(1,230)=7.90, *p*=.005), interpersonal symptoms (F(1,230)=6.75, *p*=.010), and perceived distress (F(1,230)=6.02, *p*=.015). A similar pattern of stress-hormone interactions was found for DHEA, with higher-than-usual DHEA predicting greater depressed affect symptoms at high stress (*t*(230.3) = 2.04, *p*=0.042), and lower-than-usual DHEA predicting greater symptoms at low stress (*t*(230.1) = -1.98, *p*=0.049) (Figure 8.2). At high stress, within-person elevations in DHEA predicted increased symptom ratings for low positive affect (*t*(230.3) = 2.31, *p*=0.022), interpersonal symptoms (*t*(230.3) = 3.00, *p*=0.003), perceived distress (*t*(230.5) = 2.84, *p*=0.005), and somatic symptoms (*t*(230.3) = 4.43, *p*<0.001; average: *t*(230.1) = 2.83, *p*=0.005).

8.4 Discussion

8.4.1 Summary of results

The present study used a dimensional approach with weekly measures of sex hormones and depressive symptom ratings to determine the role of recent stressful life events in modifying the relationship between hormone flux and depressive symptoms in peripubertal females. Weekly hormone (estrone, testosterone, and DHEA) deviations predicted differential changes in key constructs of adolescent depression and perceived stress. Results indicated that stress exposure provides a context that unmasks directional effects of hormone flux on affect state change in peripubertal females. Specifically, a consistent pattern emerged with within-person elevations in hormones predicting greater affective symptoms in high stress and decreases in hormones predicting greater symptom severity in low stress contexts. Stress-related hormone effects on affective symptoms appear to be hormone-specific. Namely, DHEA interacted with stressful life events to broadly impact symptom expression, whereas estrone and testosterone had more restricted effects on affective symptoms. The present study supports a diathesis-stress model of adolescent depression in which exposure to life stress proximal to the pubertal

transition modifies vulnerable neural networks, making them more sensitive to normal peripubertal hormone flux, which in turn, promotes the emergence of adolescent mood dysregulation and affective illness.

8.4.2 DHEA deviations predicted a range of depressive symptoms

DHEA interacted with stressful life events to predict all symptom outcomes except perceived coping ability, and higher levels of DHEA were associated with a greater number of stressful life events. DHEA and cortisol are both products of the HPA stress axis that begin to rise with adrenarche and progressively increase during the pubertal transition, and exert opposing physiological actions (Kamin and Kertes, 2017). Because of DHEA's reported antagonistic effects on glucocorticoids (i.e., cortisol), the preferential production of DHEA over cortisol is thought to buffer against stress-related mood impairment (Karishma and Herbert, 2002). At low stress, elevated DHEA in the present study predicted less severe symptom ratings, particularly depressed affect. This is consistent with previous reports that DHEA has antidepressant-like effects, with administration of DHEA improving depressive symptoms by over 50% in patients with midlife-onset depression (Schmidt et al., 2005). More recently, DHEA administration was found to reduce amygdala and hippocampus engagement and enhance limbic connectivity during an emotion regulation paradigm, which in turn, was associated with improved negative effect (Sripada et al., 2013). However, at high stress in the present analyses, higher-than-usual DHEA predicted greater symptom expression, including somatic and interpersonal symptoms, depressed and low positive affect, and perceived distress. The greater symptom expression with DHEA elevations in the high stress context resembles the higher DHEA levels in response to acute stress (Dutheil et al., 2021), also observed among adolescents with affective symptoms (Shirtcliff et al., 2007). Given DHEA's antiglucocorticoid properties, DHEA may be protective against the deleterious effects of cortisol during the pubertal transition, which is characterized by heightened stress exposure (Flinn et al. 2011).

DHEA relative to other hormones, including cortisol, could be investigated in future studies to advance our understanding of stress-related mood sensitivity to peripubertal DHEA changes.

8.4.3 Estrone deviations predicted depressed affect, somatic and interpersonal symptoms of depression

The interaction of estrone and stressful life events predicted somatic symptoms, depressed affect, and interpersonal depression symptoms. When probing the effects of different levels of stress, estrone surges were associated with increased interpersonal and somatic symptoms at high stress and decreased depressed affect at low stress. The relevance of estrogen fluctuations and interpersonal symptoms has also been reported in the menopausal transition (Gordon et al. 2016), similarly characterized by substantial ovarian hormone flux and heightened stress exposure. In fact, estradiol variability was found to predict greater sensitivity to rejection during psychosocial stress in perimenopausal women. Moreover, in women who had experienced a greater number of recent stressful life events, rejection sensitivity predicted the emergence of depression symptoms one year later (Gordon et al. 2016). Importantly, rejection sensitivity is a proximal risk factor for clinical depression and transdiagnostic factor shared across psychopathologies (Kupferberg, Bicks, and Hasler 2016; Owens et al. 2018; Slavich and Irwin 2014, Slavich et al. 2010). The results of the present study further support the pathophysiologic relevance of estrogens, specifically estrone, in the interpersonal symptoms of depression, and for the first time, demonstrated a relationship between estrone change and increased interpersonal depressive symptoms during the pubertal transition. The specificity of symptoms highlights the importance of conducting dimensional, symptom-specific research.

8.4.4 Divergent effect of testosterone-related affect dysregulation in the context of stress

The stress context created a divergence in testosterone-related affective symptoms, particularly for somatic symptoms and perceived distress. Participants exhibited increased symptoms with lower-than-usual testosterone levels at low stress, yet testosterone elevations

were associated with increased symptoms at high stress. The role of testosterone in depression has been investigated particularly in regard to pharmacological use (with testosterone treatment showing antidepressant effects) and primarily in male samples (Zarrouf et al., 2009). In adolescents, and especially female adolescents, the relationship between depression and testosterone is understudied. One previous longitudinal study investigated 630 female participants annually through adolescence for up to seven years (Copeland et al., 2019). Higher serum testosterone concentrations predicted diagnoses of depressive disorders significantly, even after controlling for factors such as pubertal status or age. Moreover, testosterone has been shown to have puberty-unique influences on neurofunction and neuroarchitecture in brain regions associated with the development of affective disorders (Bramen et al., 2012). We recently demonstrated that a significant proportion of female adolescents showed meaningful associations between fluctuations of testosterone and mood (Andersen et al. 2022). The results reported here take these investigations one step further and highlight the essential role of stressful life events on the testosterone-mood relationship. However, the biological mechanisms contributing to the interaction of life stress and mood sensitivity to testosterone flux have yet to be identified. Research on other sex steroids, such as progesterone, suggest that dysregulated receptor functioning can cause hormone sensitivity and HPA axis dysregulation in some individuals (as reviewed for progesterone by Gordon et al. 2015). Future research could explore this as a potential mechanism underlying sensitivity to testosterone flux.

8.4.5 The impact of stressful life events on hormone-induced affective symptoms

The results not only support the interaction of sex hormones and mood in the pubertal transition, but they also suggest that life stress exposure has an essential moderating effect on this interaction. One theory suggests that this moderation is biologically underpinned by dysregulation of the HPA axis. Early and chronic life stress can alter HPA axis functioning with long-term effects on mental health (Ho and King, 2021). These HPA axis alterations have been

specifically demonstrated for severe forms of life stress in the form of trauma exposure (Steudte-Schmiedgen et al., 2016) and for life stress experienced early in life, which is especially relevant to adolescents (Young et al., 2021). The pubertal transition is a unique window of vulnerability to the impact of stress-related mood impairment and contributes to differential stress responses following early life stress (Flannery et al., 2017; King et al., 2017; Nelson and Gabard-Durnam, 2020; Sisk and Gee, 2022). The HPA and HPG axes interact on various levels to regulate sex steroids, and in turn, sex steroids modulate HPA function (Oyola and Handa, 2017). Changes in HPA activity during adolescence (possibly through GABA-estrogen modifications in the amygdala (Walker et al., 2004) may contribute to hormonal sensitivity and precipitate depressive symptoms in susceptible individuals. Results from the present study suggest that experiencing a greater number of stressful life events proximal to the pubertal transition can modify hormone-mood relationships. However, the positive relationship between stressful life events and depressive symptoms at enrollment in the present study suggest that greater depressive symptoms at enrollment could have influenced the report of stressful life events, particularly stressful events that are more subjective (e.g., change in acceptance by peers). Consequently, it remains unclear whether the type or severity of stressor within the context of baseline depressive symptoms differentially impacts hormone-related mood dysregulation.

In summary, early life stress, via modifications of developing neural circuitry, may give rise to hormone sensitivity, thus enhancing vulnerability for affective symptoms in the context of normal peripubertal hormone fluctuations. Stress related alterations of HPA functioning and the strong interaction of HPA and HPG axes may underlie the relationship between sex hormone fluctuation and mood. However, the neurophysiological mechanisms supporting this relationship are poorly understood and remains a promising pursuit of future research.

8.4.6 Limitations

Despite the strengths of the longitudinal, dimensional approach employed in the current study, there are several limitations to address. While serum hormone measurement is typically preferred to saliva methods because of its greater detection sensitivity, salivary hormone collections offer a non-invasive alternative with superior feasibility and compliance in adolescent participants. Given the current study's objective to determine the relationship between hormone change and mood does not require the ability to detect the absolute lowest hormone level, the trade-off between ultra-low sensitivity and feasibility is appropriate. To account for potential confounds in saliva hormone measurement (time of day, food and water intake, etc.), we provided participants and their parents with detailed instructions and required sample collection to occur first thing upon awakening. Furthermore, steroid concentrations measured in saliva provide integrative information on free, unbound estradiol and testosterone and thereby measure the biologically active component of the hormone (Gao et al., 2015; Yasuda et al., 2008). The present study is also limited by the observation interval, which occurred weekly, and the relatively small sample size to examine moderation effects. While weekly observations may be sufficient to demonstrate how hormone flux can influence depressive symptoms from week to week (Gordon et al. 2016, 2020; Lozza-Fiacco et al. 2022), daily measures may more precisely asses hormone flux and should be considered in future studies.

For the same reasons that make the pubertal transition a critical developmental window for examining the impact of hormones on mental illness trajectories, the peripubertal endocrine environment is associated with unique study design challenges. It is difficult to study specific hormones of interest, particularly progesterone and its metabolites, because of the limited sensitivity of the available analysis methods for the relatively low levels expected during the first gynecological year. While valuable guidelines exist for determining ovulation status and

cycle phasing in adult participants (Schmalenberger et al., 2021), these recommendations have only limited utility for identifying menstrual cycle phase and ovulation status in peripubertal participants, in which anovulatory and irregular cycles are common (Carlson and Shaw 2019; Sharma, Deuja, and Saha 2016). Typical hormone thresholds (i.e., progesterone rise, LH peak) for determining cycle phase in adults are not applicable because of overall lower peripubertal hormone concentrations, and counting methods are not relevant when shorter luteal phases are expected (Carlson and Shaw 2019; Sun et al. 2019). Currently, there are no clear guidelines for determining ovulation status and cycle phasing during the first gynecological year, and thus, the impact of the menstrual cycle on hormone-mood relationships during the pubertal transition remains an important area of investigation.

8.4.7 Future Directions

Future research is needed to examine the neurobiological mechanisms underlying the relationship between hormone change, stress and depressive symptoms. Because sex hormones (e.g., estradiol, testosterone) influence the regulation of the HPA stress system and the abundance of sex hormone receptors in limbic brain regions engaged during emotion, the probability exists that the HPA stress response may mediate the hormone-mood relationship. Moreover, the biological mechanisms supporting the relationship between psychopathology and sex-hormones (i.e., DHEA and testosterone) and their fluctuation are poorly understood – not only in adolescents but across the female reproductive lifespan and should be a focus of future studies to close these existing research gaps.

The results from the present study confirm and further highlight the need to include stress exposure in studies linking sex hormones and affective symptoms. As shown in trauma research (Forbes et al., 2013; López-Martínez et al., 2018), not only the absolute number of stressful life events, but also the type of stressor is a promising factor to investigate (McLaughlin et al., 2021). Results also highlight the importance of using a dimensional approach. Limiting the

investigation to total depression symptom scores could dilute or mask significant effects only apparent on the symptom level. Accordingly, we recommend future studies similarly employ a dimensional and symptom-specific approach to investigate key constructs of depression in adolescents. This dimensional approach will also allow more specific exploration of the neurobiological mechanisms underlying the etiology of affective illness. Finally, the current results provide a foundation for understanding the impact of life stress on hormone-mood relationships in a low-risk sample reflecting the socioeconomic, racial and ethnic composition of the local community. A larger and more diverse sample of adolescents could, for instance, be employed to elucidate unique psychosocial and neuroendocrine features of the pubertal transition experienced by participants who identify differently from their sex assigned at birth. Including a wide range of depressive symptoms was not only important to achieve adequate variability, but it also allowed for greater generalizability of a developmental window in which elevated stress and depression are common. That said, the diathesis model tested in the present analyses could inform future investigations of the biological, social, environmental, and cultural factors that converge to make the pubertal transition a unique window of vulnerability for psychopathology.

8.4.8 Conclusion

The present study contributes to our understanding of peripubertal hormone-mood relationships by demonstrating that life stress differentially impacts hormone-related changes in distinct symptom constructs of depression. This study reveals how life stress predisposes sensitivity to hormone fluctuation in peripubertal female adolescents, ultimately resulting in depressive symptoms in response to normative hormone flux. Results indicated that sensitivity to within-person deviations in DHEA, testosterone and estrone predict affective symptoms, and that mood-sensitivity to weekly hormone deviations is stress context specific. Given the role of sex hormones in regulating neural networks involved in cognitive and emotional processing, the

combination of increased sex hormones and their variability during the pubertal transition and more frequent stress exposure may promote heightened emotional reactivity and arousal and thus, promote greater risk for psychopathology. The impact of stressful life events on the unique peripubertal neuroendocrine environment should continue to be investigated to inform advancements in the early detection and intervention of affective illness.

8.5 References for study 6

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Author Contributions for study 6

Elizabeth Andersen and Susan Girdler designed the study. Elisabeth Andersen and Kayla Baresich conducted the investigation. Elizabeth Andersen and Hannah Klusmann created the analysis plan. Hannah Klusmann prepared the data set. Elizabeth Andersen and conducted the analyses. Hannah Klusmann drafted the manuscript. All authors critically revised the manuscript.

Elizabeth Andersen and Susan Girdler supervised the study.

CHAPTER 9

9. Discussion

9.1 Summary of findings

This dissertation investigated the interaction of the menstrual cycle, depressive symptoms, and stress markers. A heuristic model was used to structure the related biological and psychological underpinnings of depression etiology. To answer the overarching scientific question (how do stress and depressive symptoms interact with the menstrual cycle?), it can be stated that 1) depressive symptoms can show perimenstrual and mid-cycle worsening with varying intensities in a subgroup of women, 2) depressive symptoms are connected to cycle irregularity, specifically higher depressive symptoms are associated with irregularity in cycle length and anovulatory cycles, 3) biological stress, in the form of HPA axis functioning, differs between the follicular and luteal phase, and 4) stressful life events moderate the interaction of hormone fluctuation and depressive symptoms. Figure 4 summarizes the findings of the six conducted studies and integrates them into the heuristic model.

The relationship between the menstrual cycle and depressive symptoms (path 1) was investigated in **study 1**, in which participants with and without current depressive disorder (n = 77) participated in a longitudinal ambulatory assessment study across one menstrual cycle. They reported depressive symptoms three times daily and used urinary ovulation tests to determine the menstrual cycle phase. Multilevel models, incorporating cosine coefficients as marker for cyclical fluctuation, were used to investigate the research questions. In this framework, depressive symptoms showed systematic differences in their intensity (i.e., degree of fluctuation) across the menstrual cycle. Thereby, the fluctuation intensity differed significantly between individual symptoms. Further, the symptom fluctuation pattern and

intensity differed significantly between participants. This study answered scientific question **Q1**, stating that depressive symptoms change systematically in some individuals and with varying intensities. The cycle-related fluctuations are symptom-specific and can show different patterns (perimenstrual vs. mid-cycle worsening).

Path 1 was further investigated by comparing depressive symptoms between regular and irregular cycling participants (**study 2**). In this study, the association between depressive symptoms and ovarian hormone fluctuation was investigated in a cross-sectional sample (n = 394), and the sample which was also investigated in study 1 (n = 77). Depressive symptoms were compared between regular and irregular cycling participants using a Welch t-test and an ANCOVA. The results revealed greater depressive symptoms in participants with irregular cycles in both samples. They addressed scientific question **Q2,** indicating that menstrual cycle irregularity related to cycle length was associated with greater depressive symptoms in this sample.

Irregular cycles were further investigated regarding occurrence of ovulation in **study 3**. A sample of peripubertal females (n = 32) reported daily affective symptoms and provided daily urine samples to analyze ovarian hormones for one menstrual cycle. A self-developed and validated algorithm to determine ovulation status and the area under the curve (AUCg) of symptoms across the menstrual cycle and t-tests were used to compare irregular and regular cycles. The results revealed greater depressive symptoms (significant for difficulty concentrating and loneliness) in irregular/anovulatory cycles, answering scientific question **Q3**. Additionally, this study provided new guidelines on how to investigate irregular and anovulatory cycles.

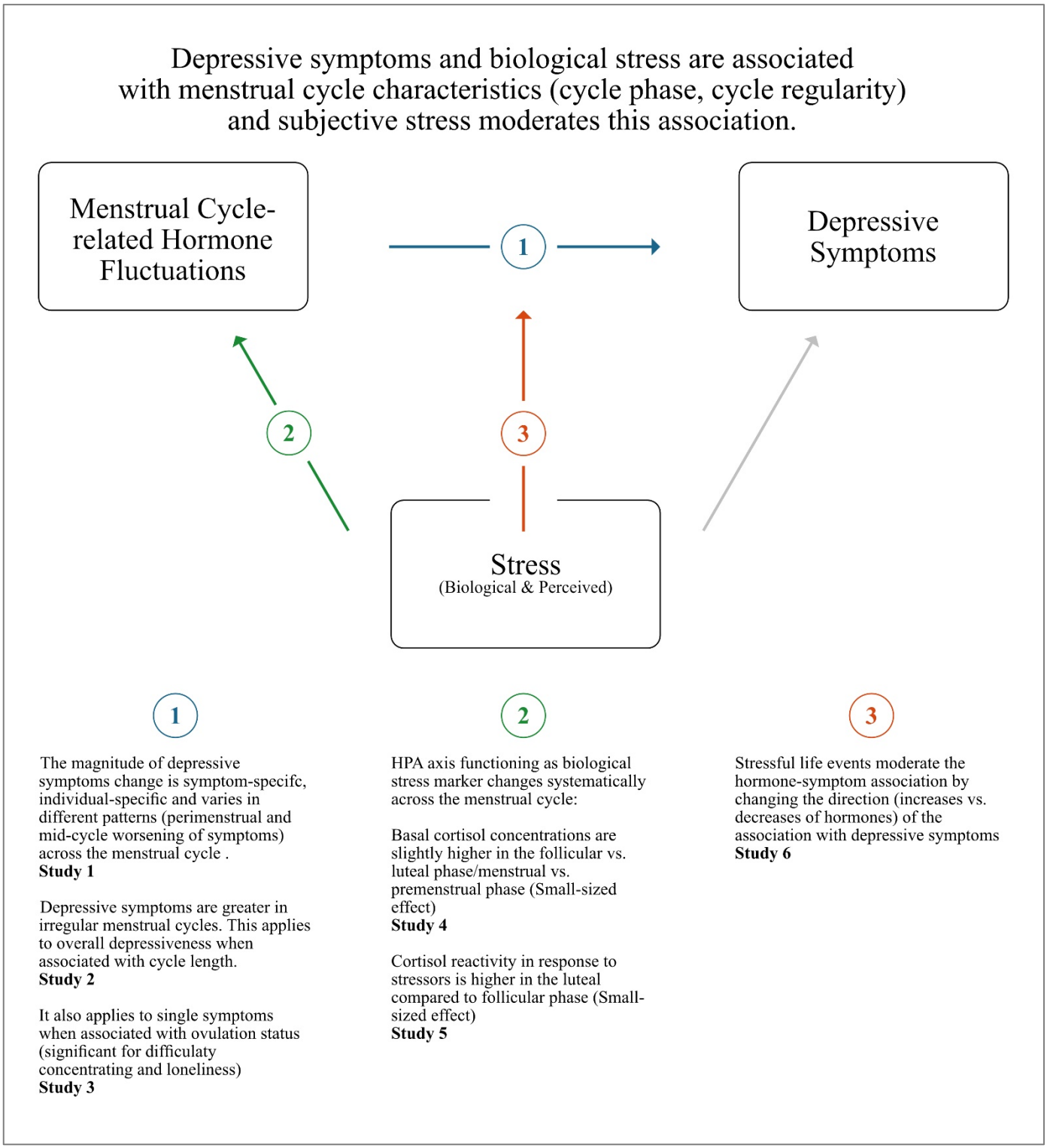
The biological underpinnings of the relationship between stress and hormone fluctuations across the menstrual cycle were meta-analytically investigated in **study 4** and **study 5**. Study 4 summarized 121 studies, including 2641 healthy, naturally-cycling

participants that measured basal cortisol concentrations as a marker for biological stress in different menstrual cycle phases. The standardized mean change was used to meta-analytically compare cortisol concentration between cycle phases. Answering scientific question **Q4**, the results indicated higher basal cortisol concentrations in the follicular compared to the luteal phase (or, more precisely, in the premenstrual compared to the menstrual phase). Study 5 systematically reviewed 12 longitudinal studies (n = 182) that assessed cortisol responses to acute physiological and psychological stressors. A similar meta-analytic comparison of three studies that assessed cortisol reactivity in the follicular and luteal phase revealed a significant, small-sized effect, indicating higher cortisol reactivity in the luteal compared to the follicular cycle phase, which answered scientific question **Q5** regarding physiological stressors. For psycho-social stressors, available data from the reviewed studies was insufficient to conduct meta-analytic comparisons between cycle phases.

Study 6 introduced stressful life events as a moderator of the relationship between hormone fluctuation and depressive symptoms in a second sample of peripubertal females (n = 35). Participants provided weekly urine samples for ovarian hormone measurement and reported depressive symptoms. Linear mixed models were used to predict weekly symptom change related to hormone change, incorporating stress as a moderator. As a result, stress changed the direction of the association between symptoms and hormone change. Specifically, a high-stress context led to a positive association of hormones (estrogens, testosterone, and DHEA), and depressive symptoms (greater depressive symptoms and increases in hormones) and a low-stress context led to a negative association (greater depressive symptoms and decreases in hormones). Scientific question **Q6** can therefore be answered by stating that life stress showed a moderating role in this study, specifically unmasking a directional effect regarding hormone surges compared to withdrawals.

Figure 4

Heuristic model of stress as moderator of hormone sensitivity to depression with results of this dissertation



9.2 Interpretation of findings

The results of this dissertation contribute to our understanding of menstrual cycle-related factors of depression. The results are incorporated in the heuristic model presented in chapter 2 and depicted in figure 4. This discussion will focus on the interpretation and possible biological underpinnings of the respective relationships between menstrual cycle-related hormone fluctuations, depressive symptoms and stress.

9.2.1 Path 1: Menstrual cycle-related hormone fluctuations and depressive symptoms

The association of menstrual cycle-related hormone fluctuations and depressive symptoms (Path 1) was investigated in multiple studies in this dissertation. Study 1 was the first longitudinal investigation of the full spectrum of depressive symptoms across the menstrual cycle incorporating multiple cyclicity patterns (perimenstrual and mid-cycle worsening of symptoms). The results confirmed that depressive symptoms fluctuate systematically across the menstrual cycle in some individuals. These findings were in line with the two most similar available studies (Fakhari et al., 2011; Hartlage et al., 2004) which also reported systematic cycle-related changes of depressive symptoms in some susceptible individuals (hormone sensitivity). Study 1 confirms the concept of hormone sensitivity of depressive symptoms in participants with and without current depressive disorder (Schweizer-Schubert et al., 2021).

Participants differed significantly in the intensity of menstrual cyclicity (i.e., systematic fluctuation across the cycle), and while some showed premenstrual exacerbation of symptoms, others reported mid-cycle worsening, and some had no cycle-related fluctuations of symptoms. This emphasizes the importance of accounting for individual differences when analyzing menstrual cycle effects. Averaging all participants can result in the underestimation of these effects, because different patterns and intensities might cancel each other out or dampen effects

when summarized (Kiesner et al., 2016). This is consistent with earlier studies that highlight individual variations in hormone sensitivity (Eisenlohr-Moul, 2019; Schmidt et al., 1998).

Moreover, hormone sensitivity, as investigated in this dissertation, has been shown to be symptom-specific, meaning different depressive symptoms can fluctuate differently in response to hormonal fluctuations. Multiple studies in this dissertation demonstrated this, revealing specified significant cycle effects for some symptoms and not others (Studies 1,3 & 4). This also aligns with prior studies, where individual symptoms exhibited heightened sensitivity to hormone fluctuation (Andersen et al., 2022; Eisenlohr-Moul et al., 2016; Kiesner et al., 2016). The results highlight the importance of investigating individual symptoms and not only summarized scores, as this can result in the loss of valuable information regarding cyclicity. Symptoms with low cyclicity may obscure the cyclicity effects of other symptoms.

The exact explanations for such cyclical symptom fluctuation are poorly understood in the current state of literature (Girdler et al., 2012; Schweizer-Schubert et al., 2021). However, some hypotheses address biological explanations of hormone sensitivity that might also inform possible the results in this dissertation. The most prominent explanatory hypotheses for hormone sensitivity are associated with the synthesis and functioning of the neurosteroid metabolite of progesterone, Allopregnanolone (ALLO). ALLO is a promising biological candidate for underlying hormone sensitivity as ALLO is metabolized from progesterone and increased in the luteal compared to the follicular phase (Girdler et al., 2001). ALLO can interact with neurotransmitter systems, specifically the $GABA_A$ receptor, to influence affective neural networks that contribute to affective functioning (Schiller et al., 2016; Walker et al., 2004a). Changes in ALLO (increases and decreases) have been shown to increase affective symptoms in individuals with PMDD but not those without. Furthermore, stabilizing ALLO in the luteal phase (by using dutasteride to prevent the conversion of progesterone), reduced PMDD symptoms significantly (Martinez et al., 2016). It is hypothesized that hormone sensitive

women might have a differential sensitivity to changes in ALLO concentrations, specifically differentiated functioning of $GABA_A$ receptors (Girdler et al., 2001). Another explanatory hypothesis for symptom sensitivity to hormone change was investigated by Wei et al. (2018), who reviewed distinguished sulfation of several neuroactive steroids in participants with PMDD, which converts the functioning of $GABA_A$ receptors from agonist to antagonist. This might provoke a differential reaction to normal sex hormone fluctuations. However, research on ALLO and possible modifications of $GABA_A$ receptors is still in its infancy, and no final conclusions on exact biological underpinnings can be made. Further, most research on the biological underpinnings of hormone sensitivity is conducted with PMDD patients, and conclusions might not be directly adaptable to depression (Wei, 2018). However, the results of this study indicate the presence of hormone sensitivity in depression, which gives reason for the further exploration of such possible biological influencing factors in future studies.

Furthermore, this dissertation gives indications that irregularity of hormone fluctuations might have an impact on depressive symptoms. Within study 2, results from the cross-sectional and longitudinal sub-studies indicated that participants with irregular menstrual cycle length, exhibit significantly higher levels of depressive symptoms compared to those with regular cycles. This effect remained, even after accounting for potential influencing factors such as stress exposure. These results complement previous studies in which menstrual cycle regularity was predicted from depressive symptom severity (Nillni et al., 2018; Yu et al., 2017). Within study 3, participants with irregular cycles, expressed through anovulation, also reported stronger affective symptoms, specifically difficulty concentrating and loneliness. All other assessed symptoms (depression, hopelessness, anxiety, mood lability, anger, low interest, sensitivity to rejection, interpersonal conflict, feeling overwhelmed) showed the same tendency, however not significantly.

The association between greater depressive symptoms and irregularity of menstrual cycle characteristics (length, ovulation status) is a novel finding with few to no studies investigating related concepts or possible biological explanations. Of note, neither study investigated HPA axis functioning, e.g., with basal cortisol assessments. It stands to reason that the interaction of HPA and HPG axes (Handa and Weiser, 2014; Oyola and Handa, 2017) might be a possible rationale for increased depressive symptoms in cycle irregularity. As stress has been shown to affect both, depressive symptoms (Hammen, 2005) and menstrual cycle regularity (Acevedo-Rodriguez et al., 2018; Ayrout et al., 2019), it can be speculated that a dysregulation of the HPG axis, resulting in irregular or anovulatory cycles, might be associated with a dysregulation of the HPA axis, resulting in increased depressive symptoms (Rothe et al., 2020), or vice versa. However, this hypothesis warrants further research and could be essential in investigating properties and risk factors of hormone sensitivity.

In summary, our results show that menstrual cyclicity of depressive symptoms is person-specific and symptom-specific and is potentially explained by a differentiated sensitivity to ALLO changes. Further, our findings indicate that in addition to the normal fluctuation of sex hormones across the menstrual cycle, irregularities in these fluctuations may contribute to the development of depressive symptoms.

9.2.2 Path 2: Menstrual cycle-related hormone fluctuations and stress

To explore the association of menstrual-cycle related hormone fluctuations and biological stress (Path 2), cortisol was investigated as a marker for HPA axis functioning across the menstrual cycle (Q4 and Q5). The results of two meta-analyses (study 4 & 5) revealed slightly lower cortisol concentrations in the luteal phase compared to the follicular phase and, more precisely, in the premenstrual compared to the menstrual phase (Q4). While some smaller-sized cross-sectional studies found null-effects for cycle phase differences of basal

cortisol (Espin et al., 2013; Kirschbaum et al., 1999), the results align with a previously conducted meta-analysis (Hamidovic et al., 2020). This study also reported lower cortisol concentrations in the luteal phase.

The novel comparison of five precise cycle phases in study 4 made it possible to relate specific hormone patterns to changes in cortisol concentrations. Precise cycle phase pairs that show significant differences in cortisol concentrations (e.g., premenstrual vs. menstrual phase) can be compared directly, and possible responsible ovarian hormone concentrations can be identified. Thereby, lower cortisol concentration could be associated with either estradiol or progesterone, as both show high concentrations in the premenstrual phase. However, if estradiol would drive a decrease in cortisol, this would have been observable in the periovulatory phase where estradiol is also elevated, and the study did not show this effect. Consequently, it can be hypothesized, that progesterone or its metabolites, such as ALLO, have a cortisol-dampening effect because they are elevated in the luteal phase only (Girdler et al., 2001). This hypothesis is supported by previous studies on ALLO and HPA axis functioning. ALLO has been identified as a modulator of cortisol synthesis and as being essential in promoting homeostasis after stress exposure (Cullinan et al., 2008; Girdler et al., 2001). More specifically, ALLO enhances Cl-ion flux on α-subunits of $GABA_A$ receptors, thereby increasing GABA-ergic transmission (Belelli and Lambert, 2005; Chisari et al., 2010; Wirth, 2011). GABA, a highly potent inhibitory neurotransmitter, has been proposed to reduce HPA axis activity after stress exposure (Cullinan et al., 2008; Decavel and Van Den Pol, 1990). Additionally, by reducing gene transcription of corticotrophin-releasing hormone (CRH), ALLO plays a significant regulatory role in HPA axis activity (Patchev and Almeida, 1996). The association of ALLO and the HPA axis is also extensively investigated by Crowley and Girdler (2014) or Morrow (2007). To summarize, the decrease in cortisol concentrations in the luteal phase may be influenced by the biological actions of progesterone's metabolites,

specifically ALLO, through enhancement of GABA-ergic transmission, leading to the inhibition of HPA axis activity.

These results and biological hypotheses might also inform the interpretation of results of study 5. HPA axis reactivity, measured through cortisol concentrations in response to a stressor, was greater in the luteal than in the follicular phase (Q6). This result is aligned with a cross-sectional study by Kirschbaum et al. (1999). It is possible that the increased reactivity is influenced by the lower basal cortisol concentrations in the luteal phase demonstrated in study 4. As HPA axis reactivity is measured through a change in cortisol concentration from baseline, a lower baseline might lead to a stronger increase of concentrations when confronted with a stressor. However, Kirschbaum et al. (1999) did not find a baseline difference in their cross-sectional study, which contradicts this hypothesis. It might also be possible that HPA axis reactivity is reinforced through ovarian steroids that increase in the luteal phase, such as progesterone, ALLO, or estradiol. However, this can only be hypothesized, and future research that assesses cortisol reactivity in response to stressors in all five precise cycle phases, as well as research examining specific ovarian hormone-related influences on cortisol reactivity, is warranted.

Aside from the results and implications derived from these meta-analyses, a noteworthy result of studies 5 and 6 was a lack of studies that specifically investigated cortisol across the menstrual cycle using high-quality assessment of the menstrual cycle and HPA axis functioning. In most studies, menstrual cycle phase was conducted as a possible confounder and not intended to be the main object of research. This limits the focus of these studies concerning specific biological underpinnings and explanations for phase effects. In general, not only the menstrual cycle, but also female-specific factors of mental health are underrepresented in research. This phenomenon, also known as the “gender data gap”, highlights the lack of data investigating sex differences and data on issues affecting females in

particular (Temin and Roca, 2016). The gender data gap has been investigated across research domains, specifically in regard to a high percentage of male-only samples in animal and human studies (e.g., 67-97% of behavioral pharmacological studies (Hughes, 2007)). This underrepresentation of female animals and humans in pharmacological research has led to a dramatic disadvantage, for example, due to drugs that would need much lower doses (McGregor, 2017) or are less effective or even harmful for women (Carey et al., 2017; Hughes, 2007; Soldin et al., 2011). The menstrual cycle (or the rodent equivalent, the estrous cycle) is often argued to be the reason for excluding females from research because the associated drastic hormonal changes might make it harder to provide stable experimental conditions (Hughes, 2007; Plevkova et al., 2021). The results of the meta-analyses show that, on the one hand, there are differences across the menstrual cycle regarding HPA axis activity and, on the other hand, that these differences can be examined, quantified and, therefore, controlled for. Consequently, the implication of the results for cortisol research are twofold: first, the menstrual cycle needs to be accounted for in cortisol research and cannot be ignored without potentially yielding biased results or failing to provide generalizable results for all individuals. Second, the menstrual cycle phases can be controlled for, and the effects are quantifiable as has been shown with this research. The meta-analyses contribute to a better understanding of menstrual cycle effects and thereby to reducing the gender data gap.

9.2.3 Path 3: Stress as a moderator on the hormone-depressive symptom relationship

The third part of the heuristic model that was investigated in this dissertation was stress as a moderator of the association between hormone fluctuations and depressive symptoms (Path 3; Q6). This was examined in study 6, which investigated how weekly hormone change was associated with change of affective symptoms in peripubertal females with different exposure to stressful life events such as “parents’ divorce” or “end of a romantic relationship”. The results revealed a directional moderating effect of stress exposure to the hormone – affect

change relationship. Broadly speaking, in a high-stress context, increases in hormone concentrations within individuals predicted increased affective symptoms, while in a low-stress context, decreases in hormone concentrations predicted increased affective symptoms. This pattern was similar for estrone, testosterone and DHEA fluctuations, but not all of the depressive symptoms assessed showed significant effects. A possible biological underpinning of this moderating effect might stem from the association of the HPA and HPG axes. The HPA axis regulates the physiological response to stressors (Kudielka and Kirschbaum, 2005) and affects depressive symptoms (Rothe et al., 2020) and the HPG axis regulates hormone fluctuations. Thereby, HPA axis functioning might be altered through HPG activity which in turn might contribute to hormone sensitivity and an increased risk of reproductive mood disorders. This susceptibility to hormone change, possibly influenced by HPA axis dysregulation, might be especially relevant for long-term mental health when chronic or traumatic stress is experienced in early life (Pilver et al., 2011; Yang et al., 2022).

On a more detailed level, there have been several complimenting proposals on how ovarian hormone regulation and stress response interact and contribute to the development of reproductive mood disorders. In a neurocognitive model, Newhouse and Albert (2015) argued that estradiol could modify brain areas involved in cognitive and emotional processing of stressors, such as the amygdala or the dorsomedial prefrontal cortex. Further, estrogen exposure has the ability to modify the GABAergic system, changing stress responsivity and thereby susceptibility to depressive symptoms (Walker et al., 2004b). Aside from the role of estrogen, progesterone and its metabolite ALLO has been associated with the possible moderating role of stress and HPA axis dysfunction, as described in previous sections. Under conditions of stress, progesterone can be converted into cortisol, resulting in heightened stress responses and impaired emotional processing abilities (Handy et al., 2022; Sundström-Poromaa et al., 2020). Schweizer-Schubert et al. (2021) highlighted the role of HPA axis

functioning and stressor response in reproductive mood disorders by expanding the concept of hormone sensitivity to “steroid hormone sensitivity”. This expanded concepts aims to include corticosteroids such as cortisol and neurosteroids such as ALLO and the GABA-A Receptor complex in the etiology of reproductive mood disorders.

To summarize, the relationship between depressive symptoms and sex hormone fluctuation may be influenced by stress-induced changes in the functioning of the HPA axis, as well as the strong interaction between the HPA and HPG- axes. However, the specific neurophysiological mechanisms underlying this relationship remain poorly understood, presenting an intriguing area for future research.

9.3 Strengths and limitations

This dissertation presents a set of strengths but also limitations. Strengths and limitations specific to the studies are discussed in chapters 2 and 7. The strengths and limitations relevant to the dissertation overall are presented below.

The diversity and comprehensiveness of study methods, investigated parameters, and populations is a strength of this dissertation. Regarding the study methods, biological (e.g., ovarian hormone concentrations) and subjective variables (e.g., assessment of stressful live events) were investigated and related to each other, which is especially important in this research field that links physiological and psychological aspects. The dissertation further provides a combination of study methods, yielding a variety of advantages that come with these methods. The application of various study methods also results in a detailed and more comprehensive investigation of the interaction of depressive symptoms, stress, and the menstrual cycle. Specifically, ambulatory assessment studies (studies 1 and 2) and longitudinal studies with daily assessments (studies 3 and 4) provided prospective data which allowed for a unique linkage of hormone change and depressive symptoms that would not be possible with

retrospective or infrequent measuring. Thereby, the investigations incorporated various biological markers, specifically estrogens, progesterone, LH, and cortisol. Meta-analytic investigations (studies 4 and 5) provided information based on high numbers of included participants, improving the statistical power and reliability of results. Lastly, methodological guidelines for studying the menstrual cycle were provided for specific groups, including knowledge from experts and through analyses of information-dense data sets (study 3 and 4). Thereby, the dissertation yields not only clinical implications and knowledge but also advancing the field of menstrual cycle research with improved analysis methods and algorithms. Furthermore, the studies included in this dissertation followed overall quality guidelines for research, e.g., by preregistering studies and review protocols, publishing studies in peer-reviewed journals, and providing openly accessible R scripts to replicate analyses. Additionally, quality guidelines for the respective research topics and methods were adhered to, for example, the PRISMA guidelines for systematic reviews and meta-analyses or the guidelines to study the menstrual cycle by Schmalenberger et al. (2021). This is not self-evident, as many studies that fail to comply by such guidelines yielded strongly biased results (e.g., through retrospective rating of menstrual cycle change or not measuring ovulation; Henz et al., 2018). Lastly, the investigated samples differed widely across the reproductive life span. Including adults and adolescents widens the reliability and generalizability of the results on symptom sensitivity to hormone change. Of note, peripubertal females are especially underrepresented in biopsychological research (Andersen et al., 2022), and the studies investigating peripubertal samples thereby provide rare results.

Overall, the diversity and comprehensiveness of methods, investigated parameters, and populations allow for a multidimensional investigation of the proposed model linking depressive symptoms, the menstrual cycle, and stress. Such a broad investigation from different

angles on the same topic allows for more general results (e.g., across age groups) and minimizes biases (e.g., through meta-analytic investigations and primary studies).

However, several limitations are important to keep in mind when regarding and interpreting the results of this dissertation.

First, ambulatory assessment is time-consuming for participants and expensive to carry out, especially when hormones are assessed and analyzed, so the primary studies' sample sizes were rather small. This was addressed by conducting meta-science (studies 5 & 6) that incorporated very large participant numbers. However, more primary studies with larger sample sizes are necessary to strengthen and validate the results of this dissertation, especially when investigating hormone sensitive subgroups across the menstrual cycle. Further, due to the burden on participants and extensive cost, the primary studies in this dissertation investigated only one cycle per participant. To obtain a more reliable picture of menstrual-cycle related changes in symptoms, hormone changes and cycle characteristics, at least two study cycles are recommended and would have improved the results (Schmalenberger et al., 2021). However, the highly frequent data collection (up to three assessments each day) increases the power and reliability of the present results and is novel compared to other investigations with more investigated cycles but less frequent assessments. Further, due to the planning of assessment schedules according to specified cycle phases, the start of investigation was always at the start of menses rather than counter-balanced. The statistical disadvantages that come with starting assessments in the same cycle phase for all participants were addressed by incorporating buffer days prior to expected menses onset to account for habituation effects. These buffer days were later discarded from the analyses.

Second, there were limitations concerning the incorporated samples in the study. Due to the small sample sizes, confounding factors other than the investigated research variables needed to be kept to a minimum. Therefore, the primary studies (especially studies 1 and 2)

had rigorous exclusion criteria, including no psychotropic medication and no medication or chronic illness that might affect the HPA axis or ovarian hormones. Only participants with highly regular cycles (+/- 2 days) between 26 and 30 days were included. While this reduced noise in the data set, it also decreases the generalizability of the results, as many individuals have more irregular cycles, take medications, or have chronic illnesses. Furthermore, individuals with active suicidal ideation were excluded, which limited the informative value of the results on the cyclicity of suicidality. Implications from the results need to be replicated with broader samples. This does also apply for transgender individuals with a menstrual cycle. While studies 3 and 6 did not exclude transgender or non-binary individuals, there were not specifically recruited for, and only one non-binary participant was included in the study. Therefore, it was not possible to investigate specific menstrual cycle effects for transgender or non-binary individuals. Conducting such investigations is an important subject for future research, especially because an increased prevalence of mental health problems exists in this population (Carmel and Erickson, 2016; Safer, 2021). It is imperative to not only strengthen the inclusion of female participation in research but also of trans- and non-binary people who might also be affected by endogenous or medication-caused hormone changes.

Lastly, regarding the proposed heuristic model some limitations that were not investigated in the studies in this dissertation need to be pointed out. Especially, there were no investigations of subjective stress across the menstrual cycle (path 2) and biological stress markers as a moderator for the relationship between hormonal fluctuations and depressive symptoms (path 3). The two meta-analyses investigated fluctuations of HPA axis functioning as biological stress marker, but perceived stress was not included in the study. As HPA axis functioning and perceived stress do not necessarily correlate (Weckesser et al., 2019), it is important to address the fluctuation of perceived stress across the menstrual cycle in future studies – ideally using frequent measuring as done in studies 1-3 and 6. Additionally, other

important biological stress markers, such as alpha-amylase, need to be considered (Nater et al., 2005; Nater and Rohleder, 2009). Furthermore, the moderating effect of stress on the hormone and depressive symptom relationship was only investigated for the experience of stressful life events and not for biological stress markers. The exact interplay of biological stress markers as a moderator for hormone sensitivity is an important subject for future research.

9.4 Implications for future research

The results of this dissertation provide specific implications for future research. The results emphasize that menstrual cycle-related fluctuations of depressive symptoms occur, but only in some hormone sensitive patients, with different patterns and differently across depressive symptoms. Therefore, future research must assess and analyze cyclicity effects symptom-specifically, ideally with multiple validated items per individual symptom (e.g., multiple items for concentration difficulties). This needs to be analyzed while allowing for individual effects (e.g., through multilevel models including random effects) and various cyclicity patterns (e.g., mid-cycle or perimenstrual worsening). As only a subset of susceptible individuals experiences hormone sensitivity, it is essential to identify these individuals as quickly as possible in future studies to reach the required sample sizes with hormone sensitivity efficiently. A screening tool for Premenstrual disorders (PSST) is available (Steiner et al., 2003); however, such retrospective screening tools have been shown to misdiagnose both individuals with and without premenstrual disorders compared to prospective ratings (Eisenlohr-Moul et al., 2017). Consequently, more accurate screening tools, biomarkers, and indicators for hormone sensitive individuals must be developed and investigated. Possible screening methods could include external assessments of symptoms, for example from partners or family members. Alternatively, smartphone-based passive assessment of affect correlates or movement change as a marker for increasing symptoms could be used as indicators for hormone sensitivity (Eisenlohr-Moul, 2019). Furthermore, future studies can replicate the

strengths of this thesis when assessing cyclicity of depressive symptoms, for example, by measuring daily ovarian hormones in dried urine, assessing affective symptoms daily, or using meta-analyses to combine studies with smaller sample sizes. Future research can avoid the limitations of this thesis, for example, by assessing multiple cycles with larger sample sizes.

Further, the results raise scientific questions and call for explanations for the observed phenomena, such as biological explanations of hormone sensitivity and essential psychological mechanisms in PME. Rigorous longitudinal studies are necessary to identify risk factors and biological markers associated with hormone sensitivity in depression across the life span (e.g., puberty, menstrual cycle, pregnancy, menopause). Future studies can be informed by biological risk factors for PMDD, e.g. differently functioning of $GABA_A$ receptors (Girdler et al., 2001). Additionally, other possible explanations for this hormone sensitivity need to be considered, as PME of depression might have different biological causes than PMDD (Eisenlohr-Moul, 2019). This includes the integration of psychosocial and biological stress and resilience factors that may exacerbate the risk for hormone sensitivity and PME of depression (Schweizer-Schubert et al., 2021). Investigating these factors will significantly enrich the understanding of the etiology of depression in general. Identifying which individuals are sensitive to hormone fluctuation is essential to develop new and improved pharmacological and psychotherapeutic treatment options for depression influenced by ovarian hormones. Thereby, it is also relevant to identify the psychological mechanism of PME, for example, which symptoms appear first, which mechanisms (e.g., self-focused attention, impulsivity, or rumination) drive the expression of other symptoms, and which behaviors accompany self-reported symptoms (Eisenlohr-Moul, 2019). Such mechanisms can then be targeted in psychotherapeutic interventions.

Furthermore, other mental disorders might show cyclical fluctuations and are understudied. For example, some smaller studies have investigated and indicated the

occurrence of PME in posttraumatic stress disorder (Nillni et al., 2015), borderline personality disorder; Eisenlohr-Moul et al., 2015; T. A. Eisenlohr-Moul et al., 2018), obsessive-compulsive disorder (Vulink et al., 2006) or substance abuse (Barone et al., 2023; Martel et al., 2017). However, epidemiological studies estimating the prevalence of PME in these disorders, or biological explanations, are lacking (Eisenlohr-Moul, 2019).

This implies the ultimate aim of the research on PME of depression: to investigate and develop prevention and treatment options for patients with menstrual cycle-related forms of depression and other disorders. While there are some guidelines for treating PMDD (Hantsoo and Epperson, 2015; Kleinstäuber et al., 2016), the research on individualized treatment of PME of depression is in its infancy. Recent studies have demonstrated that some specific treatments for PMDD are not effective for PME, for example, GnRH analogues (Freeman et al., 1993) or the $GABA_A$ antagonist isoallopregnanolone (Bixo et al., 2017), making treatment options rarer and more complicated to investigate. A small clinical study by Eisenlohr-Moul et al. (2018) indicated a treatment effect of ovarian hormone supplementation that prevents hormone withdrawal in patients with PME of depression. Additional research on pharmacotherapy or psychotherapy to treat PME of depression is scarce and highly needed.

All in all, the results of this dissertation provide implications for the improved assessment and understanding of individual and symptom-based research on PME of depression. As a research agenda, the next steps in investigating PME of depression are to clarify biological underpinnings and key psychological mechanisms, improve assessment methods and improve and develop psychotherapeutic and pharmacological interventions.

9.5 Conclusion

This dissertation presented a collection of studies investigating the interconnection between stress, depressive symptoms, and the menstrual cycle (see figure 4). The results reveal that depressive symptoms can be exacerbated systematically across the menstrual cycle and that these exacerbations are better described on a symptom-specific and individual level compared to aggregated measures or across multiple individuals. The results also provide promising new indications that menstrual cycle irregularity seems to be a risk factor for increased depressive symptoms. Further, biological and perceived stress markers have shown to be relevant for menstrual cycle-related depression, specifically indicating cycle phase differences of HPA axis functioning and a moderating effect of the menstrual cycle and depressive symptom relationship.

Therefore, it is essential to include the menstrual cycle and ovarian hormone fluctuations in future research on depression and stress. This has been neglected in the majority of previous studies, leading to a lack of data investigating sex differences and data on issues affecting females in particular – the so-called gender data gap (Temin and Roca, 2016). Enriching the data and identifying such influencing factors for the etiology of depression provides a target point for developing individualized and more effective treatment – both pharmacologically and psychotherapeutic. Importantly, the available knowledge and future treatment options on reproductive mood disorders or women's mental health need to be anchored in the training and education of current and future clinicians and psychotherapists. This is currently rarely the case, shown by an absence of these topics in education curricula for Bachelor's and Master's degrees for psychotherapists in Germany ("Approbationsordnung für Psychotherapeutinnen und Psychotherapeuten," 2020) or medical schools in the US (Henrich and Viscoli, 2006; Song et al., 2016). Even large German training institutes for psychotherapists, such as the German Society for Behavior Therapy (DGVT), do not offer

seminars or training on topics such as PMDD or postpartum depression in the regular training program for psychotherapists (“DGVT - Theoretische Ausbildung,” 2023) nor in their advanced training program for experienced psychotherapists (“Themenseminare - DGVT Fort- und Weiterbildung,” 2023). Therapists and clinicians need to have knowledge of reproductive mood disorders such as PMDD, postpartum depression, or depression during menopause to provide understanding, psychoeducation, and optimal care to affected patients.

In summary, females and female-specific factors are underrepresented in basic science, treatment development, and education curricula, despite the important role of the menstrual cycle and ovarian hormones that was demonstrated with the results of this dissertation. It is imperative to evolve further from the disregard of sex- and gender-related differences in mental health to an adequate representation of female-specific factors in science. The interconnection between depression, stress, and the menstrual cycle presented in this dissertation deepens our understanding of female-specific factors of depression. This ultimately provides great potential to improve the treatment of depression with tailored interventions, inform future studies, and advance mental health care for depression.

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CHAPTER 10

10.1 Eigenständigkeitserklärung

Hiermit erkläre ich, die vorliegende Dissertation selbstständig verfasst und ohne unerlaubte Hilfe angefertigt habe.

Alle Hilfsmittel, die verwendet wurden, habe ich angegeben. Die Dissertation ist in keinem früheren Promotionsverfahren angenommen oder abgelehnt worden.

Berlin, 07. Juli 2023

Hannah Klusmann

10.2 List of publications

Articles in peer reviewed journals

Klusmann, H., Brose, A., Schulze, L., Engel, S., Laufer, S., Bücklein, E., Knaevelsrud, C., & Schumacher, S. (under review). Menstrual cycle related depressive symptoms and their diurnal fluctuations – an ambulatory assessment study. *Depression and Anxiety.*

Klusmann, H., Kapp, C.., Engel, S., Laufer, S., Schumacher, T., Bücklein, E., Knaevelsrud, C., & Schumacher, S. (under review). Higher depressive symptoms in irregular menstrual cycles - converging evidence from cross-sectional and prospective assessments. *Psychopathology.*

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Haering, S., Schulze, L., Geiling, A., Meyer, C., **Klusmann, H**., Schumacher, S., Knaevelsrud, C. & Engel, S.,(submitted). Higher risk – less data: A systematic review and meta-analysis on the role of sex and gender in trauma research. *Clinical Psychological Science.*

Engel, S., Laufer, S., **Klusmann, H.**, Schulze, L., Schumacher, S., Knaevelsrud, C., 2023. Cortisol response to traumatic stress to predict PTSD symptom development – a systematic review and meta-analysis of experimental studies. *European Journal of Psychotraumatology.* (IF: 5.78).

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Laufer, S, Schulze, L., Engel, S., **Klusmann, H.**, Skoluda, N., Nater, U., Knaevelsrud, C. & Schumacher, S. (2023). The effect of an internet-based intervention for depression on cortisol and alpha-amylase. *Psychoneuroendocrinology.* (IF: 4,7).

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*geteilte Erstautorenschaft

Conference contributions

Blecker, M.K., **Klusmann, H.,** Engel, S., Haering, S., Schumacher S. & Knaevelsrud, C. (2023). Traumatische Kindheitserfahrungen als Risikofaktor für PTBS Symptome und Stresserleben nach einer unbeabsichtigten Schwangerschaft - ein Studiendesign. Jahrestagung der deutschsprachigen Gesellschaft für Psychotraumatologie (DeGPT), Zürich, Schweiz, 16. – 18. Februar 2023.

Brose, A., **Klusmann, H.,** Heinrich, M., Zagorscak, P., Bohn, J., S., Schumacher S. & Knaevelsrud, C. (2023). Die Messung depressiver Symptome in EMA Studien. 2. Psychotherapie Kongress (DPK), Berlin, Deutschland, 10.-13.Mai 2023.

Bücklein, E., **Klusmann, H.,** Schulze, L., Engel, S., Knaevelsrud, C. S. & Schumacher S. (2023). HPA-Achsen-Aktivität während des Menstruationszyklus - eine systematische Übersicht und Meta-Analyse von Längsschnittstudien. 2. Psychotherapie Kongress (DPK), Berlin, Deutschland, 10.-13.Mai 2023.

Klusmann, H., Brose, A., Engel, S., Schumacher S. & Knaevelsrud, C. (2022). Characterizing the menstrual cycle in adolescents – challenges and guidelines for psychological research. Conference of International Assosciation of Psychoneuroendocrinology (ISPNE), Chicago, USA, 8.–10. September 2022.

Haering S., **Klusmann H.,** Schumacher S., Knaevelsrud, C., Engel, S. (2022) Doing Gender in der PTBS Forschung? Ein systematisches Review zur Berücksichtigung von Geschlecht in prospektiven Studien zu PTBS Risiko. 1. Deutscher Psychotherapiekongress, Berlin, 7.–11. Juni 2022.

Klusmann, H., Schulze, L., Engel, S., Knaevelsrud, C., Schumacher, S. HPA axis activity in the course of the menstrual cycle – a meta-analysis of longitudinal studies. 3rd International Congress of the World Association for Stress Related and Anxiety Disorders (WASAD), Vienna, Austria. 20 - 22.09.2021

10.3 Supplemental Material

Supplemental material study 1

Appendix 1A: Detailed exclusion criteria (chronic disease and medication)

Appendix 1B: Modified PHQ-9 adapted for ambulatory assessment

Appendix 1C: Procedure for determining standardized cycle days

Appendix 1D: Flowchart of participant inclusion

Appendix 1E: Results of pairwise t-test to compare cyclicity of depressive symptoms

Appendix 1A

Excluded chronic diseases and medication.

Chronic disease or condition
Psoriasis
Lymph node impairment
Endometriosis
Diabetes Type 1 / 2
Neurodermatitis
Asthma
AV Block
Polycystic Ovaries
Crohn's disease
Hashimoto
Coeliac disease
Lichen sclerosus
Glaucoma
Hay fever
Irritable bowel syndrome
Grave's disease
Sinusitis
Anemia
Menier's disease
Pain syndrome
Blood clotting disorders
Gastritis
Epilepsy
Alopecia areata

Medication
Glucocorticoids / hydrocortisone (continuous intake)
All psychotropic drugs
Anticonvulsants
Sedatives
Orraycea

Appendix 1B

Modified PHQ-9 adapted for ambulatory assessment

German original:

Wie sehr fühlen Sie sich **GERADE** durch die folgenden Beschwerden beeinträchtigt?
1) Wenig Interesse oder Freude an Ihren Tätigkeiten
2) Niedergeschlagenheit, Schwermut oder Hoffnungslosigkeit
3) Schwierigkeiten ein- oder durchzuschlafen oder vermehrter Schlaf
4) Müdigkeit oder Gefühl, keine Energie zu haben
5) Verminderter Appetit oder übermäßiges Bedürfnis zu essen
6) Schlechte Meinung von sich selbst; Gefühl, ein Versager zu sein oder die Familie enttäuscht zu haben
7) Schwierigkeiten, sich auf etwas zu konzentrieren, z.B. beim Zeitunglesen oder Fernsehen?
8) Sind Ihre Bewegungen oder Ihre Sprache so verlangsamt, dass es auch anderen auffallen würde? Oder sind Sie im Gegenteil „zappelig“ oder ruhelos und hatten dadurch einen stärkeren Bewegungsdrang als sonst?
9) Gedanken, dass Sie lieber tot wären oder sich Leid zufügen möchten

gar nicht 0	1	2	3	4	Sehr stark 5
o	o	o	o	o	o

English translation:

How much do you feel affected by the following symptoms RIGHT NOW?
1) Little interest or pleasure in your activities
2) Low spirits, melancholy or hopelessness
3) Difficulty falling asleep/staying asleep or increased sleep
4) Fatigue or feeling of having no energy
5) Decreased appetite or excessive need to eat
6) Poor opinion of self; feeling like a failure or having let family down
7) Difficulty concentrating on something, such as reading the newspaper or watching television
8) Are your movements or speech so slowed down that others would notice? Or, on the contrary, are you "fidgety" or restless and thus had a stronger urge to move than usual?
9) Thoughts that you would rather be dead or harm yourself

Not at all 0	1	2	3	4	Very strongly 5
o	o	o	o	o	o

Appendix 1C

Procedure for determining standardized cycle days

First, we assigned a forward count and a backward count variable to each assessment day of each participant as described by Schmalenberger et al. (2021): *forward count* is defined as the respective assessment date minus the previous cycle start date (start_cycle_1), therefore it typically ranges from 1 to 28. *Backward count* is defined as the assessment date minus the next cycle start plus 1 (start_cycle_2), therefore it typically ranges from -28 to -1. We further assigned an *ovulation count* variable to each day, with the day of positive ovulation test referred to as day zero, the prior days as -1, -2, -3 etc. and the subsequent days as 1,2,3 etc.

Second, we assigned one of the four cycle phases to respective assessment days: mid-follicular (days -7 to -3 before positive lh test or days 4 to 7 after menstrual onset (forward count)), periovulatory (days -2 to +1 around positive lh test or, days -15 to -12 based on backward count), mid-luteal (days -9 to -5 before menstrual onset (backwardcount) or premenstrual phase[6](three days before onset of menses (based on backwardcount)). For more background see the openly accessible R script, or the outstanding guideline paper by Schmalenberger et al. (2021).

Third, we selected 27 uniform days (c0 – c26) from each participant to be able to compare between and within effects of cyclicity.

For days c0 – c9, we selected the first ten days of the assessment cycle to represent the menstrual and mid-follicular hormone pattern (low estrogen and progesterone).

For days c10 – c16, we selected the seven days surrounding a positive LH test to represent the periovulatory hormone pattern (strong rise and fall of oestradiol and LH). If no ovulation test result was available, we estimated the periovulatory hormone pattern by using days -17 to -11 counting back from the onset of next menses.

For days c17 – c26, we used the last 10 days of the assessment cycle before onset of next menses, representing the mid-luteal and premenstrual hormone pattern (strong rise and fall of progesterone, slight rise and fall of estrogen). If a participant had a short cycle resulting in cycle days being assigned to multiple standardized days, the days reflecting the periovulatory phase (c10 – c16) were chosen over other phases. In summary, this resulted in a dataset of 27 cycle days per participant (newly assigned as c0 – c26), where the cycle days fell into comparable phases.

[6] As we assessed only one cycle, starting with the first days of menses, we did not assess the perimenstrual phase (which measures the 5 days sourrounding each menstrual start, but the premenstrual phase at the end of the assessment)

Three examples for determining standardized cycle days

28 day cycle with positive LH test on day 14/-15

Forward	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Backward	-28	-27	-26	-25	-24	-23	-22	-21	-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1
Ovulation											-3	-2	-1	0	1	2	3											
Cycle day	c0	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16		C17	C18	C19	C20	C21	C22	C23	C24	C25	C26

32 day cycle with positive LH test on day 18/-15

Forward	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Backward	-32	-31	-30	-29	-28	-27	-26	-25	-24	-23	-22	-21	-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1
Ovulation															-3	-2	-1	0	1	2	3											
Cycle day	c0	C1	C2	C3	C4	C5	C6	C7	C8	C9					C10	C11	C12	C13	C14	C15	C16		C17	C18	C19	C20	C21	C22	C23	C24	C25	C26

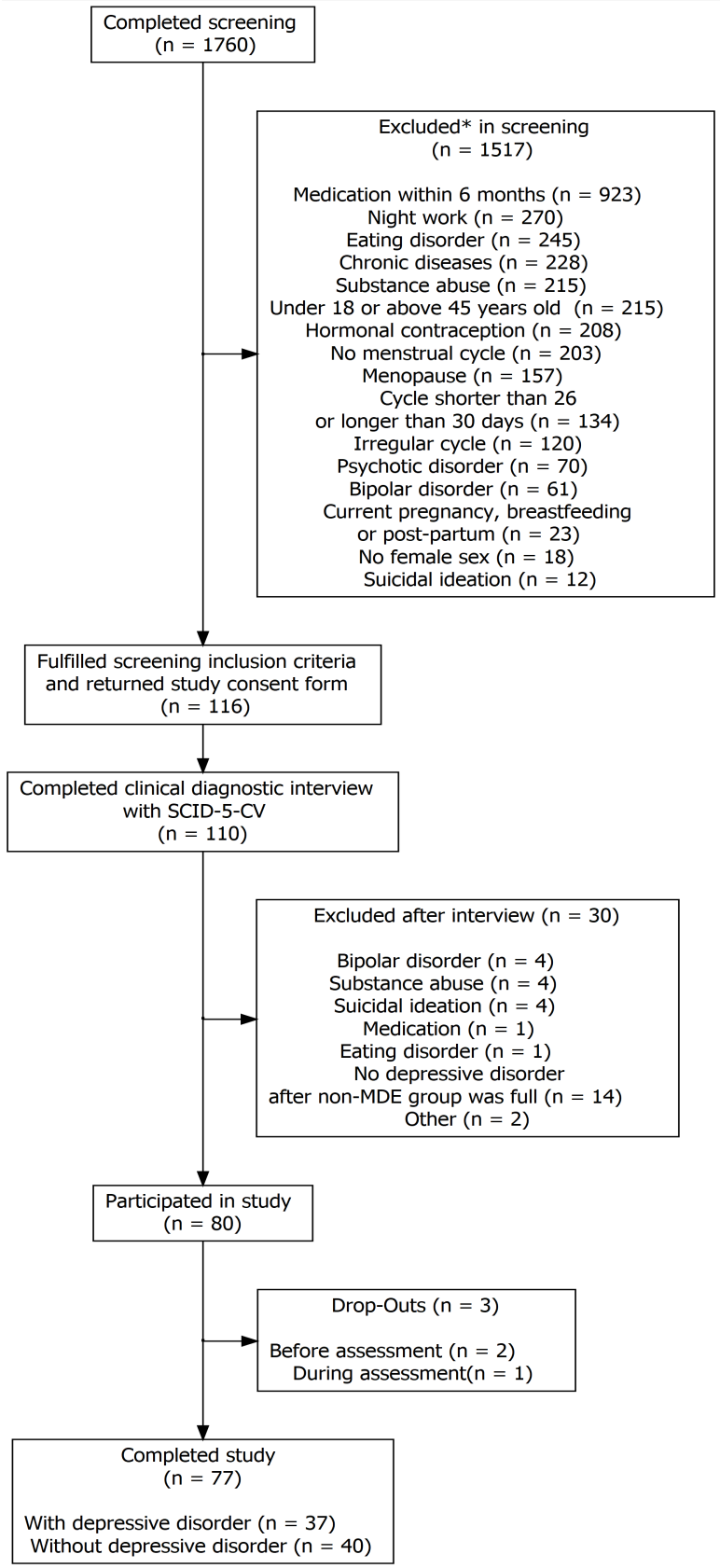
25 day cycle with positive LH test on day 12/-14

Forward	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Backward	-25	-24	-23	-22	-21	-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1
Ovulation									-3	-2	-1	0	1	2	3										
Cycle day	c0	C1	C2	C3	C4	C5	C6	C7	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26

→ this person would have no values for days C8 and C9 because of a shorter follicular phase

Appendix 1D

Flowchart of participant inclusion



Appendix 1E

Results of pairwise t-test to compare cyclicity of depressive symptoms

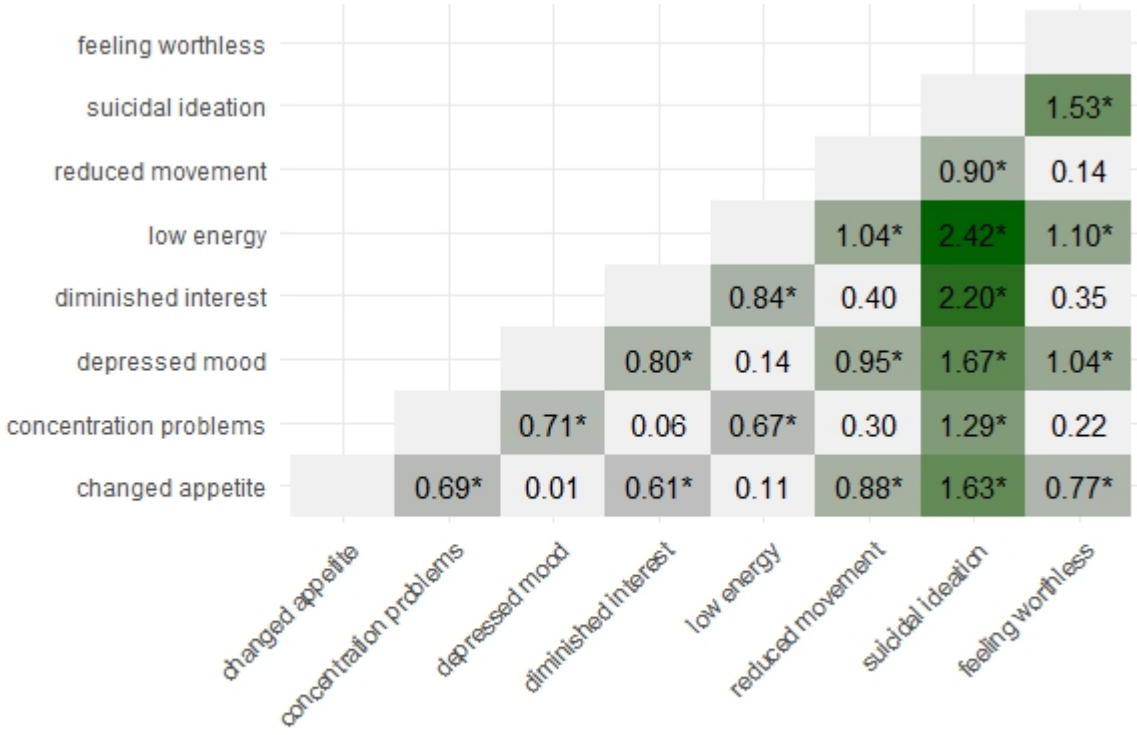
Symptom 1	Symptom 2	Pairwise comparison	Adjusted p-value	Significance adjustet p-value	Mean cc symptom 1	Mean cc symptom 2
Changed appetite	Concentration problems	t(76)=4.294 , $p < .001$	>0.01	**	0.01	0.08
Changed appetite	Depressed mood	t(76)=-0.033 , $p = .974$	1.00	ns	0.01	0.32
Changed appetite	Diminished interest	t(76)=3.796 , $p < .001$	0.01	**	0.01	0.08
Changed appetite	Low energy	t(76)=0.684 , $p = .496$	1.00	ns	0.01	0.19
Changed appetite	Reduced movement/Restlessness	t(76)=5.437 , $p < .001$	>0.01	****	0.01	0.13
Changed appetite	Suicidal ideation	t(76)=10.093 , $p < .001$	>0.01	****	0.01	0.06
Changed appetite	Feeling worthless	t(76)=4.751 , $p < .001$	>0.01	***	0.01	0.12
Concentration problems	Depressed mood	t(76)=-4.385 , $p < .001$	>0.01	**	0.08	0.32
Concentration problems	Diminished interest	t(76)=-0.362 , $p = .718$	1.00	ns	0.08	0.08
Concentration problems	Low energy	t(76)=-4.135 , $p < .001$	>0.01	**	0.08	0.19
Concentration problems	Reduced movement/Restlessness	t(76)=1.886 , $p = .063$	1.00	ns	0.08	0.13

Symptom 1	Symptom 2	Pairwise comparison	Adjusted p-value	Significance adjustet p-value	Mean cc symptom 1	Mean cc symptom 2
Concentration problems	Suicidal ideation	t(76)=8.029 , $p < .001$	>0.01	****	0.08	0.06
Concentration problems	Feeling worthless	t(76)=1.369 , $p = .175$	1.00	ns	0.08	0.12
Depressed mood	Diminished interest	t(76)=4.965 , $p < .001$	>0.01	***	0.32	0.08
Depressed mood	Low energy	t(76)=0.843 , $p = .402$	1.00	ns	0.32	0.19
Depressed mood	Reduced movement/Restlessness	t(76)=5.921 , $p < .001$	>0.01	****	0.32	0.13
Depressed mood	Suicidal ideation	t(76)=10.353 , $p < .001$	>0.01	****	0.32	0.06
Depressed mood	Feeling worthless	t(76)=6.431 , $p < .001$	>0.01	****	0.32	0.12
Diminished interest	Low energy	t(76)=-5.216 , $p < .001$	>0.01	****	0.08	0.19
Diminished interest	Reduced movement/Restlessness	t(76)=2.506 , $p = .014$	0.40	ns	0.08	0.13
Diminished interest	Suicidal ideation	t(76)=13.632 , $p < .001$	>0.01	****	0.08	0.06
Diminished interest	Feeling worthless	t(76)=2.178 , $p = 0.032$	0.91	ns	0.08	0.12
Low energy	Reduced movement/Restlessness	t(76)=6.438 , $p < .001$	>0.01	****	0.19	0.13
Low energy	Suicidal ideation	t(76)=14.991 , $p < .001$	>0.01	****	0.19	0.06

Symptom 1	Symptom 2	Pairwise comparison	Adjusted p-value	Significance adjustet p-value	Mean cc symptom 1	Mean cc symptom 2
Low energy	Feeling worthless	t(76)=6.799 , $p < .001$	>0.01	****	0.19	0.12
Reduced movement/Restlessness	Suicidal ideation	t(76)=5.563 , $p < .001$	>0.01	****	0.13	0.06
Reduced movement/Restlessness	Feeling worthless	t(76)=-0.896 , $p = 0.373$	1.00	ns	0.13	0.12
Suicidal ideation	Feeling worthless	t(76)=-9.515 , $p < .001$	>0.01	****	0.06	0.12

Note: CC = cosine coefficient

Tile plot of visualizing effect sizes of pairwise comparisons



Note: *significant comparisons marked with "*", darker green displays larger effect sizes

Supplemental material study 2

Supplemental Material 2A

Excluded chronic diseases.

Chronic disease or condition
Psoriasis
Lymph node impairment
Fibromyalgia*
Endometriosis
Diabetes Type 1 / 2
Neurodermatitis
Asthma
AV Block
Polycystic Ovaries
Crohn's disease
Hashimoto
Coeliac disease
Lichen sclerosus
Glaucoma
Hay fever
Irritable bowel syndrome
Grave's disease
Sinusitis
Anemia
Menier's disease
Pain syndrome
Blood clotting disorders
Gastritis
Epilepsy
Alopecia areata
Obesity

Note. *Fibromyalgia was included in the longitudinal analysis.

Supplemental Material 2B

Influence of COVID-19 pandemic

In December 2019, during the data collection, an airborne virus (SARS-Cov-2) was identified as highly contagious and fast spreading causing a global pandemic. Political measures to contain the virus caused miscellaneous impairments in everyday life of people in Germany and worldwide. From the beginning of the German "lockdown" at the end of March 2020, five items were included in the questionnaire asking participants specifically about their constraints, worries and stressors at the current state of the pandemic whilst study participation.

The following table shows the results of the ANCOVA for the longitudinal study including the variable for worries about COVID-19 and using full-case analysis.

Table

Results of the ANCOVA for longitudinal study including worries about COVID-19

	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	-0.33	0.40	-0.34	.408
WHS_{occur}	0.61	0.17	3.47	.001*
Cycle irregularity	0.38	0.21	1.81	.076
COVID	-0.01	0.06	-0.1	.924

Notes. Std. Error: standard error; WHS_{occur}: unstandardized regression weight for Weekly Hassles Scale (frequency of occurred stressors); Cycle irregularity: unstandardized regression weight for menstrual cycle irregularity; COVID: unstandardized regression weight for sum score of five items describing stressful COVID-19-pandemic experiences; *: $p < 0.05$

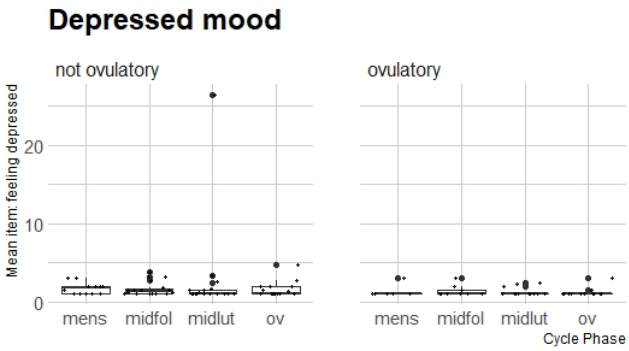
Supplemental material study 3

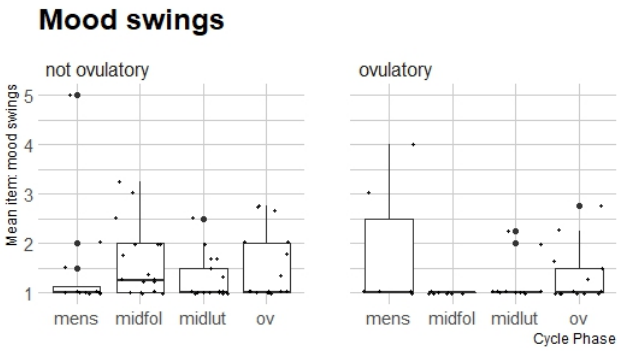
Appendix 3A: Comparison of affective symptoms by ovulatory status

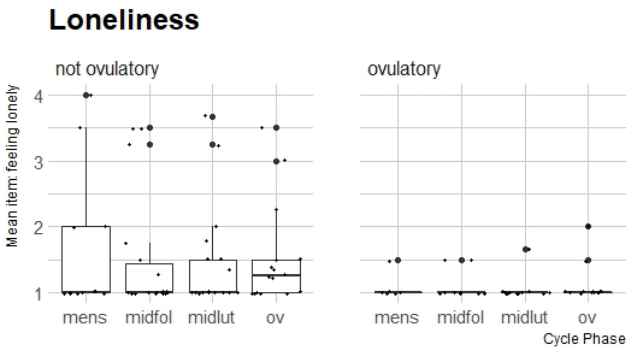
	not ovulatory (N=19)	ovulatory (N=13)	p-value
Feeling depressed			
AUCg (Mean ± SD)	2.09 ± 2.10	1.31 ± 0.375	0.13
Feeling lonely			
AUCg (Mean ± SD)	1.68 (0.895)	1.19 (0.198)	**0.032***
Feeling hopeless			
AUCg (Mean ± SD)	1.53 (0.792)	1.12 (0.308)	0.055
Feeling anxious			
AUCg (Mean ± SD)	1.85 (0.919)	1.49 (0.563)	0.178
Moodswings			
AUCg (Mean ± SD)	1.76 (0.822)	1.40 (0.535)	0.153
Rejection sensitivity			
AUCg (Mean ± SD)	1.76 (0.972)	1.37 (0.576)	0.17
Feeling angry			
AUCg (Mean ± SD)	2.05 (1.12)	1.65 (0.630)	0.218
Low interest			
AUCg (Mean ± SD)	1.48 (0.548)	1.37 (0.664)	0.636
Difficulty concentrating			
AUCg (Mean ± SD)	2.00 (1.01)	1.37 (0.461)	**0.025***
Interpersonal conflict			
AUCg (Mean ± SD)	1.54 (0.736)	1.32 (0.390)	0.275
Feeling overwhelmed			
AUCg (Mean ± SD)	1.72 (0.814)	1.34 (0.491)	0.114
DRSP Sum score[1]			
AUCg (Mean ± SD)	16.0 (6.90)	12.30 (3.31)	0.057

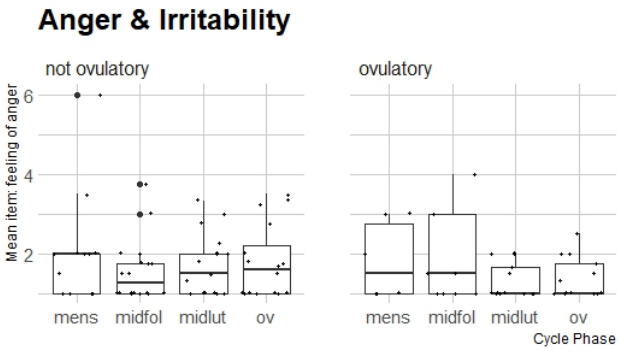
[1]Sum score was calculated excluding the loneliness item

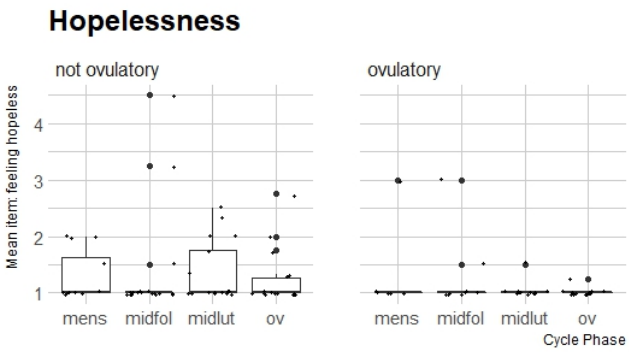
Appendix 3B: Graphs of affective symptoms across the menstrual cycle by ovulatory status

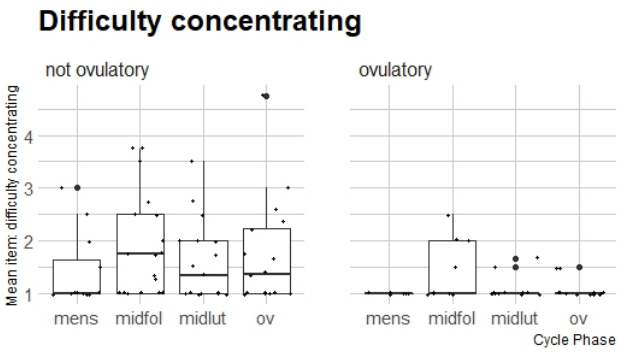




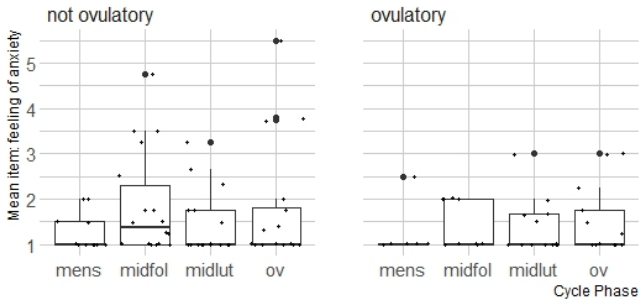




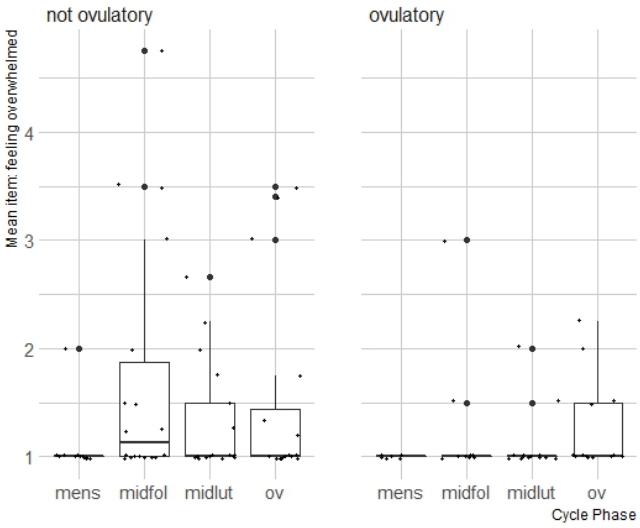




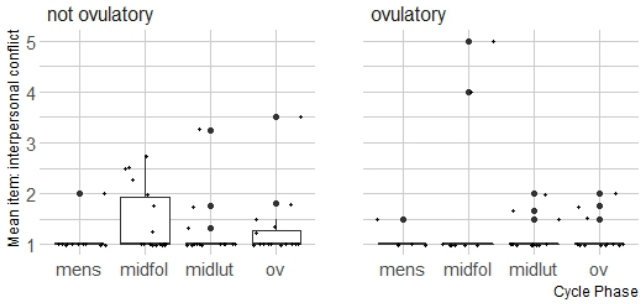
Anxiety



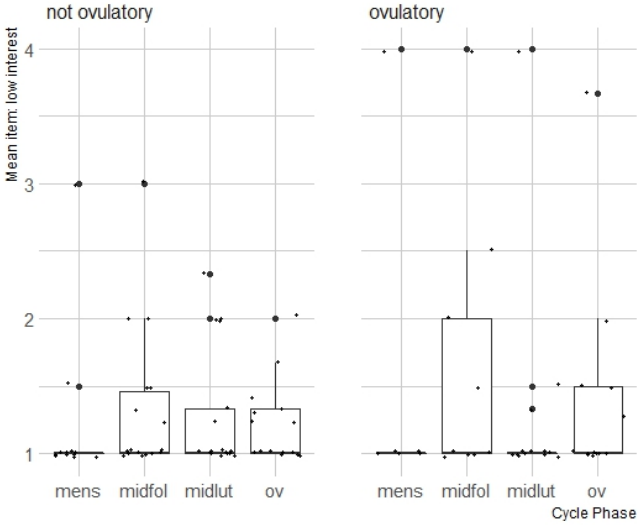
Overwhelmed



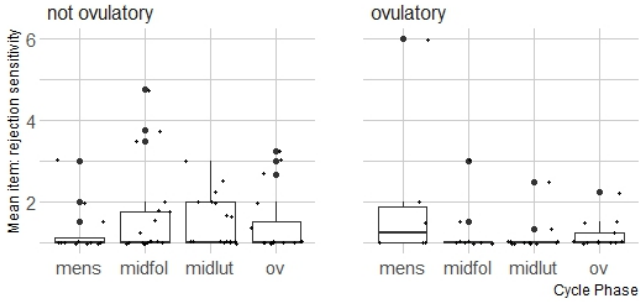
Interpersonal conflict



Low interest



Rejection sensitivity

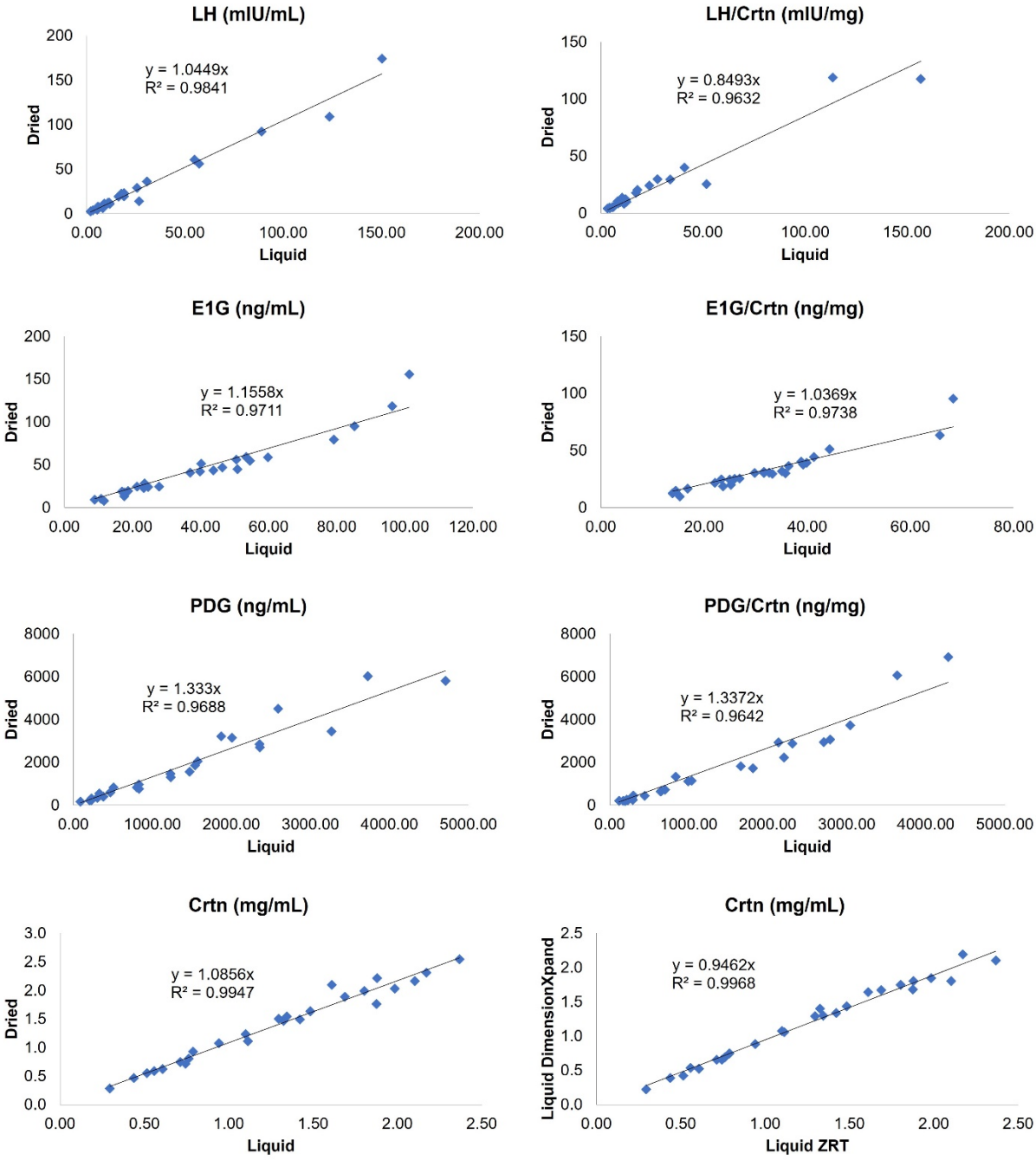


Appendix 3C: Checklist for studying the menstrual cycle in adolescents

Recommended procedures for studying the peripubertal menstrual cycle	
Assessing menarche	Assess menarche dates from both participant and parent. Ask participant if they told their parents that they started their period at that time or not/later. Assess contextual information at time of menarche (age it occurred, season of the year, nearest holiday) to confirm date accuracy. If parent and participant disagree by more than 3 months, facilitate a discussion to attempt to come to a consensus.
Length of assessment period	Allow at least 45 days of daily sampling for assessment of one menstrual cycle.
Sampling frequency	Ideally, collect hormone samples daily to aid formation of habit and analyze hormone peaks. If that is not feasible, collect hormones in high frequency (e.g. every second day) or use ovulation tests (LH strips) daily starting within the first week after menses onset.
Identifying menses	Confirm multiple days of bleeding before determining menses onset and assess heaviness of bleeding (bleeding vs. spotting) to exclude possibility of misinterpreting mid-cycle spotting as menses.
Measuring ovulation status	When vaginal ultrasound is inaccessible or unfeasible, ovulation status may be determined from daily hormone measurements based on the occurrence of an LH peak and PdG rise in relation to the rest of the cycle. Relational methods and not absolute thresholds are important in peripuberty. Methods are provided in the manuscript.
Determining cycle phase	The date of the onset of menses and the date of the LH peak may be used as anchor points to determine cycle phase as such: *Perimenstrual:* Days -2 to +2 before and after menses onset. *Mid-follicular:* Days -7 to -3 before LH peak. *Periovulatory:* Days -2 to +1 before and after LH peak. *Mid-luteal:* Individual phase length (35% of luteal phase) around midway point between LH peak and onset of menses If no ovulation measure is available: *Perimenstrual:* Days -2 to +2 before and after menses onset. *Mid-follicular:* +4 to +7 after menses onset. *Periovulatory:* not recommended. *Mid-luteal:* -6 to -3 before onset of menses.

	➔ Attention: use with caution when no ovulation measure is available See manuscript for more details
Daily compliance checks	Participants complete a daily survey, entering the time and date of their daily sample for study staff to monitor compliance.
Sample assessment	Participants receive instructions for sample collection and storage, and a study checklist to keep track of daily samples and surveys. They receive a large binder clip to dry urine card in open air before storage in freezer. To increase compliance, participants send a picture of sample card at the time of sample assessment to the study team. Urine cards are labeled with freezer-save print or marker.

Appendix 3D: Validation studies comparing liquid and dried urine methodologies.



Supplemental material study 4

not included in the online version due to copyright reasons, see Chapter 6

Supplemental material study 5

not included in the online version due to copyright reasons, see Chapter 7