# Menstrual cycle monitoring on eumenorrheic endurance athlete

*Identification of changes in physiological and perceptual response across menstrual cycle phases using wearable device, urine luteinizing hormone detection kits and self-reported measures.*

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submitted by

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## Abstract

**Introduction:** Female athlete is subject to natural hormones fluctuation from puberty to menopause that have various effects on physiological systems. Further research on specific effects, recommendations and tools for monitoring menstrual cycle are yet to be established. The aim of the present work is to investigate menstrual cycle monitoring by identifying changes in physiological and perceptual features across the menstrual cycle on female endurance athletes.

**Method:** Nine endurance-trained female athletes, aged between 20 and 35 years old, tracked their physiological activity (distal body temperature, heart rate, heart rate variability) using OURA multi-sensor ring, nightly across 3 menstrual cycles. They performed morning urine Luteinizing hormone (LH) test and completed daily questionnaire to assess perceptual response (wellness indicators, perceived readiness and motivation to train, pre-/menstrual symptoms, rated perceived exertion). Distal Body temperature (DBT) and LH-test were used to identify four menstrual cycle windows of interest (perimenstrual, mid-follicular, periovulatory, mid-luteal). Analysis of variance (ANOVA) and additional post-hoc test was performed to determine the significance of differences between means.

**Results:** An ovulatory window was identified for all recorded cycles. 35.7% of cycle interest windows were defined based on a nadir in DBT confirmed with LH-positive test, 53.6% was based only on nadir in DBT and 10.7% only with LH-positive result. The biphasic DBT pattern was statistically verified ($F_{3,72}$ = 43.8, $p<0.001$, $\eta^2_G=0.497$) as well as changes in nocturnal heart rate ($F_{3,\,75}$ = 6.07, $p < 0.001$, $\eta^2_G = 0.141$).

**Discussion** The low rate of positive LH tests obtained could be explained by methodological deficiency. Body temperature, on the other hand, enabled the identification of ovulatory window, which supports the robustness of overnight DBT as an indicator. The use of nadir alone for the application of the menstrual phasing method requires scientific validation. The resulting analysis must be taken with care. A lower nightly heart rate during mid-follicular and periovulatory phases compared to mid-luteal was recorded. A trend was identified in mid-follicular and periovulatory phases, during which individuals perceived higher readiness and motivation to train/compete.

**Conclusion:** Further research on the validation of the nadir in DBT to monitor ovulation is necessary. However, DBT data results are supporting the robust and convenience measures of wearable devices to retrospectively determine ovulation. Based on the wide possible effects of ovarian hormones on different physiological systems, this study presents an outline of self-reported and objective measurements for athletes to monitor across menstrual cycle.

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# 1 Introduction

## 1.1 Context

In sport sciences, the male body has long been the reference in research. Studies conducted on female athletes still only represent a small percentage of literature (Bruinvels et al., 2017). The origin of this literature gap is multifactorial, but despite women sport participation approaching parity with 48% of women at Tokyo 2020 Olympic Games, evidence-based training practice is still mostly based on male physiology (Elliott-Sale et al. 2021). Gender physiology has numbers of similarities, but women are naturally subject to cyclical hormonal fluctuations from puberty to menopause. The natural hormonal cycle has not only been ignored but also considered detrimental to performance (Höök et al., 2021) or controlled by various contraceptive means. The fluctuation of hormones has a physiological effect on cardiovascular, respiratory, metabolic, and neurologic systems. However, there is no evidence so far that physical performance is impacted by the menstrual cycle (McNulty et al., 2020). Since 2019, the number of studies on the topic "menstrual cycle" and "exercise" has considerably increased, showing a real need for answers on the interaction of the menstrual cycle with exercise (Carmichael et al., 2021). Despite growing interest on this vast subject, research on the topic is still insufficient and remains unable to provide guidelines (McNulty et al., 2020). Research must embrace the challenges of working in this field and adopt high quality methodology guidelines (Smith et al., 2022). A deeper understanding of menstrual cycle physiology, as well as the potential impact of estrogens and progestins on exercise physiology are key components to understand the goal of this thesis.

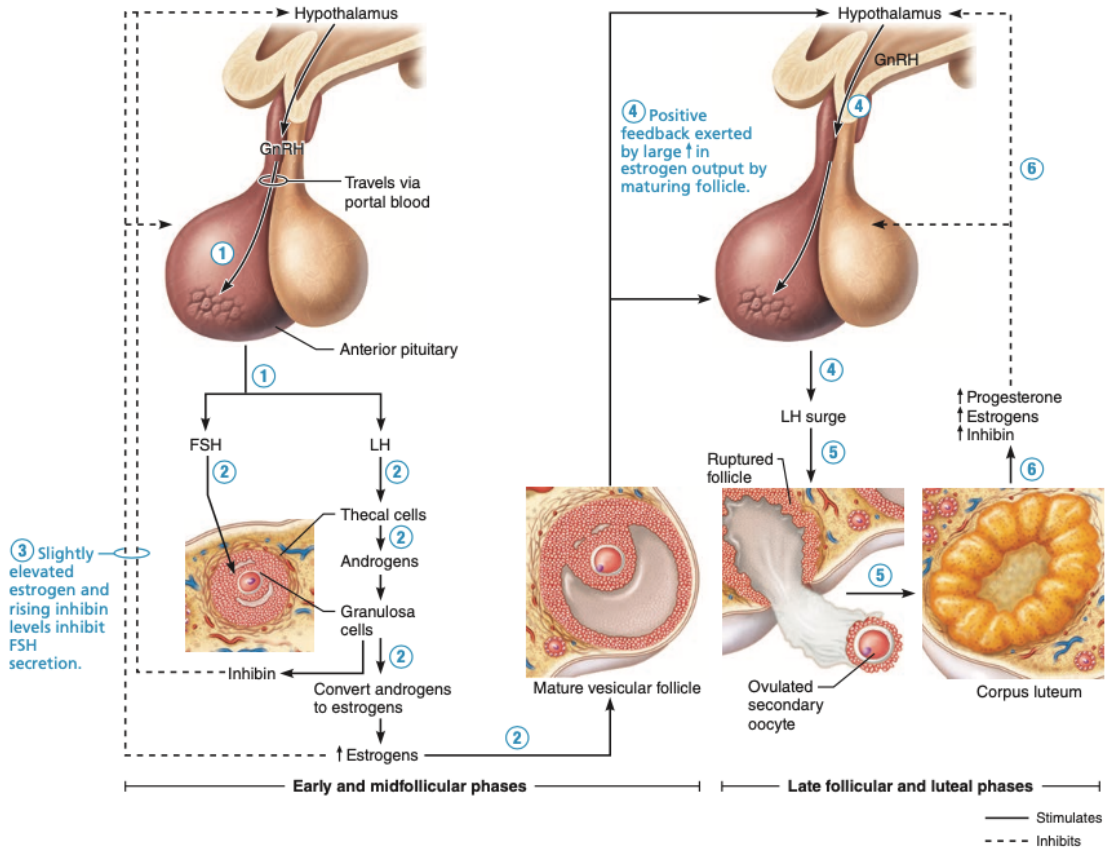
## 1.2 Menstrual cycle physiology

### *1.2.1 Hypothalamic-pituitary system*

The menstrual cycle is part of female reproductive system consisting of complex interactions between endocrine feedback loops between the hypothalamus, pituitary, and ovary (HPO axis) (Figure 1). The signal begins in the hypothalamus, where gonadotropin-releasing hormone (GnRH) is released into blood stream to reach the anterior-pituitary gland. The pituitary responds by releasing luteinizing hormone (LH) and follicle stimulating hormone (FSH). FSH and LH binds to ovarian receptors, which induces the production and secretion of both estrogen and progesterone (Sam & Frohman, 2008).

**Figure 1**

*Feedback loops of the HPO axis*



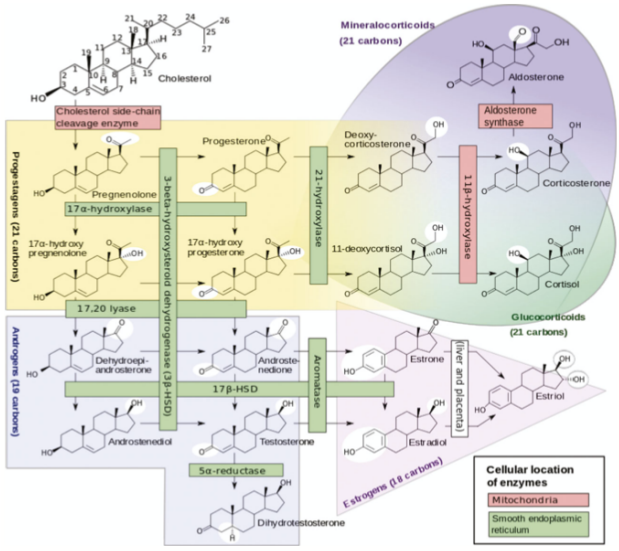

*Note*. Regulation of the ovarian cycle with anterior pituitary gonadotropins (FSH and LH) and ovarian hormones inducing positive and negative feedbacks. (1) GnRH secreted by the hypothalamus stimulates the anterior pituitary inducing LH and FSH secretion. (2) FSH and LH stimulate follicles growth. FSH stimulates the granulosa cells of vesicular follicles to release estrogens. LH stimulates thecal cells to release androgens which granulosa cells convert to estrogens. (3) As estrogen levels in plasma rise, it exerts negative feedback on the hypothalamus and anterior pituitary, inhibiting release of FSH and LH. Inhibin secreted by the granulosa cells, also causes negative feedback on FSH. (4) Once estrogens reach a critical blood concentration, they exert positive feedback on the anterior pituitary (5) High estrones level sets a cascade of events. The sudden burstlike release of LH. LH surge causes the rupture of the follicle releasing the oocyte (ovulation). LH surge also transform the ruptures follicle info a corpus luteum producing large amounts of progesterone. (6) Rising progesterone and estrogens exert negative feedback on LH and FSH release. (Marieb & Hoehn, 2015, p. 1079)

### *1.2.2 Sex steroid hormones*

Estrogens and progestins are steroid hormones, and their biosynthetic pathway (Figure 2) is the same regardless of the releasing organ. Estrogen is a group made up of estrone, estriol and estradiol-β-17. Estrone and estriol are produced locally in target tissues such as adipose cells and the liver while estradiol-β-17 is produced at the ovaries. Progesterone is a progestogen produced by the ovaries, but also produced locally in some tissues (Taraborrelli, 2015). Sex steroid hormones are known for their modulation role in reproductive functions. Research data have shown that other tissues are target of sex hormones including functions of nervous system, metabolism, soft tissues, immune system, and bone structure (Wierman, 2007), suggesting possible interactions between exercise physiology and sex hormones (McNulty et al., 2020).

**Figure 2**

*Steroidogenesis overview*



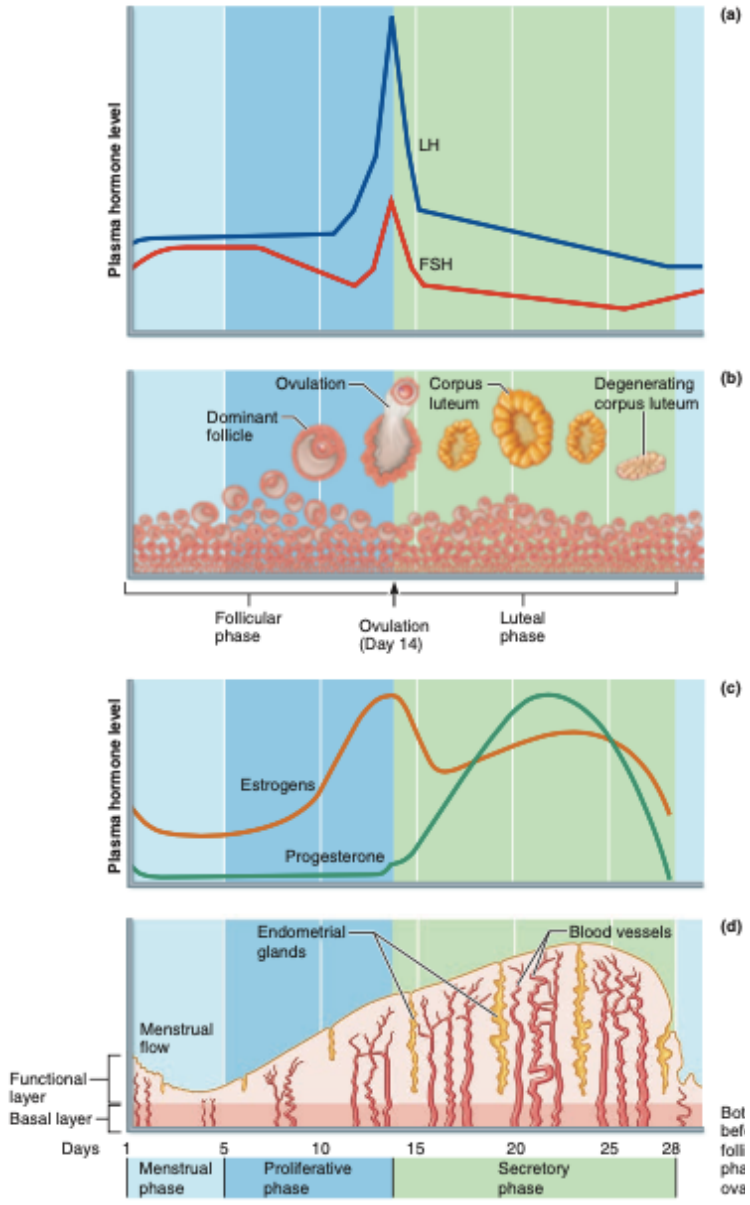

*Note*. According to the number of carbon atoms, sex steroids are divided into progestins (21-carbon), androgens (19-carbon), estrogens (18-carbon). Cholesterol is the precursor of all steroid hormones. Regardless of the steroid-generating organ, the biosynthetic pathways are the same. The type and amount of secreted steroids is related to the enzymatic expression. Enzymes located in the mitochondria are in red and those located in smooth endoplasmic retinaculum are in green (Taraborrelli, 2015).

### *1.2.3 Menstrual cycle phases*

The menstrual cycle (MC) is an important biological rhythm known as cyclic fluctuations of reproductive hormones (Davis & Hackney, 2017). MC length vary approximately from 21 to 35 days (eumenorrhea) and occurs from menarche, the first onset of menstruation, to menopause, defined as one year without menstruation (Mihm et al., 2011). MC can be divided into two to six distinct phases (Bruinvels et al., 2022). The first phase is the follicular phase starting the day of menstruation until ovulation. This phase can be divided into early- and mid- follicular and is characterized by the ovarian follicle development, a low basal body temperature and the progressive rise in estrogen and low progesterone levels. The late follicular or peri-ovulatory phase is characterized by high estrogen levels. At a certain level estrogen provides positive feedback on the pituitary gland, which causes release of high levels of LH and FSH. LH and FSH surge (Figure 3) cause the release of the oocyte (ovulation). Following ovulation, the follicle becomes corpus luteum releasing progesterone, estrogen, and inhibin A. This phase called "luteal phase" is characterized by the dominance of progesterone, a higher basal body temperature (BBT) compared to the follicular phase (+ 0.3 - 0.6 °C) and a stabilization of endometrial lining. If no egg is fertilized, progesterone and estrogen sharply decline (late luteal phase) causing the shedding of endometrial wall: a new cycle begins (Davis & Hackney, 2017; Reed et al., 2000; Thijssen et al., 2014). Serum concentration of steroid hormones during menstrual cycle vary widely from woman to woman and between cycles for the same woman (Stricker et al., 2006). This intra- and inter- individual variability of sex hormones production causes the variability of the length of the phases and the different symptoms associated. The greatest intra-individual variability occurs at the extremity of the fertility life span (menarche and menopause). According to Fehring et al., 2006 inter individual cycle length vary between 22 and 36 days (mean = 28.9). Inter individual variability of the follicular phase length ranges from 10 to 22 days (mean = 16.5) and the luteal phase ranges from 9 to 16 days (mean = 12.4). The length of menstruation is between 3 to 8 days (mean = 5.8).

**Figure 3**

*Ovarian and anterior pituitary hormones correlation with changes of the uterus and ovary*




*Note*. (a) Fluctuation of gonadotropin levels (LH/FSH) in the blood. (b) Structural changes in ovarian follicles and corpus luteum composing the ovarian cycle. (c) Fluctuating levels of estrogen and progesterone. Estrogen levels are related to the LH/FHS levels and progesterone is secreted by the corpus luteum (d) The three phases of the uterine cycle are the menstrual phase where a layer of the endometrium is shed, the proliferative phase where a layer of the endometrium is rebuilt, the secretory phase begins after ovulation preparing the endometrium to receive an embryo.(Marieb & Hoehn, 2015, p. 1080)

## 1.3 Sex hormones and possible implication for exercise physiology

In addition to the primary reproductive function of estrogen and progesterone, the changing concentration of these hormones affects multiple physiological systems like substrate metabolism, cardiovascular system, nervous system and soft tissues (McNulty et al., 2020). Suggested mechanisms that could have implications for exercise physiology are not yet fully understood (Meignié et al., 2021). Bruinvels et al., 2022 highlighted that research needs to consider the importance of both phases and the transitions between phases, as drastic changes in hormone concentration challenge the maintenance of homeostasis. Smith et al., 2022 presented the importance to follow higher quality methodology. To date, many studies have followed a poor methodology to determine the phases of the cycle, and there are several differences in the definition of the phases. Therefore, stronger conclusions about the impact of sex hormones on exercise physiology are yet to be drawn but there are several supposed physiological effects related to the variation of estrogen and progesterone.

### *1.3.1 Estrogen*

Estrogen is known for its anabolic effects (Lowe et al., 2010). On substrate metabolism, estrogen can increase lipolysis and inhibit glycogen utilization during rest and acute exercise (Oosthuyse & Bosch, 2010). Another potential effect of estrogen is its membrane-stabilizing and antioxidant properties which may offer protection against exercise-induced muscle damage. Estrogen also facilitates calcium uptake and decreases osteoclast activity, thus helping sustain bone mass density (Boisseau & Isacco, 2021; Minahan et al., 2015). Estrogen is thought to have neuroexcitatory effects, i.e. it reduces inhibition and increases voluntary muscle activation, which might have impacts on force production for maximal and submaximal efforts (Ansdell et al., 2019). Finally, estrogen peak might have an impact on ligament laxity, making the athlete potentially more prone to injury (Herzberg et al., 2017).

### *1.3.2 Progesterone*

Progesterone, better known for its catabolic action, is thought to have anti-estrogenic effects. At high concentration, progesterone inhibits estrogen binding sites and causes the conversion of estradiol-β-17 to a less active form of estrogen: estrone (Rylance et al., 1985). It can be assumed that the beneficial effects of the estrogens are likely to be greater at the end of the follicular phase and during the ovulatory phase. Progesterone inhibits cortical excitability (Smith et al., 2002). This finding supports the hypothesis that phases where progesterone remains low is favorable to greater strength and power production. In fact, progesterone has major impact on the brain by binding to the GABA-ergic receptors, inducing sedative, anxiolytic, anticonvulsant, neuroprotector and memory-impairing effects (Melcangi et al., 2011; Sundström-Poromaa et al., 2020). Elevated progesterone is associated with a shift in thermoregulatory set point. In long-endurance performance, increased basal body temperature could induce more cardiovascular and thermoregulation strain (de Jonge, 2003). Another possible mechanism of interaction between progesterone and exercise is the change in body composition. Body water is increasing in presence of progesterone (Szmuilowicz et al., 2006). Progesterone acts as a vasodilator, thus contributing to reducing blood pressure (Rylance et al., 1985). Changes in appetite and food consumption in relation with high progesterone have also been observed (Akturk et al., 2013).

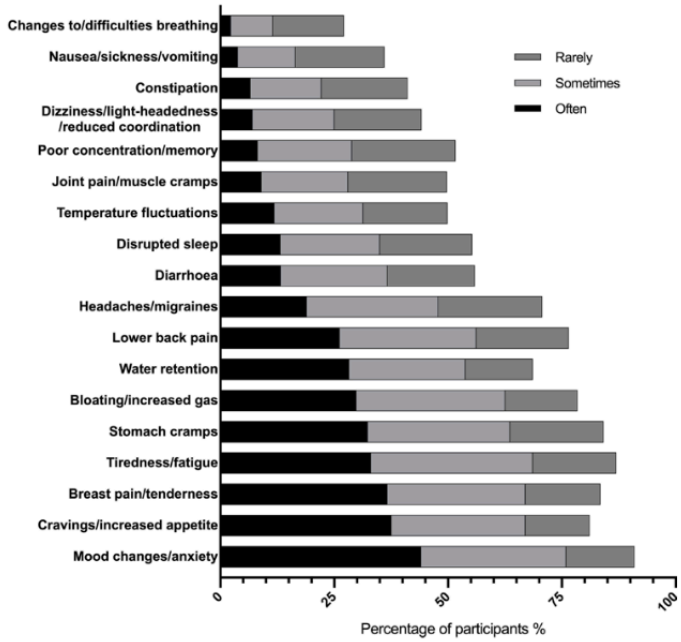
### *1.3.3 Premenstrual and menstrual symptoms*

The sharp decline in estrogen and progesterone that occurs in the late luteal and early follicular phase can cause various symptoms experienced by around 90% of woman of reproductive ages (Hylan et al., 1999). Premenstrual symptoms have been observed to be physical (e.g abdominal cramps, headaches, reduced energy level) and psychological (e.g., worry, distraction, negative mood states) (Read et al., 2014). Most prevalent premenstrual symptoms among eumenorrheic (regular menstruating) athletes are increased fatigue, tension-anxiety and pain (abdominal pain, headache) (Figure 4) (Bruinvels et al., 2021). A possible explanation for increased fatigue and tension-anxiety is that low estrogen levels might be responsible for low serotonin levels. Serotonin is known to be a major neuromodulator in the central nervous system involved in most physiological functions including mood disturbance, appetite regulation, sexual arousal, sleep regulation and motor control (Bakay et al., 2018). The most widely accepted explanation for pain is the overproduction of uterine prostaglandins (PGs) during endometrial sloughing. PG have a range of biological effects including pain and inflammation (Iacovides et al., 2015).

Physical activity was proven to be an effective way to reduce pre-menstrual symptoms (López-Liria et al., 2021; Minuzzi et al., 2022). Premenstrual syndrome (PMS) is a common disorder that could affect up to half of woman of reproductive age (Direkvand-Moghadam et al., 2014). PMS is characterized by at least one physical, emotional, or behavioral symptom appearing in the luteal phase and resolving shortly after menses onset (Yonkers et al., 2008). PMS are generally considered normal, but some individuals experiment severe and disruptive conditions in their daily life. In this case, the condition is often referred to as premenstrual dysphoric disorder (PMDD), which is a more severe form of PMS, characterized by intense emotional and physical symptoms. PMDD was recently (2019) coded as a gynecological diagnosis. It requires the presence of at least one mood symptom (affective liability, irritability, depressed mood, anxiety or tension) within a group of at least five (fatigue, food cravings, insomnia or hypersomnia, loss of interest, …)(Tiranini & Nappi, 2022). These symptoms should occur during the luteal phase of the majority of menstrual cycles, over several years.

**Figure 4**

*Most prevalent premenstrual symptoms*



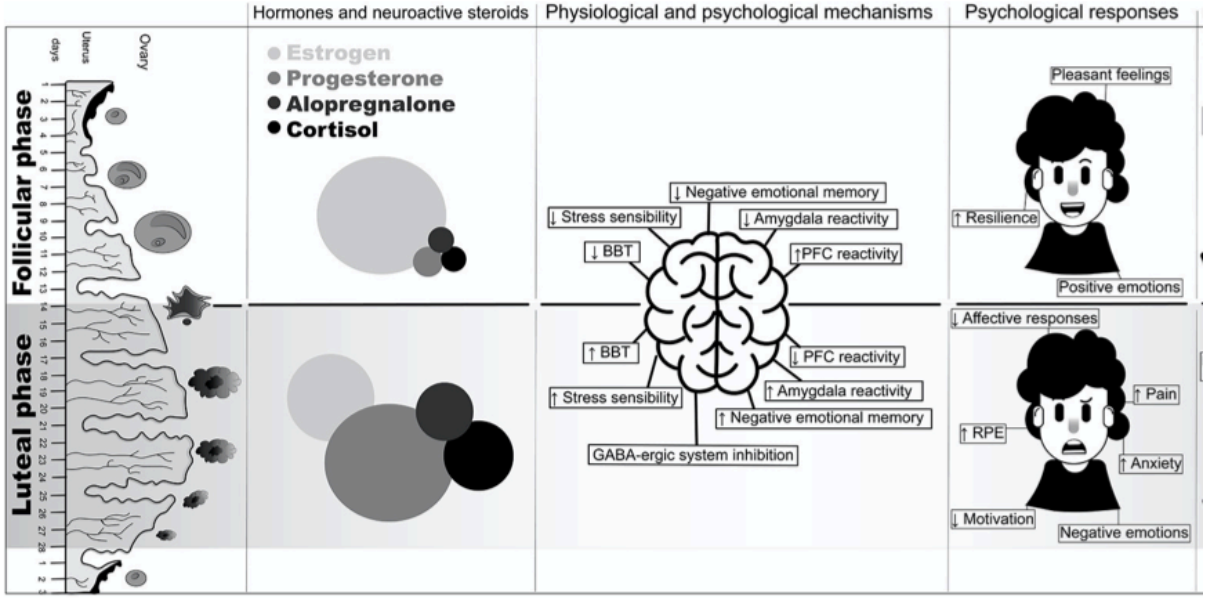

*Note*. Stacked bar chart of the most prevalent types of premenstrual symptoms experienced by exercising women (N = 6812) and the frequency of the occurrence of symptoms. Mood changes, tiredness, cravings, breast pain and stomach cramps are the most common symptoms recorded (Bruinvels et al., 2021).

### *1.3.4 Perceptual response*

The perceptual response refers to the subjective perception and experience of various physiological and environmental factors for an individual. In the context of the menstrual cycle, perceptual responses can encompass a range of psychological and emotional experiences. As previously mentioned, estrogen and progesterone play an important role in the brain activity and emotional outcome by modulating the sensibility level of neural receptors (Prado et al., 2021). Due to the current limited number of studies and heterogeneous methodology used, the possible changes in athlete's subjective responses across the menstrual cycle is still a sensitive area to interpret (Paludo et al., 2022). It is important to consider perceptual response, as complex multifactorial mechanisms and interactions modulated by sensibility levels of neural receptors, concentration of hormones and neuroactive steroids and the individual capacity to cushion negative impacts (Davydov et al., 2004). However, Toffoletto et al., 2014 systematic review concluded that there are different brain activation patterns between follicular and luteal phases. Several potential physiological and psychological mechanism described in Figure 5 try to summary the modulation systems related to changes in hormonal levels across the menstrual cycle. The late follicular and ovulation period characterized by rises of estrogen and testosterone levels has been observed to play a role in positive response to motivation and readiness to train and compete (Cook et al., 2018). Late luteal and early follicular phases, defined by a drastic decrease is estrogen and progesterone, could be related to negative mood response, as an increase in mood disturbance, pain, and menstrual symptoms disturbance. This tendency of negative perceptual outcome increasing strain and monotony might impact athletes on their ability to train or compete (Cristina-Souza et al., 2019). Significant changes in self-reported sleep, stress, muscle soreness, fatigue, and perceived effort in relation to menstrual cycle phases have not yet been demonstrated (Paludo et al., 2022).

**Figure 5**

*Possible physiological and psychological modulation mechanisms related to hormonal levels*




*Note*. From left to right: The uterus and ovary system; hormone and neuroactive steroids proportion; psychological responses; during the follicular (upper part) and luteal phase (lower part). The higher level of progesterone, cortisol and allopregnanolone in luteal phase has been related to decreased prefrontal cortex (PFC) reactivity increasing amygdala reactivity. These mechanisms are thought to increased negative emotional memory and stress sensibility. GABA-ergic system inhibition might also be increased in luteal phase (Prado et al., 2021).

### *1.3.5 Cardiac vagal activity*

The interest in cardiac vagal activity (CVA; e.g., parasympathetically-mediated heart rate variability) in sport has increased in recent years. CVA, also known as vagal tone, refers to the influence of the vagus nerve on the heart's rhythm and function. The vagus nerve is a key component of the parasympathetic nervous system, playing a significant role in regulating heart rate and maintaining cardiovascular homeostasis. Vagal activity exerts a calming effect on the heart, slowing down the heart rate (HR) and promoting a relaxation state. It is responsible for the heart rate variability (HRV), which is the natural variation in time intervals between successive heartbeats. Higher HRV, often associated with increased vagal activity, is generally considered as a marker of good cardiovascular health and resilience to stress (Manresa-Rocamora et al., 2021). Schmalenberger et al., 2019 concluded in a meta-analysis the presence of CVA fluctuation

across menstrual cycle. CVA is acknowledged to be a biomarker for physiological and psychological functions. In psychopathology, decreased levels of CVA is associated with poorer emotional regulation, resilience, adaptability, and social engagement (Balzarotti et al., 2017). In physical health, due to its association with peripheral immune dysregulation and inflammation, as well as glucose dysregulation, decreased levels of CVA have been connected to a higher risk of cardiovascular and metabolic diseases, as well as certain types of cancers (Schuster et al., 2016). Since estrogen and progesterone affect brain function including areas of the central-autonomic network (CAN) such as the hypothalamus, limbic system and prefrontal cortex, CVA may be influenced by the menstrual cycle. A statistically significant and physiologically relevant decrease in CVA from the follicular to the luteal phase has been observed (Schmalenberger et al., 2019). The large effect size is seen from menstrual to premenstrual and from mid-to-late follicular to premenstrual phases. The underling mechanism could be explained by the dopamine-enhancing effects of elevated estrogen in the prefrontal cortex (Jacobs & D'Esposito, 2011). Progesterone is metabolized into soothing GABAergic neuroactive steroid metabolites (allopregnanolone). The sharp premenstrual withdraw of progesterone and estrogen levels may cause a critical GABAergic withdrawal in brain regions responsible for CVA maintenance as well as a decrease in choline uptake and acetylcholine synthesis, the primary vagal neurotransmitter (Thayer et al., 2012).

### *1.3.6 Menstrual cycle dysfunction*

Menstrual cycle dysfunction refers to irregularities in the typical patterns of MC. There are several types of menstrual cycle dysfunction. Menstrual irregularities have been reported to be higher for athletes, especially at the professional level (Gimunová et al., 2022). Amenorrhea is the absence of menstruation for an extended period, which can be categorized as primary (menstruation never starting after the age of 16) or secondary (absence of menstruation for at least three months for an individual who previously experienced regular cycles)(Ackerman & Misra, 2018). The long-term consequences include infertility, delayed puberty, deficiency in bone mineral density, or cardiovascular consequences. Oligomenorrhea refers to low frequency of menstruation occurrence. Menorrhagia involves abnormally heavy or prolonged bleeding during menstruation, while dysmenorrhea is marked by severe menstrual cramps that can significantly impact life quality (Gimunová et al., 2022). Polycystic ovary syndrome (PCOS) is a common hormonal disorder that can cause irregular periods along with other symptoms.

In addition, endometriosis, uterine fibroids, and thyroid disorders can also contribute to menstrual cycle dysfunction. Menstrual cycle dysfunction can arise from a variety of factors, including hormonal imbalances, stress, extreme body composition changes and medications (Song et al., 2022). Vigorous and frequent physical activity is known to be a stressor and potential energy drainer. When a disbalance between energy expenditure and dietary intake is extended, it can lead to a neuroendocrine adaptation to caloric deficit. Low leptin levels and release of large amounts of stress hormones thought to affect HPO-axis function have been observed on amenorrhoeic athletes (Warren & Perlroth, 2001). This phenomenon is called Functional hypothalamic amenorrhea (FHA). It is one of the main causes of secondary amenorrhea and it is characterized by the suppression of the HPO axis (Roberts et al., 2020). However, Ahrens et al., 2014 conducted a study on 259 women moderate to highly active for up to two menstrual cycle, which found no significant change in hormone levels in relation to physical activity level. The Hakimi & Cameron, 2017 systematic review states that the incidence of amenorrhea on highly trained female is related to a Low Energy Availability (LEA) state. LEA underpins the concept of Relative Energy Deficiency in Sport (RED-S). RED-S in female athlete has been well described. It refers to a complex multifactorial syndrome resulting in impaired physiological function caused by relative energy deficiency. It involves impairments of metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular health (Mountjoy et al., 2018).

### 1.4 Menstrual cycle monitoring

MC monitoring involves tracking and analyzing the physiological, hormonal, and perceptual changes that occur throughout MC. This process provides insights into health and hormonal balance. For athletes, MC could help to tailor training, recovery strategies, and overall approach to optimize performance and well-being. MC monitoring can include various elements such as tracking start and end dates, body temperature, blood and urine hormone levels, cervical mucus observation, symptom recording and perceptual features. The first step is to identify MC phases. As menstrual cycle phases length is subject to individual variability, a calendar-counting method based on a 28 day cycle has been demonstrated to provide low accuracy (Setton et al., 2016). Although establishing individual hormonal profiles based on serum measurements over several cycles was used as a high-quality methodological tool for research, it is a tedious and costly method. To provide a simple, fast, and widely applicable method to objectively identify the key points of menstrual cycle, a combination of self-measurable attributes might be sufficient (Smith et al., 2022). Menstrual cycle end and start is the onset of menstruation.

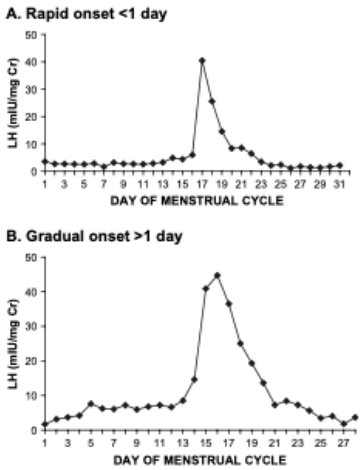
The periovulatory phase without serum measurement can be assessed using a combination of daily body temperature measurements, LH detection tests and cervical mucus observation (Ecochard et al., 2001). The start of the luteal phase is characterized by a rise in BBT that can be identified with the three-over-six-rule: an upward trend in temperature for three consecutive days, compared to the six preceding days (Shilaih et al., 2018).

### *1.4.1 Ovulation detection*

Monitoring of ovulation occurrence has long been practiced. Standard reference examination is made by ultrasonography to determine the maximum growth of the dominant follicle. Detection of LH surge in serum or urine is a very sensitive and specific measure. LH surge is an abrupt secretion of LH by the anterior pituitary gland that occurs approximatively 10-12 hours before the ovulation (Park et al., 2002). It is important to notice that, although the rise in LH in urine is known to occur near the time of ovulation, it may not be strictly one or two days before ovulation and may remain high just after ovulation (Leiva et al., 2017). In an observational study of 43 women, it was found that LH surge cannot be characterized as a single type. Three types of LH surge were categorized: spiking (41.9%), biphasic (44.2%) and plateau (13.9%). In addition, two women demonstrated LH surge without ovulation. This common phenome is called Luteinized Unruptured Follicles (LUFs) (Park et al., 2007). In fact, anovulation can occur in over a third of clinically normal menstrual cycles (Prior et al., 2015). LH surges are variable in configuration Figure 7 , amplitude, and duration Figure 6. Urinary LH surge concentrations range from 20 to 100mlU/ml and commercially LH kits can detect concentration as low as 20mlU/ml. Although urinary LH kits are a widely used tool, at-home realization might be poorly performed and the LH kit results can be misinterpreted (Su et al., 2017). Leiva et al., 2017 stated that LH test should not be sole method to predict ovulation and mentioned the use of complementary markers, such as cervical mucus or other urinary hormonal metabolites.

**Figure 6**

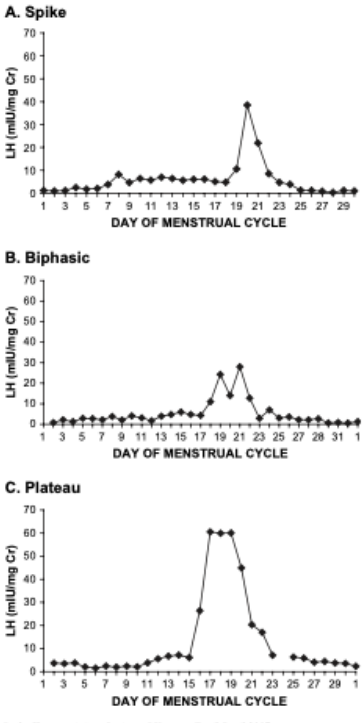
*Type of onset of the luteinizing hormone surge*




*Note*. The horizontal axis represents the day of MC, vertical axis the LH concentration. A. rapid onset of one day duration, B. gradual onset over several days (Park et al., 2007).

**Figure 7**

*Types of LH surge configurations*




*Note.* A. Spike, B. Biphasic, C. plateau (Park et al., 2007).

### *1.4.2 Basal body temperature*

The basal body temperature is the lowest body temperature during rest phase. Monitoring BBT is a simple and widely used ovulatory window detection method. The lowest BBT point occurs across follicular phase (nadir), increases at ovulation, and remains high in the luteal phase due to thermogenic properties of progesterone. Daily point oral or vaginal temperature measurement recorded shorty upon waking is a common method but has been demonstrated to be sensitive to missing values and strongly influenced by lifestyle factors (Bauman, 1981). Several environmental factors can influence BBT readings and potentially affect the accuracy of measurements such as sleep patterns, alcohol consumption, illness and inflammation, physical activity, travel, or room temperature. Continuous distal skin temperature during sleep has been demonstrated to show objective temperature shift in 82% of the cases (Goodale et al., 2019; Shilaih et al., 2018). Zhu et al., 2021 results showed that continuously measured wrist skin temperature had a higher sensitivity for detecting ovulation and to determine fertile window than morning BBT. Nocturnal finger skin temperature (Distal Body Temperature; DBT) recorded by OURA-ring device have also showed potential for menstrual cycle monitoring (Maijala et al., 2019).

### *1.4.3 Physiological response*

Monitoring physiological response enable to understand how the body responds to a stimulus. Various physiological features can be monitored such as cardio-vascular activity, respiration rate, oxygen consumption or hormonal levels. CVA has been identified as a biomarker for psychological and physical health (Beauchaine & Thayer, 2015; Pavlov et al., 2018). Nocturnal Heat Rate (HR) and Heart Rate Variability (HRV) are related to CVA and have been demonstrated to have a good reliability to monitor an acutely ongoing recovery status (Nuuttila et al., 2022). HR is the number of heartbeats per unit time while HRV is the variability of the heart rate between two successive beats in milliseconds. Electrocardiogram (ECG) is the reference measurement tool for HR and HRV. However, wearable technology using photoplethysmography (PPG) measure on wrist or finger offer a promising tool to monitor HR and HRV(Charlton et al., 2022). Only recent research confirmed significant decrease in CVA from mid-luteal compared to mid-follicular phase in relation to progesterone level that must be taken in consideration (Schmalenberger et al., 2020).

### *1.4.4 Perceptual response*

Monitoring perceptual responses during MC involves keeping track of emotional and psychological experiences through different cycle phases. Phases with major ovarian hormones concentration being in favor of positive perceptual response is still an ambitious topic to interpret. Individual monitoring of subjective response such as wellness indicators, perceived readiness and motivation to train/compete and menstrual cycle symptoms has been integrated by professional female teams and organizations to develop individual training strategies to the possible cyclic changes across menstrual cycle phase (Carmichael et al., 2021; Paludo et al., 2022). To date, motivation (Cook et al., 2018), readiness (Rael et al., 2021), competitiveness (Crewther & Cook, 2018), stress , muscle soreness (Chaffin et al., 2011; Graja et al., 2022) , perceived effort, menstrual symptoms (Cristina-Souza et al., 2019) and mood (Martínez-Cantó et al., 2018) are the main subjective outcomes monitored across menstrual cycle phases.

Scientific research agrees on the fact that female athletes are subject to hormones fluctuation that have various effects on physiological systems. It is now well established that it is necessary to consider the menstrual cycle in performance sport. Further research on specific effects such as athletic performance, recovery, nutritional need, injury and psychological factors is yet to be established to draw conclusions for training planning. However, the great intra and inter individual variability might always result in conflicting results. Therefore the development of individual monitoring techniques and technologies seems to play an important role. Schmalenberger et al., 2021 provided recommendations and tools for to study menstrual cycle. However practical, widely applicable recommendations for athletes to monitor menstrual cycle are yet to be established. To manage both athlete's performance, health and wellbeing, Paludo et al., 2022 mentioned the need for developing a battery of daily perceptual and physiological measurements across the whole menstrual cycle, together with training and competition that could provide efficient feedback to coaches and athletes. Based on the possible changes in physiological and perceptual features across the menstrual cycle, daily tracking with wearable devices such as OURA-ring, recording physiological data (DBT, HR, HVR) combined with daily perceptual, training related, information seems to form a complete battery of features to monitor.

### 1.5 Objectives

The aim of this work was to investigate changes in physiological and perceptual features across the menstrual cycle on female athlete. To accomplish this goal, it is important to first determine whether the DBT recorded with OURA-ring combined with LH-tests provides sufficient data to determine menstrual cycle phases. And if so, to identify individual cyclic changes across MC.-The concrete issues are:

1) Is the combination of DBT recorded with OURA-ring and LH-tests on three consecutive menstrual cycle sufficient to determine menstrual cycle phase following Schmalenberger et al., 2021 phasing method?
2) Is there cyclic changes in self-reported wellness (energy level, mood, stress level, muscle soreness), perceived readiness and motivation to train/compete across MC phases?
3) Is there cyclic variations of OURA-ring measures in nocturnal HR and HRV across MC phases?

The following hypotheses were formulated to scientifically examine the three issues presented above:

1) Hypothesis on the determination of menstrual cycle phases:

   $H_10$: OURA-ring does not objective a DBT shifts corresponding to the obtention of a LH positive test result that allow to determine menstrual cycle phases.

   $H_11$: OURA-ring objective a DBT shifts corresponding to the obtention of a LH positive test result that allow to determine menstrual cycle phases.

2) Hypothesis on changes in perceptual response (energy level, mood, stress level, muscle soreness readiness and motivation to train/compete):

   $H_20$: There is no individual cyclic variation of self-reported wellness, perceived readiness and motivation to train/compete across the phases of three MC.

   $H_21$: It is possible to observe individual cyclic variation of self-reported wellness, perceived readiness and motivation to train/compete across the phases of three MC.

3) Hypothesis on changes in nocturnal HR and HRV recorded with OURA-ring:

   $H_30$: There is no changes in nocturnal HR and HRV across MC phases.

   $H_31$: There is changes in nocturnal HR and HRV across MC phases.

# 2 Method

## 2.1 Participants

Ten eumenorrheic woman between 18 and 35 years old were recruited and nine constituted the final sample (N=9), as one participant was excluded during the study for amenorrhea. Participants were recruited in Romandie (French-speaking part of Switzerland) via social media advertisement and selected according to following inclusion criteria: cycle length between 21 and 35 days, no contraceptive in the last 3 months, practicing a minimum of 5 sport sessions in the week including 3 endurance sport specific sessions for a weekly average of 9 hours. According to McKay et al., 2021 participants were retrospectively classified as tiers 2 “Trained/Developmental” “Endurance/Long distance” “independent sport” based on their recorded activity (type of sport and time duration of the session). All participants took part to an information session to learn about the process and submitted a declaration of consent. The study was ethically approved by the CER-VD (req-2022- 01550).

## 2.2 Instruments

Physiological activity was tracked using OURA ring (Ōura Health, Oulu, Finland). This multisensor ring measures HR, HRV, respiration rate via infrared photoplethysmography (PPG), every 5 minutes through the night. Distal body temperature (DBT) is measured every minute using negative temperature coefficient (NTC). Thermistor and movement are detected by a 3-D accelerometer. Sensors are located on the inside of the ring (Alzueta et al., 2022).

**Figure 8**

*Technical illustration of OURA-ring*

*Note*. Left is the titanium cover, middle is the battery, power handling circuit, double core processor, memory, two LEDs, photosensor, infrared PPG sensor, NTC thermistor, 3-D accelerometer and Bluetooth connectivity to smartphone app and right the waterproof cover (Altini & Kinnunen, 2021).

Ovulation was assessed using ovulation predictor-kit by EVIAL. EVIAL home self-checking strips detect LH concentration in urine with sensitivity of 20mlU/ml.

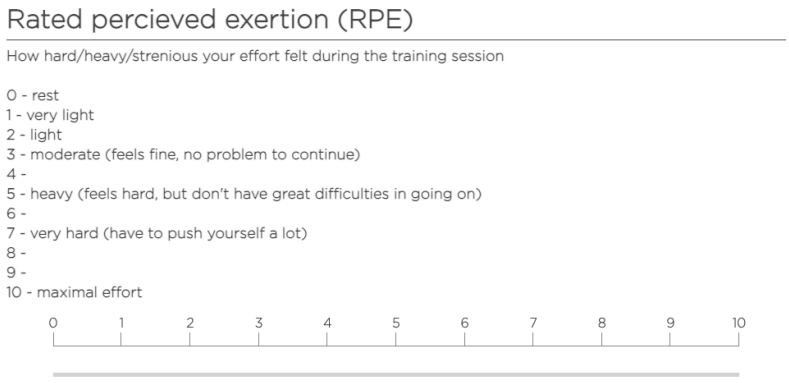
Customized online surveys hosted on docs.gooogle.com were used to collect all self-reported data. *Energy level, mood, stress level, muscle soreness* were each rated on a 5-point likert scale (1 = very low to 5 = very high), reverse scored for negative item (Hooper & Mackinnon, 1995). An overall score was obtained by summing all items (Matos et al., 2019; Rabbani et al., 2019). *Readiness to train/compete* and *motivation to train/compete* were assessed on a 5-point likert scale (1 = not at all to 5 = completely/extremely) (Karu et al., 2000; Saw et al., 2016).

**Figure 9**
*5-point likert scale*


*Note.* Example of the 5-point likert scale used to assess readiness and motivation to train or compete

Exercise intensity was measured by using the rate of perceived exertion (RPE) CR-10 Borg's scale (Borg, 1998) Figure 10. Session-RPE (sRPE) was obtained by multiplying the RPE score with de session duration (Foster et al., 2001).

**Figure 10**
*Borg CR-10 scale*




*Note.* Exercise intensity scale used in the online questionnaire.

Inspired by the 11-item Daily Record of Severity of Problems (DRSP) (Endicott et al., 2006) and the Premenstrual Symptoms Screening Tool (PSST) (Steiner et al., 2003) it was decided to assess following pre-/menstrual items: *menstrual flow, pain and aches, gastrointestinal disorder, appetite change, change in body composition/perception*. All items were assessed on a 5-points training impairment scale (Endicott et al., 2006) 1 – Not at all, 2 – Mild, 3 – Moderate, 4 – Severe, 5 – Extreme. The DRSP and PSST *sleep-quality, mood, energy level, stress level, muscular soreness* related item already covered by the *wellness index* were removed (Pearce et al., 2020) (Table 1).

**Table 1**

*Example of symptoms according to categories*

| **Categories** | **Examples** |
|---|---|
| Pain and aches | Uterine cramps, headache, joint pain, low back pain, tender breasts, ... |
| Gastrointestinal disorder | Bloating, diarrhea, constipation, gas, ... |
| Appetite change | Food cravings, nausea, decreased appetite, ... |
| Change in body composition/perception | Water retention, increased breathing, heavy legs, ... |

### 2.3 Procedure

Data were collected between the 15.04.2023 and 07.08.2023. This observational study consisted of a familiarization and an experimental phase. The familiarization phase lasted minimum 5 days for participants to accommodate to the daily measures and prevent individual questions. The experimental phase war performed during 3 menstrual cycles for each participant. From the familiarization phase to the end of the third menstrual cycle (fourth onset of bleeding), participants were asked to track physiological activity using OURA-ring, complete ovulation self-tests and to fill daily questionnaire about wellness, training, and menstrual cycle symptoms. Participants were encouraged to keep their regular training and sleep routines.

An OURA-ring (Horizon, 3$^{rd}$ generation) was provided to all participants. Fitting on their index finger of the non-dominant hand the size was determined using the Oura test kit. Each participant created an anonymized Oura account and downloaded Oura mobile Application. For the analysis, nightly recorded data were used. Data were accessible to the participant with Oura App and to the investigator through the Oura online service. When needed, investigator sent instructions and reminders to ensure data quality and acquisition. Participants were asked to charge the ring daily to ensure battery level before bedtime.

EVIAL ovulation predictor-kit was performed on the first morning urine, 11 days after the onset of bleeding, and until 3 days after obtention of a positive result (de Jonge, 2003). After the first recorded menstrual cycle, the use of ovulation predictor kit was individualized to 5 days prior to the expected day of ovulation (Schmalenberger et al., 2021). Participants received a personal ID to log the ovulation test result hosted on docs.google.com. In case of unclear results, participants were asked to send a picture of the test result to the investigator.

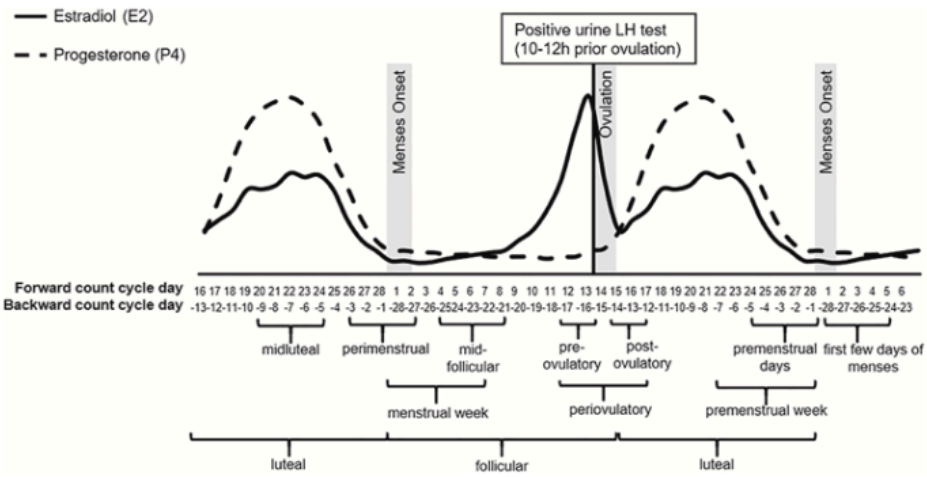
Participants used their assigned ID to fill the online questionnaire daily. They were asked to complete wellness questionnaire between 8 and 12 am, readiness and motivation around 30 minutes before the training session or competition, RPE 30min after the training/competition, Athlete were also asked to report the type of sport and the time of the session to obtain the sRPE. Participants reported their Pre-/menstrual symptoms at any time during their cycle and if needed they were able to add other symptoms than the four presented categories.

**2.4 Data analysis**

Menstrual cycle phase was determined following (Schmalenberger et al., 2021) method. *Periovulatory Phase* can be determined by using LH-testing and nadir in BBT, as ovulation typically lasts for 24 – 36 hours. *Mid-luteal* phase is defined as day +6 to +10 following the positive LH-test or nadir in BBT. *Perimenstrual Phase,* characterized by active withdrawal in estrogen and progesterone levels, can be defined with a combination of backward and forward counting form cycle days –3 to +3. *Mid-follicular Phase* is described by a rise in estrogen and very low progesterone starting at day 1 and ending between day 8 (in 21-day cycle) and day 21 (in 35-day cycle) (de Jonge, 2003). This phase can be determined with backward counting –7 to –3 days prior to LH surge or nadir in BBT. Additionally, according to the three-over-six rule, shift in DBT over 0.2°C compared to six preceding days and steady elevated temperature for three days were marked as high basal body temperature (HBBT). Such temperature shift is useful to retrospectively confirm the occurrence of ovulation and the beginning of the mid-luteal phase (Shilaih et al., 2018). Four windows of interest were selected: Perimenstrual (PM), mid-follicular (MID-FOLL), periovulatory (POV), mid-luteal (MID-LUT).

**Figure 11**

*Graphical overview of the menstrual cycle and its phases*




*Note.* Graphical overview of the menstrual cycle and its phases. The horizontal axis shows the forward and backward count and the corresponding phase. The vertical axis draws the fluctuation of ovarian hormones. (Schmalenberger et al., 2021)

DBT, HR, HRV measured with the OURA-ring were extracted from the Oura online platform. As reported by the manufacturer, the nocturnal temperature is registered every minute, but raw data are not provided by the manufacturer. Temperature deviation was calculated as an average of the absolute distal body temperature, a measurement directly provided by the Oura company. HRV was calculated by the company as the square root of the mean squared difference of successive NN intervals (rMSSD). HR and HRV was recorded every 5 minutes through the night and gathered in averages, the lowest resting heart rate (Lowest HR) was also identified. HR and HRV measures were normalized to the related participant measures, using Z-score ($Z - score = \frac{(x-\mu)}{\sigma}$). Self-reported energy level, mood, stress level, muscle soreness, RPE, perceived readiness and motivation were gathered on averages according to predefined menstrual cycle phases and standard deviation (STD) was calculated. All averages and STD were plotted according to the corresponding menstrual cycle phase. All processing and analysis were conducted using excel for descriptive statistics and Python (v3.7.3) for visual representations. Analysis of variance (ANOVA) and additional post-hoc test was performed with Jamovi (v2.3.0) to determine the significance of differences between means. For all the p-values of the statistical tests, the level of significance was $p < 0.05$. The effect size was defined by the following generalized eta-squared values: small effect if $\eta^2_G < 0.3$, moderate effect if $\eta^2_G < 0.5$ and marked effect if $\eta^2_G > 0.5$.

## 3 Results

An ovulatory window was identified for all recorded cycles. 35.7% were based on a nadir in DBT confirmed with LH-positive test. 53.6% were based only on nadir in DBT and 10.7% only with LH-positive result. Overall, 4 participants over 9 obtained at least one positive LH-result during the study and only 2 participants obtained LH-positive results for all cycles.

The biphasic DBT pattern of menstrual cycle has been individually observed in 85.7% of the recorded cycles with a mean HBBT of 0.23 ± 0.08 °C. A repeated measure ANOVA revealed significant change in DBT across menstrual cycle phases ($F_{3,72}$ = 43.8, p<0.001, $\eta^2_G$=0.497). an additional post-hoc comparison determined the most effect between mid-follicular ($t_{24}$=-9.137, p<.001), periovulatory ($t_{24}$= -11.311 p<.001) compared to mid-luteal (see Table 4). A nadir in DBT was identified on 96.4% (-0.47 ± 0.24 °C) of the cycles and was followed 5.0 ± 2.1 days later by a HBBT in 85.7% of the cycles. The nadir in DBT occurred -1.2 ± 1.4 days before the obtention of a LH-positive result.

An overview of individual length of cycles, ovulation day, temperature shifts for each participant is presented in Table 2. The length of cycles ranged from 29.3 ± 4 days, the day of ovulation was 16 ± 3.2, the duration of the follicular phase 15 ± 3.2 days and 14.3 ± 2.2 days for the luteal phase. The length of menstruation was 5.2 ± 1.2 days and the start of premenstrual symptoms -2.9 ± 1.3 days.

**Table 2**

*Overview of MC length and temperature shifts per participant*

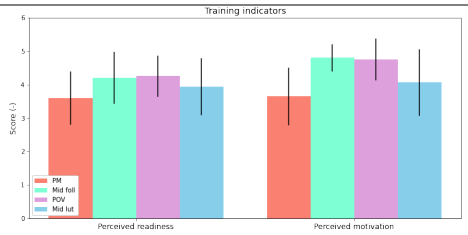
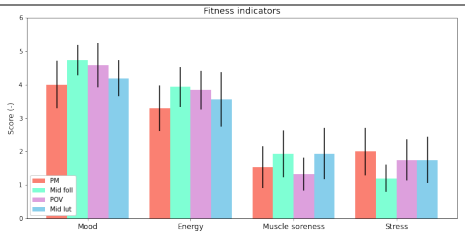
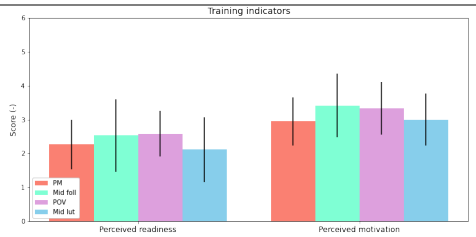
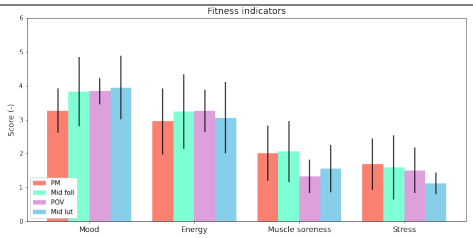
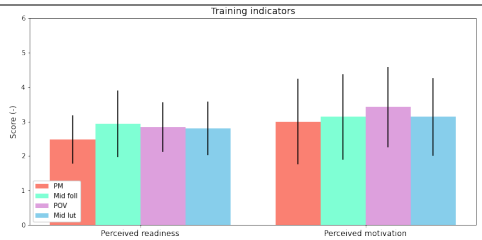
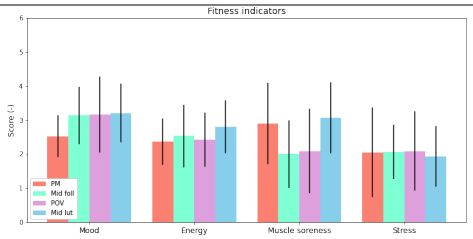
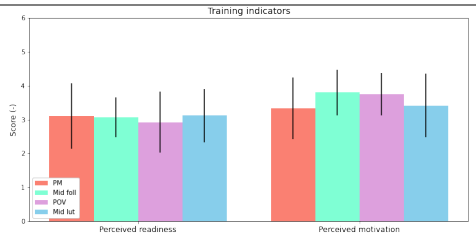
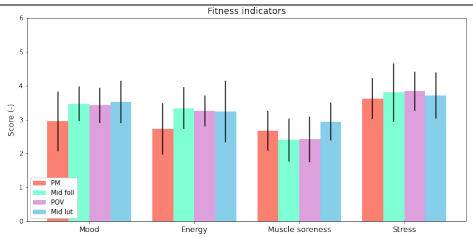
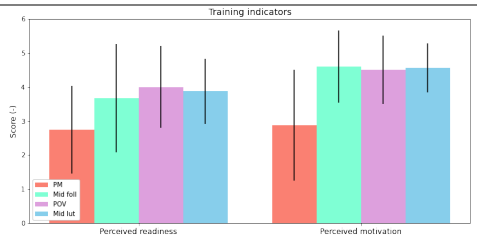
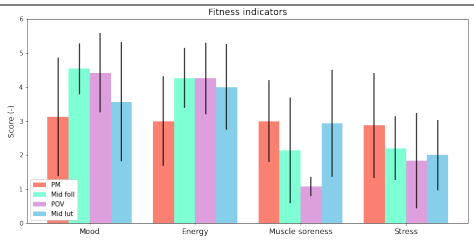
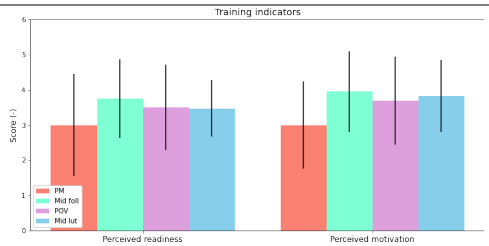
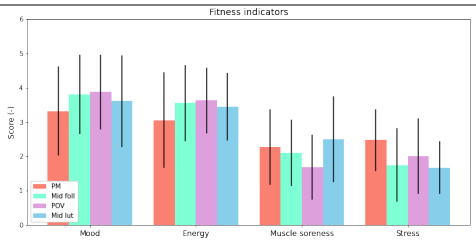
| Participant | Cycle length M ± STD (days) | Pre-ovulatory M ± STD (days) | Post-ovulatory M ± STD (days) | Ovulation M ± STD (day) | Nadir M ± STD (°C) | HBBT M ± STD (°C) |
|---|---|---|---|---|---|---|
| **1** | 30 ± 1.0 | 16 ± 1.0 | 14 ± 1.7 | 17 ± 0.3 | -0.58 ± 0.28 | 0.26 ± 0.1 (N=2) |
| **2** | 34.3 ± 4.9 | 19.3 ± 1.15 | 15 ± 4.6 | 20.3 ± 1.1 | -0.49 ± 0.21 | 0.22 ± 0.05 |
| **3** | 34 ± 3.0 | 19.3 ± 2.9 | 14.7 ± 1.5 | 20.3 ± 2.9 | -0.29 ± 0.08 | 0.31 ± 0.04 |
| **4** | 28.3 ±1.5 | 14.3 ± 0.6 | 14.0 ± 1.0 | 15.3 ± 0.6 | -0.27 ± 0.10 | 0.32 ± 0.10 (N=2) |
| **5** | 29.3 ± 2.5 | 14.7 ± 1.5 | 14.7 ± 3.8 | 15.7 ± 1.5 | -0.77 ± 0.02 | 0.23 ± 0.02 |
| **6** | 23.3 ± 0.5 (N=4) | 11.3 ± 0.8 | 12.0 ± 0.5 | 12.3 ± 0.8 | -0.63 ± 0.25 (N=3) | 0.15 ± 0.03 |
| **7** | 28.7 ± 3.2 | 13 ± 2.6 | 15.7 ± 0.6 | 14 ± 2.6 | -0.42 ± 0.11 | 0.24 ± 0.05 |
| **8** | 29.7 ± 3.2 | 13.3 ± 3.8 | 16.3 ± 0.6 | 14.3 ± 3.8 | -0.31 ± 0.03 | 0.19 ± 0.08 |
| **9** | 28.3 ± 2.5 | 15 ± 1.0 | 13.3 ± 1.5 | 16 ± 1.0 | -0.51 ± 0.42 | 0.18 ± 0.13 (N=2) |
| **All** | **29.3 ± 4.0 (N= 28)** | **15.0 ± 3.2** | **14.3 ± 2.2** | **16 ± 3.2** | **-0.47 ± 0.24 (N=27)** | **0.23 ± 0.08 (N=25)** |

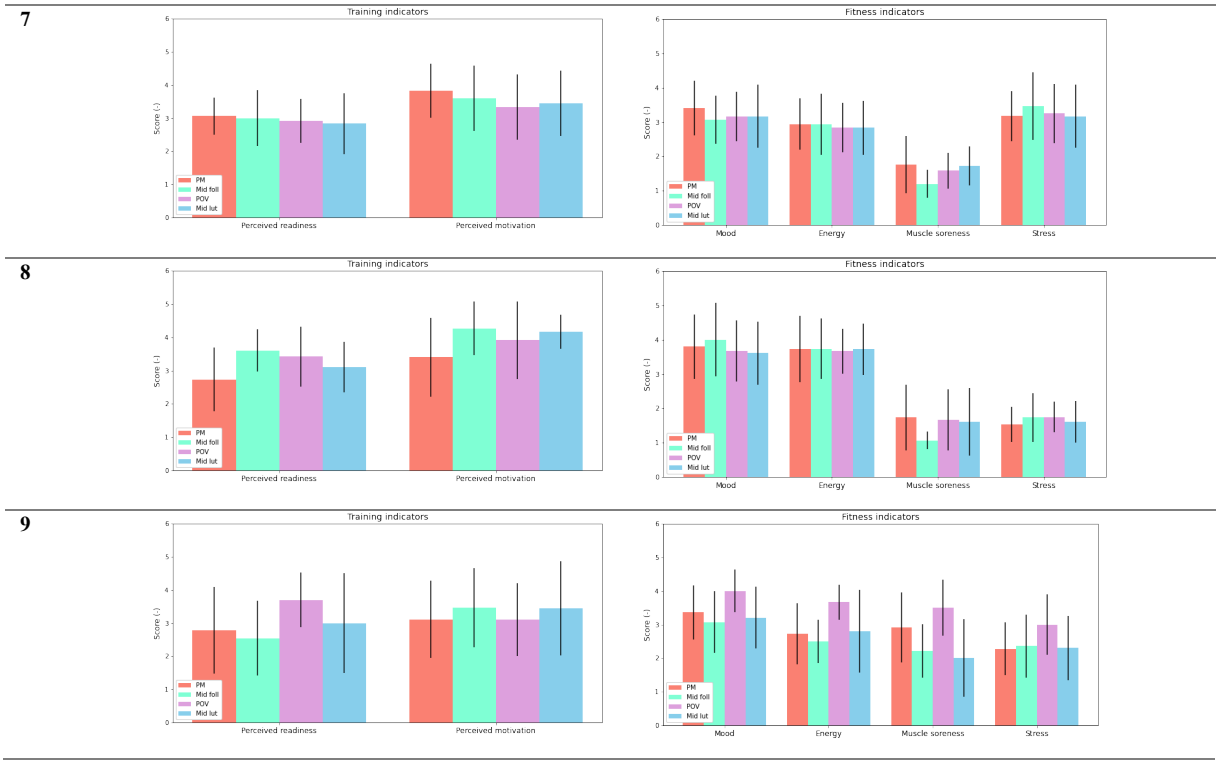
*Note.* Mean (M) and related standard deviation (STD) for each participant over three MC.

The perimenstrual (PM), mid-follicular (Mid-foll), periovulatory (POV), mid-luteal (Mid-lut) phases were determined for 16 cycles using nadir in DBT as reference day of ovulation and for 12 cycles based on the first LH-positive results. This to visually represent individual bar plot of the training and wellness indicator means and standard deviation (Table 3) over three menstrual cycles. Pre/-menstrual cycle symptoms were not subject to analysis.

## Table 3

*Perceptual features (training and fitness indicators) per participant and per phases.*

| Participant | Training indicators (Perceived readiness and motivation) | Fitness indicators (mood, energy, muscle soreness, stress) |
|---|---|---|
| 1 |  |  |
| 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |
| 6 |  |  |




*Note.* Individual presentation of training (perceived readiness and motivation) and fitness indicators (mood, energy level, muscle soreness, stress). Bars represent the average score and the black line corresponds to the standard deviation for each phase (PM = perimenstrual, Mid foll = mid-follicular, POV = periovulatory, Mid lut = Mid-luteal) over three MC.

An overview of the mean differences between MC phases for lowest HR, average HR in beats per minutes (bpm) and average HRV in milliseconds (ms) recorded with OURA-ring is presented in Table 4. According to the conducted repeated measure ANOVA, nocturnal average HR ($F_{3, 75} = 6.07$, $p < 0.001$, $\eta^2_G = 0.141$) and lowest HR ($F_{3, 75} = 5.11$, $p = 0.003$, $\eta^2_G = 0.119$) varied across menstrual cycle phases. Additional post hoc comparison showed a decrease in periovulatory phase ($t_{\text{average HR}}(25) = -4.365$, $p < .001$, $t_{\text{lowest HR}} = -4.014$, $p < .001$ ) compared to mid-luteal as well as mid-follicular compared to mid-luteal ($t_{\text{average HR}}(25) = -4.032$, $p < .001$, $t_{\text{lowest HR}}(25) = -4.354$ $p < 0.001$). There was no significant difference in nocturnal average HRV.

**Table 4**

*Overview of OURA-ring physiological data comparisons*

| Comparison | | | | | |
|---|---|---|---|---|---|
| **Cycle phase** | **Cycle phase** | Lowest HR MD±SE (bpm) | Average HR MD±SE (bpm) | Average HRV MD ± SE (ms) | T° deviation MD ± SE (°C) |
| **PM** | **Mid foll** | 0.3230 ± 0.181 | 0.3732 ± 0.173 | 0.0616 ± 0.171 | 0.2317 ±0.045 |
| | **POV** | 0.2515 ± 0.210 | 0.3571 ± 0.195 | -0.1039 ± 0.173 | 0.2408 ±0.044 |
| | **Mid lut** | -0.3009 ± 0.222 | -0.2525 ± 0.226 | 0.2946 ± 0.191 | -0.1506 ± 0.043 |
| **Mid foll** | **POV** | -0.0716 ± 0.148 | -0.0162 ± 0.133 | -0.1655 ± 0.125 | 0.0090 ± 0.033 |
| | **Mid lut** | -0.6239 ± 0.143*** | -0.6267 ± 0.155*** | 0.2330 ± 0.0.168 | -0.3823 ± 0.042*** |
| **POV** | **Mid lut** | -0.5524 ± 0.138*** | -0.6096 ± 0.140*** | 0.3985 ± 0.163 | -0.3914 ± 0.035*** |

*Note.* Between cycle phases Mean Differences (MD) and related Standard Error (SE) for Lowest Heart Rate (HR), average HR, Temperature (T°) deviation.

*p < 0.05, **p < 0.01, ***p < 0.001

PM = perimenstrual, Mid foll = mid-follicular, POV = periovulatory, Mid lut = Mid-luteal

# 4 Discussion

The aim of this work was to investigate changes in physiological and perceptual features across the menstrual cycle on female athletes. To achieve this, it was important to first determine whether the combination of DBT recorded with OURA-ring and LH-test provides sufficient data to monitor menstrual cycle phases. The results presented in the previous section reveal the individuality and complexity of menstrual cycle monitoring in athletes. The main outcomes related to the monitoring of the MC phase is that combining DBT with LH-tests did not provided advantages. The secondary outcomes showed a decrease in nocturnal HR in mid-follicular and periovulatory phase compared to mid-luteal phase as well as tendencies to favorable perceptual response in mid-follicular and periovulatory.

The biphasic BBT rhythm across MC has been confirmed since the 1960's (Marshall, 1968). The strengths of overnight skin temperature using OURA-ring is that the continuous measurement is less subject to environmental factors influence, solving the problematic of relatively tedious measure of BBT with thermometer, upon waking up (Uchida & Izumizaki, 2022) and the automatic load of the data on computerized programs less prone to misinterpretation of the results. In this study, DBT biphasic pattern was identified in 85.7% of the cycle. Shilaih et al., 2018 showed similar results, with 82% of wrist skin biphasic pattern on 437 recorded menstrual cycles. The results of this study are in the line with Zhu et al., 2020, supporting the high sensitivity of continuous DBT measures to retrospectively determine a fertile window. Nadir in DBT were identified in 89.3% of the cycles and 53.6% were used for determination of the periovulatory phase without LH-confirmation. These result might be carefully presented as Shilaih et al., 2018 detected a wrist skin nadir occurring in the fertile window on only 41% of cycles and mentioned a prior study observing temperature shifts occurrence -8 to +4 days from ovulation. Further research is required to determine the relevance of the nadir in DBT to monitor ovulation, using serum measurement or ultrasound as reference measure.

In research, commercially available home urinary LH-test are frequently used. LH-test are used to determine if a menstrual cycle is ovulatory and when ovulation occurs. The mean time interval after a positive urinary LH test to ovulation detected by sonography was reported to be 20 +/- 3 hours (Guermandi et al., 2001). However, the results of the present study have shown inconclusive results, ovulation was confirmed by positive LH test in only 46.4% of the cycles. A possible explanation to this might be the poor practical execution of the test by participants

and misinterpretation of the results. To increase reliability, participants were asked to send pictures of unclear results to the investigator. However, no pictures were submitted by participants during the study. Schmalenberger et al., 2021 ; Johnson et al., 2011 recommend LH-test using a computerized result to increase reliability. The EVIAL LH-test used in the present study only detect urinary-LH (sensitivity, 20mlU/ml). Behre et al., 2000 showed that Clearblue digital ovulation test (CB-DOT) had high concordance with ultrasound determination of ovulation (90-100% accurate). However, CB-DOT tests use a combination of detection of urinary LH and estrone-3-glucoronide (E1G), which also indicates ovulation (Wegrzynowicz et al., 2022 ). In addition, the difference in LH configuration, amplitude and duration might also be taken in consideration to justify the inconsistency of the LH-tests results as well as the fact that sporadic anovulatory cycle are not uncommon among healthy, regularly menstruating women (Hambridge et al., 2013).

The phasing method was applied to 100% of the recorded cycle, 53.6% using the nadir in temperature recorded with OURA-ring, 35.7% based on nadir confirmed by LH-positive test and 10.7% with LH-tests. On this sample, the detected ovulation ranged from day 16 ± 3.2 and length of the phases ranged from 14.1 ± 1.4 days for follicular and 15.2 ± 1.9 for luteal. These results are in line with Fehring et al., 2006, confirming the importance of monitoring ovulation, as ovulation day can widely vary. In addition to the questionable use of nadir only to determine ovulation, the nadir in DBT occurred -1.2 ± 1.4 days before the obtention of a LH-positive result. The mean time interval after a positive urinary LH test to ovulation detected by sonography was reported to be 20 +/- 3 hours (Guermandi et al., 2001). Thus, it might have been possible to miss the exact ovulation window. It appears important to point out that the forward and backward counting method could sometimes cause an overlap of MC phase. In cases of short luteal phase, the mid-luteal could overlap the perimenstrual phase. However, OURA-ring detects HBBT and this DBT shift might be used to adjust or confirm mid-luteal phase (Shilaih et al., 2018). Thereby, the first null hypothesis ($H_10$: OURA-ring does not objective a DBT shifts corresponding to the obtention of a LH positive test result that allow to determine menstrual cycle phases.) cannot be rejected. Starting from this assertion, the phasing used for the result of the following physiological and perceptual features must be carefully taken in consideration.

The link between menstrual cycle phases and perceptual response is still a difficult topic to interpret (Paludo et al., 2022). Athletes are subject to a plethora of internal and external stressor that can affect subjective response. Individual capacity to cushion negative impacts must be considered (Davydov et al., 2004). The results presented insufficient power to identify effects. The null hypothesis $H_20$ (There is no individual cyclic variation of self-reported wellness, perceived readiness and motivation to train/compete across the phases of three MC) can neither be rejected, nor accepted. However, it was possible to represent individual tendencies (Table 3 ). The most recurrent visually identified tendency was a decreased perceived readiness and motivation on perimenstrual phases compared to the other. Perceived wellness indicators tend to be slightly favorable in mid-follicular and periovulatory phase. Paludo et al., 2022 identified statistically significant results for favorable tendency of perceptual response from mid-follicular to periovulatory phase. In addition to the possible effect of MC on the brain activity and emotional outcome by modulating the sensibility level of neural receptors (Prado et al., 2021). Perimenstrual symptoms causing pain and aches, gastrointestinal disorder, appetite change and change in body composition might also play an important role in perceptual response. However, the lack of consistency in data collection did not lead to an analysis. In fact, it was noted that participants poorly completed this questionnaire, mainly on an intensity scale rather than a degree of disturbance for training scale. Perimenstrual symptoms are commonly investigated as "occurrence of symptoms" or "intensity" but not yet been investigated as "level of disturbance for training" that might provide more specific feedback for training adaptations. This point reinforces the need of establishing tools to assess menstrual cycle symptoms for athletes as the literature is to date non-existent.

CVA being a biomarker for psychological and physical health (Beauchaine & Thayer, 2015; Pavlov et al., 2018), HR and HRV are promising measurements in training planification for endurance sports (Düking et al., 2021). It was important to assess the possible change of HR and HRV recorded with OURA-ring across menstrual cycle phases. HR was significantly higher in mid-luteal phase compared to mid-follicular and periovulatory these results correspond to Goodale et al., 2019 findings supporting a significant increase of nightly wrist measure of HR in follicular compared to luteal phase with 237 woman. Goodale et al., 2019 mentioned significant effect on HRV but there was no significant difference in HRV in the presented results. Resting HR and HRV based training planification should probably take menstrual cycle phases in consideration. Therefore, the $H_30$ null hypothesis (There is no changes in nocturnal HR and HRV across MC phases) can be rejected but with caution.

The limitations of this study are primarily related to the use of nadir only as a method to identify ovulatory window, which requires further scientific validation. The results of changes in physiological and perceptual measurements across MC phases must be carefully taken. Secondly, it is important to mention the small sample size as well as the fact that most of the participants were between 26 and 28 years old, and probably had a higher awareness and longer experience of their MC. Thirdly, the first recorded cycle was used for analysis. The data provided by the manufacturer is the variation of temperature from the baseline. Oura developer mentioned a requirement of up to 30 days to calibrate this baseline and do not mention a retrospective adjustment. The minimum required 5 days familiarization phase for this study might be insufficient. Further research using OURA-ring should extend the follow-up for a minimum of 4-5 cycles and ignore the first 30 days. To determine whether a cyclic variation can be individually observed, it might be relevant to follow participants across 6-12 cycles and establish intra-individual correlations. However, one of the strengths of this study was the combination of daily physiological and perceptual measurements. In addition, the adherence to wearing the ring and filling in the daily wellness and perceived readiness/motivation questionnaires was good, thus demonstrating that the daily monitoring approach was not particularly constraining.

For practical use, high accuracy of identification of ovulation day for athlete is a disputable topic. In fact, it is less important for athletes than for scientific research to accurately verify MC phases. Based on the results it cannot be determined whether some participants responded better to one ovulation detection method than another. DBT recorded with wearable device presents a relevant, non-invasive tool for athletes with a stable daily routine to retrospectively identify an approximative ovulatory window as one cannot identify a temperature nadir until the subsequent post-ovulation temperature rise. The temperature curves must be carefully observed, and identification of nadir might require knowledge and experience. The advantage of LH-tests is the obtention of a prospective result. Using LH-kits to monitor ovulation is less convenient than DBT but can be used as a complement to DBT or recommended for athlete whose lifestyle does not allow to obtain consistent body temperature data (regular change of time zone, sleep environment and competition or training over 24h). Athletes and coach could first benefit from the identification of a periovulatory window to clarify follicular and luteal phase length tendency. Second, the length of the perimenstrual phase could be adapted according to the occurrence/disturbance of symptoms to be more appropriate for training and competition purpose.

Finally, the approximation of the mid-follicular and mid-luteal phases could serve to identify individual patterns of increase or decrease in motivation, readiness during high and stable hormones phases. MC is an important health sign that should be further discussed and studied in the sport field as a normal biological rhythm, it should be noted that one of the participants dropped out because of amenorrhea, following the start of the study. MC must be individually considered and monitored in its entirety. Disturbance related to changes in hormones should be identified to open the discussion between athletes and coaches to make the most of the athlete's ability to train and, above all, to optimize load and recovery. Further scientific validation of tools to monitor MC is necessary; first, to provide individual self-awareness and self-knowledge across female athlete hormonal lifespan, and second to integrate these elements to the performance optimization equation.

# 5 Conclusion

The goal of this work was to investigate changes in physiological and perceptual features across the menstrual cycle on female endurance athletes. The main outcome related to the methodology was that the use of LH-tests in addition to the DBT measurement did not provide advantages to monitor menstrual cycle phases. This was most likely caused by poor realization of the LH-tests. LH-tests kits are a constraining factor for daily use and should be carefully selected and instructed. However, DBT biphasic pattern has been significantly verified and nadir in DBT were used to determine the fertile window. These results are supporting the robustness and convenience of DBT recorded by wearable devices to retrospectively determine ovulation. Further research on the validation of OURA-ring as a MC monitoring mean with ultra-sound or serum measurement is necessary. Therefore, changes in physiological and perceptual measurement results must be carefully taken. Significant changes in nocturnal HR in mid-follicular and periovulatory phase compared to mid-luteal phase were identified. Tendencies to favorable perceptual response in mid-follicular and periovulatory were observed. Based on the wide possible effect of ovarian hormones on different physiological systems, this study presents an outline of physiological and perceptual measurements for athletes to monitor across the menstrual cycle. This battery of subjective and objective measurements provides a definitive direction for practical use. MC should be considered and monitored in its entirety to provide individual self-awareness and self-knowledge across female athlete hormonal lifespan.

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# 7 Appendix

Declaration of consent for participant

EHSM
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Hochschule
für Sport
Magglingen

**Consentement écrit de la participante**

- Veuillez lire attentivement ce formulaire.
- Veuillez compléter les informations surlignées en jaune vous concernant dans le formulaire et le signer.
- N'hésitez pas à poser des questions si vous ne comprenez pas ou si vous souhaitez d'autres renseignements.

| | | |
|---|---|---|
| **Titre du projet** | | **Menstrual cycle monitoring on well trained eumenorrheic female endurance athletes. Identification of relevant parameters for training planification.** |
| **Emplacement:** | | Fribourg |
| **Responsable du projet:** | Prénom et Nom | Mirjam Hintermann |
| **Participante:** | Prénom et Nom: | |
| | Date de naissance: | |
| | Sport pratiqué: | |
| | Nombre de séances et d'heures d'entrainement hebdomadaire : | |

- Nous déclarons avoir été informés des objectifs et du déroulement de l'enquête.
- Nous certifions avoir lu et compris l'information écrite qui nous a été remise sur l'enquête précitée. Nous avons reçu des réponses satisfaisantes aux questions que nous avons posées en relation avec la participation à cette enquête.
- Nous sommes informés qu'une assurance a été souscrite pour couvrir les dommages éventuels découlant de l'enquête.
- Nous savons que les données personnelles ne seront transmises que sous une forme anonyme à des institutions externes.

  Nous prenons part de façon volontaire à cette enquête. Nous pouvons à tout moment et sans avoir à fournir de justification, révoquer notre consentement à participer à cette enquête.

.....

Lieu, Date Signature de la participante

BASPO
2532 Macolin

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Bundesamt für Sport BASPO

EHSM
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Hochschule
für Sport
Magglingen

# Informations aux participantes

**Titre du projet:** Menstrual cycle monitoring on well trained eumenorrheic female endurance athletes. Identification of relevant parameters for training planification.

Chère participante,

## 1. Sélection des participantes

Nous vous demandons si vous souhaitez participer à ce projet d'étude sur le monitoring du cycle hormonal parce que vous entrez dans les critères de l'étude. Les critères sont les suivants :

- Avoir un cycle hormonal régulier (occurrence tous les 21-35 jours)
- Sans prise de contraceptif hormonal depuis au minimum 3 mois
- Pratiquer un sport d'endurance (trail running, cyclisme, ski alpinisme, ski de fond)
- Réaliser 5 séances ou plus hebdomadaires pour un total de minimum 5 heures d'entrainement

## 2. But du projet

Il existe une grande variabilité inter- et intra-personnelle du taux hormonal et de la durée des phases du cycle menstruel. L'expérience de la variation des taux hormonaux en fonction des phases varie d'une personne à l'autre et parfois d'un cycle à l'autre. Il est donc judicieux d'établir un suivi journalier et individualisé. L'objectif du projet est d'identifier les paramètres pertinents de la surveillance et du suivi du cycle menstruel pour la planification de l'entrainement dans les sports d'endurance. Pour ce faire il s'agira de fournir, trois cycles durant, une évaluation perceptive journalière d'informations relatives à l'entrainement, une mesure de température quotidienne (durant la nuit) et une série d'autotests d'ovulation. Une période familiarisation avec la méthode de récolte de données sera effectuée avant le premier cycle évalué.

## 3. Informations générales

Le projet est réalisé dans le cadre d'un travail de master en science du sport à l'université de Fribourg en collaboration avec la haute école de sport de Macolin. En sciences du sport, le corps masculin a longtemps été la référence en matière de recherche. Les études menées sur les athlètes féminines ne représentent encore qu'un faible pourcentage de la littérature (Costello et al., 2014). La physiologie masculine présente un certain nombre de similitudes, mais les femmes sont naturellement sujettes à des fluctuations hormonales cycliques de la puberté à la ménopause. La fluctuation des hormones a un effet physiologique sur les systèmes cardiovasculaire, respiratoire, métabolique et neuromusculaire (McNulty et al, 2020). Cependant, il n'existe pas d'évidences que les performances physiques sont affectées par le cycle menstruel. Il existe une grande variabilité inter- et intra- individu. La tendance actuelle est donc l'individualisation et au suivi individuel et journalier du cycle menstruel.

## 4. Participation volontaire

La participation à ce projet est volontaire. Vous avez à tout moment la possibilité de révoquer un consentement donné sans avoir à vous justifier.

## 5. Déroulement

Les mesures seront effectuées trois cycles durant. Une période de familiarisation à la méthode de récolte de données sera observée avant le premier cycle enregistré, valable si le début de la phase de familiarisation commence au minimum 7 jours avant le début du premier des trois cycles enregistrés. Les mesures évaluées seront celles de trois cycles complets (entre 70 et 100 jours). La tâche sera de fournir des informations journalières à un moment précis de la journée :

1. Une évaluation perceptive quantitative de son niveau de forme physique à remplir **chaque jour**. (*sleep, mood, energy level, stress level, muscular freshness*)
2. Une évaluation perceptive quantitative journalière liée à l'entrainement à remplir **avant l'entrainement**. (*percieved readiness for training/competition, motivation to train/compete*)
3. Un évaluation perceptive quantitative de l'intensité de l'entrainement à remplir **après l'entrainement** (*RPE*)
4. L'inscription de symptômes en lien avec le cycle menstruel et leur **niveau de désagrément** sur l'entrainement. (*menstrual flow, pain and aches, gastrointestinal disorders, appetite change, change in body composition*)
5. La mesure de température basale, variabilité de fréquence cardiaque et fréquence cardiaque au repos, sera enregistrée **chaque nuit** à l'aide d'un anneau porté à la base de l'index équipé de capteurs photopléthysmographiques.
6. Une série de tests d'ovulation (autotests de détection du taux de LH dans l'urine) également **au réveil à partir du 10ème jour du cycle** (à compter du premier jour de règle) jusqu'à obtention d'un résultat positif (+3jours).

## 6. Avantages pour les participantes

La réalisation de ce projet permet d'obtenir des connaissances importantes sur la pertinence des informations récoltées lors du monitoring du cycle hormonal. La participation à ce projet peut vous permettre d'obtenir des informations importantes sur votre cycle menstruel qui pourraient vous aider à agencer la charge d'entrainement de manière optimale dans un objectif de progression ou de performance. Le projet vise également à informer et lever un tabou sur le sujet du cycle menstruel dans le monde du sport.

## 7. Risques et inconvénients

La participation à ce projet n'entraîne aucun risque ou inconvénient. La charge de travail correspond à un investissement minimal de temps journalier. Le capteur de mesure porté sur l'index est léger et indolore.

## 8. Confidentialité des données

Dans le cadre de ce projet, vos données personnelles sont collectées. Ces données sont anonymes. Votre nom ne sera en aucun cas publié dans les rapports ou publication et seront utilisées exclusivement en rapport avec le projet.

## 9. Coût

La participation à ce projet est **gratuite,** les outils de mesure vous seront fournis. Il vous sera demandé de **rendre le matériel** de mesure en **état de fonction** à la fin de l'étude (Oura ring, tests d'ovulation non utilisés).

## 10. Garantie en cas de dommage

L'Office fédéral du sport assume la responsabilité civile, pour tous les cas qui peuvent survenir en relation avec le projet.

## 11. Personne de contact

Si vous avez des questions, vous pouvez toujours vous adresser à la personne de contact ci-dessous :

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