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Research article

Impact of The Menstrual Cycle on Physical Recovery after Small-Sided Games: A Crossover Study in Women's Soccer Players

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Abstract

The aim of this study was twofold: (i) to compare potential variations in reactive strength index (RSI), interleukin-6 (IL-6), and delayed onset muscle soreness (DOMS) between the early follicular and mid-luteal phases in response to small-sided games (SSGs); and (ii) to analyze potential interactions in the magnitude of responses to different SSG formats, specifically 1v1 and 5v5. A crossover study design was employed, involving 20 amateur female soccer players (age: 21.4 ± 1.8 years) who were randomly assigned to two groups. With a 15-day interval between sessions, participants underwent repeated assessments following 1v1 and 5v5 formats across two menstrual cycle phases (based on calendar estimates). Participants completed 1v1 and 5v5 sessions and were evaluated at four time points: at rest, immediately post-session, 24 hours post, and 48 hours post. A three-way repeated measures ANOVA was used to assess the effects of play format (1v1, 5v5), hormonal phase (early follicular, mid-luteal), and time (rest, post-exercise, 24h, 48h). RSI was assessed using a drop jump test, IL-6 was measured via salivary analysis, and DOMS was rated using a Likert scale. To control for potential confounders like nutrition, sleep, and training load, participants maintained their regular diet and training during the study, though individual variation in these factors could still affect the results. Significant interactions were found between menstrual cycle phase, format, and time for RSI ($p = 0.040$; $\eta p^2 = 0.154$) and IL-6 ($p < 0.001$; $\eta p^2 = 0.773$), but not DOMS ($p = 0.121$; $\eta p^2 = 0.283$). RSI was significantly lower and IL-6 significantly higher in the mid-luteal phase, especially in 1v1 sessions (RSI: $p < 0.001$; IL-6: $p < 0.001$). These findings suggest that neuromuscular fatigue and inflammatory responses to SSGs are modulated by menstrual cycle phase, with potential implications for optimizing training and recovery strategies in female athletes.

Key words: Female, woman, muscle function, muscle inflammation, football.

Introduction

The menstrual cycle (MC) is a complex physiological process marked by hormonal fluctuations and regular bleeding. It typically spans 28 days, though cycles between 21 and 35 days are considered normal in adults (Bull et al., 2019; Itriyeva, 2022). The cycle is divided into two main phases: the follicular phase, averaging 16.9 days, and the luteal phase, about 12.4 days (Bull et al., 2019). The follicular phase begins with menstruation, marked by rising estrogen levels and follicle development, while the luteal phase follows ovulation and is characterized by elevated progesterone (Hedayat et al., 2020). Hormones central to cycle regulation include FSH, LH, estrogen, and progesterone (Pierson and Pagidas, 2018). Ovulation typically occurs 16 - 48 hours after the LH surge (Carmichael et al., 2021). These hormonal shifts influence various physiological processes, including muscle recovery and inflammation, which are central to the study of exercise responses in women (Berga, 2020). Understanding these hormonal variations is essential for interpreting how menstrual phases impact training and recovery.

Research indicates that the MC significantly affects physical fitness, recovery, and performance in female athletes. Hormonal fluctuations across the cycle influence cardiorespiratory capacity, strength, pain thresholds, and physiological responses to exercise (Hackney et al., 2019; Recacha-Ponce et al., 2023). Athletes often report reduced performance during menstruation, while the post-menstrual phase is perceived as more favorable (Solli et al., 2020). Studies show increased cardiorespiratory fitness in the mid- and late-luteal phases compared to the early follicular phase (Recacha-Ponce et al., 2023), and higher markers of muscle stress - such as creatine kinase and interleukin-6 - are observed during recovery in the follicular phase (Hackney et al., 2019). Training monotony and strain also tend to be higher in this phase, with longer technical sessions occurring in both the follicular and luteal phases (Cristina-Souza et al., 2019). Despite this, many athletes lack awareness of how the MC affects their training and rarely discuss it with coaches (Solli et al., 2020). Incorporating MC-informed training plans can help reduce menstrual symptoms like dysmenorrhea and premenstrual discomfort, optimize performance, and support recovery (Koushkie Jahromi et al., 2008; Aguilar Macías et al., 2017; Kubica et al., 2023).

The biomarkers interleukin-6 (IL-6), delayed onset muscle soreness (DOMS), and the reactive strength index (RSI) provide complementary insights into physical recovery following exercise. IL-6, a cytokine released during exercise, reflects inflammatory and metabolic responses to

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muscle activity, with levels increasing up to 100-fold depending on exercise intensity, duration, and the extent of muscle damage (Reihmane and Dela, 2014; Silva Vasconcelos and Fernanda Salla, 2018). Acting as an energy sensor, IL-6 facilitates glycogenolysis in the liver and lipolysis in adipose tissue to meet the increased energy demands of exercise, while also modulating immune and metabolic responses (Reihmane and Dela, 2014). Regular training may attenuate both basal IL-6 levels and the acute IL-6 response, reflecting positive training adaptations such as reductions in visceral and epicardial fat mass (Peppler et al., 2020). DOMS is characterized by muscle pain and stiffness that occurs 12 to 48 hours after unaccustomed or intense eccentric exercise (Schutte and Lambert, 2001; Coudreuse et al., 2004). Although traditionally attributed to muscle damage, recent evidence suggests it may also result from neural microdamage within muscle spindles (Sonkodi et al., 2020). DOMS can impair performance by reducing strength, movement economy, and power output, potentially increasing injury risk (Smith, 1992). However, it may also contribute to muscle adaptation, as demonstrated by the "repeated bout effect," whereby a single exposure to eccentric exercise provides protection against subsequent DOMS for weeks (Schutte and Lambert, 2001). RSI measures an athlete's ability to rapidly transition from eccentric to concentric muscle action, often evaluated through rebound jump tasks (Jarvis et al., 2022; Egesoy, 2023). It is associated with key athletic qualities such as strength, speed, and agility and is influenced by factors including relative strength and individual performance strategies, such as prioritizing contact time versus jump height (Healy et al., 2019; Southey et al., 2024). Together, IL-6, DOMS, and RSI offer valuable physiological and neuromuscular markers to monitor recovery and guide individualized training and rehabilitation strategies.

Small-sided games (SSGs) in soccer impose significant physiological and physical demands on players, with the intensity largely influenced by the number of participants, game format, and pitch size. Generally, SSGs with fewer players, such as 1v1, result in greater physiological stress, as evidenced by elevated heart rates, perceived exertion, and blood lactate concentrations compared to games with more players (Köklü et al., 2011; Goliński et al., 2016). Possession-based formats also tend to be more demanding than goal-oriented games (Castellano et al., 2013). While 1v1 games elicit the highest individual intensity, 5v5 formats strike a balance between physical and technical demands, showing higher metabolic power output than both 3v3 and 10v10 games (Zlojutro et al., 2023). Moreover, 5v5 games are particularly effective for technical development, promoting more frequent actions such as passes, dribbles, and shots (Owen et al., 2013; Sannicandro and Cofano, 2017). Pitch size also modulates physical output, with larger playing areas associated with increased total distance and high-speed running (Gaudino et al., 2014). Overall, the design of SSGs should consider these variables to effectively target specific physical and technical outcomes.

Considering the influence of physiological processes related to the menstrual cycle on recovery in women, the present study aimed to investigate the impact of two distinct training stimuli - small-sided games with different demands (1v1 and 5v5) - during two specific phases of the menstrual cycle: the early follicular and mid-luteal phases. Despite increasing interest in menstrual cycle-informed training, few studies have explored how different SSG formats interact with menstrual phases in terms of neuromuscular and inflammatory recovery markers. RSI, IL-6, and DOMS analysis were included for their relevance to assessing neuromuscular performance and inflammatory recovery, which are central to understanding the impact of menstrual cycle phases on exercise recovery. It was hypothesized that recovery would be slower during the luteal phase compared to the mid-luteal phase, and that the 1v1 condition would induce greater physiological stress and muscle damage than the 5v5 format.

Methods

Study design

The current study employed a crossover design in which the same players participated in both 1v1 and 5v5 training sessions, with different sequencing to control for sequencing effects. Additionally, players were tested and compared during two distinct phases of the menstrual cycle: early follicular and mid-luteal. In both menstrual phases, participants completed both training formats. One group began with the mid-luteal phase, then completed the same experiment during the early follicular phase 15 days later. The other group started in the early follicular phase and repeated the experiment during the mid-luteal phase after 15 days. The experimental design is represented in Figure 1. Throughout the study, participants trained twice per week, as they were amateur athletes competing at the local-level representation.

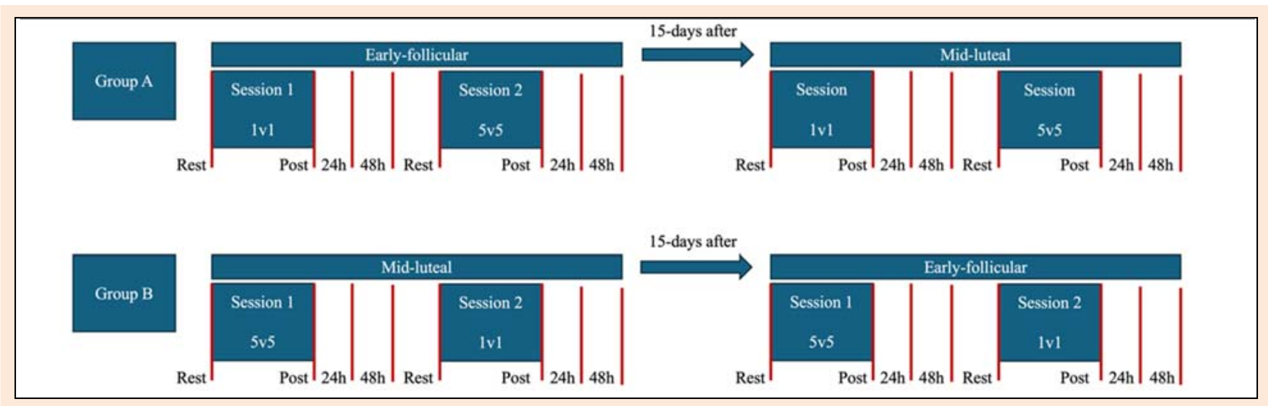

Figure 1. Study design.

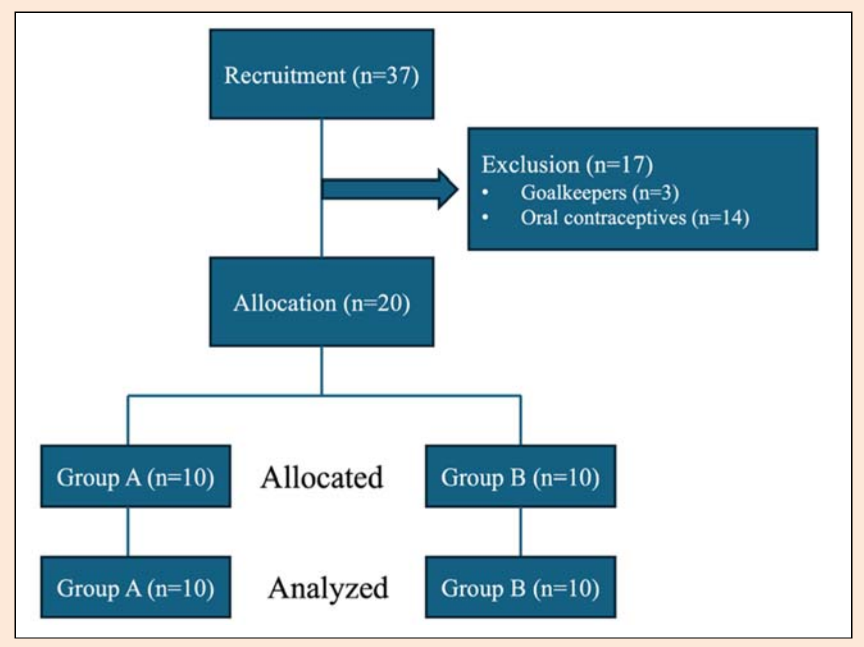

Figure 2. Representation of the players' allocation.

Participants

The inclusion criteria were established a priori, with the minimum requirements being: medical clearance to engage in soccer training; a menstrual cycle of approximately 28 days (with a ±5-day variation); a history of regular menstrual cycles over the past three months, verified through menstrual tracking; age over 18 years; and at least two years of experience in soccer. Exclusion criteria included the use of hormonal contraceptives or any additional hormonal supplements for sports performance and the goalkeepers. Recruitment was carried out through convenience sampling, specifically by inviting local amateur teams. Two teams expressed willingness to participate in the study, and its players were then invited to take part on a voluntary basis.

Out of the 37 volunteers, 3 were excluded for playing as goalkeepers, and 14 were excluded due to regular use of hormonal contraceptives. As a result, 20 players were included in the final sample (Figure 2). These 20 players were then assigned to Group A and Group B based on the similarity of their menstrual cycles, i.e. they cycle length. This process was carried out manually, based on the responses obtained after asking the athletes about the characteristics of their menstrual cycle.

The participants' mean age was 21.4 years (±1.8), with an average height of 158.2 cm (±4.7), body mass of 53.5 kg (±3.1), and a body mass index (BMI) of 20.8 kg/m² (±0.9). They had an average of 3.4 years (±0.9) of training experience. Menstrual cycles lasted an average of 5.3 days (±0.4), with a typical total cycle duration of 27.3 days (±0.9).

The study was reviewed and approved by the Ethics Committee of Instituto Politécnico de Viana do Castelo under the code number CECSVS2024/10/ii on October 21, 2024. Prior to the start of the study, participants were informed about the study's objectives, design, potential risks, and benefits. Subsequently, they provided written informed consent, which included details regarding data protection and their right to withdraw from the study at any time.

Small-sided games sessions

The SSG sessions were conducted under similar conditions, specifically on synthetic turf and outdoors. The average temperature was 15.4 ± 2.3°C, with a relative humidity of 64.9 ± 5.9%. Each session began at 7 pm, with a warm-up protocol consisting of 5 minutes of jogging around the field, followed by dynamic stretching of the lower limbs. The dynamic stretching included 12 repetitions per limb, targeting the anterior and posterior thighs, abductors, adductors, and the gastrocnemius. Participants then performed 10 repetitions of unilateral balance exercises, followed by jumps to balance. Lastly, they completed 5 accelerations of 10 meters. After the warm-up, they engaged in a 5v5 ball possession match. Following the warm-up, participants rested for 3 minutes before proceeding to the main part of the session. A brief rest period of 3 minutes was deemed appropriate to allow for the restoration of energy stores (such as phosphocreatine) and to help prevent fatigue that might impair performance during the main activity.

Players assigned to the 1v1 condition were randomly paired with opponents from the same group. The 1v1 games were played with small goals positioned at the center of the end line. The field dimensions were 18 × 12 meters, providing 108 m² of space per player. The 1v1 format, places significant demands on agility, speed, and explosive power. Players need to rapidly change direction and accelerate to beat their opponent or recover defensively. While the overall intensity may be lower compared to larger team formats, the individual effort required for constant movement, quick sprints, and close ball control is high. Recovery times between bursts of activity may be longer, but the focus is on short, high-intensity efforts that test a player's ability to perform under pressure in one-on-one duels. During the games, whenever the ball went out of bounds, the player was required to restart from their goal. Balls were placed around the field to expedite the process of ball retrieval. The 1v1 sessions consisted of 4 sets of 6 repetitions, each lasting 1 minute, with a 2-minute rest between repetitions and a 3-minute rest between sets, totaling 24 minutes of active work.

In the case of 5v5, the composition of the teams was based on playing positions, allowing each team has a balanced number of defender, midfielders and forwards. The 5v5 games also followed the rule of scoring in small goals positioned in the center of the endline. The field dimensions were 40 × 25 meters, providing 100 m² of space per player. In this format a greater player density led to a much higher overall intensity. The physical demands are focused on aerobic endurance and muscular endurance, as players must constantly move, perform quick sprints, and recover rapidly between high-intensity bursts. The game requires continuous involvement in both attacking and defending, placing a premium on stamina, speed, and quick recovery. With more players and less space, the physical demands are more sustained, and the ability to maintain high-intensity efforts throughout the game is key. During the games, whenever the ball went out of bounds, the player was required to restart from the location where the ball went out, and no offside rule was applied. Balls were placed around the field to expedite the process of ball retrieval. The 5v5 sessions consisted of 4 sets, each lasting 6 minutes, with a 3-minute rest between sets, totaling 24 minutes of active play.

Verbal encouragement was provided by the research team and field staff to promote maximal effort during the 1v1 and 5v5 games.

Measurements

Muscle damage, soreness, and function were measured during each session, specifically at rest (before the SSGs session), immediately after the SSGs session, and at 24 hours and 48 hours post-session. Between sessions, no structured training was conducted. Participants were instructed to follow their regular physical activity routines, but to avoid engaging in any structured exercise. Measurements were taken in a dedicated room, maintained at a temperature of 21°C using air conditioning, in the evening. The sequence of assessments was as follows: identification of the menstrual cycle phase, administration of a delayed onset muscle soreness (DOMS) questionnaire, measurement of Interleukin-6 (IL-6) levels, and finally, performance of a drop jump to assess the reactive strength index (RSI).

Salivary IL-6 (Interleukin-6)

Saliva samples were collected from participants using Salimetrics Saliva Collection Devices (Salimetrics, USA. A total of 2 mL of saliva was collected per participant to allow for the assessment of IL-6. Samples were promptly transported to the laboratory, where they were centrifuged at 4,000 g for 10 minutes at 4°C using a standard laboratory centrifuge. IL-6 concentrations (pg/mL) were measured using the Salimetrics IL-6 ELISA Kit (Salimetrics, USA), following the manufacturer's instructions. The assay was conducted in triplicate to minimize inter-assay variability.

Reactive Strength Index (RSI)

To assess lower limb reactive strength, the Reactive Strength Index (RSI) (Healy et al., 2018) was used, with measurements taken from a Force Decks ground reaction force plate (Vald-Performance, Australia, 2012). Participants performed a jump from a 50 cm box, immediately executing a maximal vertical jump upon landing on the force plate. Before testing, participants underwent dynamic lower-limb stretching followed by a set of 10 preparatory jumps. During the familiarization phase, a research assistant provided consistent jumping technique instructions. Afterward, participants completed three test trials, with 30-second rest periods between each. The highest RSI value recorded across the trials was used for analysis. RSI (m/s) was determined by dividing the jump height by the contact time, with the result expressed in meters per second (Flanagan et al., 2008).

Delayed Onset Muscle Soreness (DOMS)

The 7-point Likert scale was employed to assess lower limb delayed onset muscle soreness (DOMS). The scale consisted of seven categories ranging from 0 (complete absence of soreness) to 6 (severe pain that limits movement). Each point on the scale was anchored with specific descriptors to guide participants in their assessment, ensuring a consistent response. The scale was validated in previous research, showing convergent evidence with the VAS, and was found to have similar sensitivity in detecting muscle soreness induced by eccentric contractions during the first 96 hours post-exercise (Impellizzeri and Maffiuletti, 2007). The scores obtained from the Likert scale (A.U.) were used in subsequent data analysis, facilitating the analysis of soreness patterns and recovery dynamics.

Training load monitoring

Training load monitoring was conducted using the session Rating of Perceived Exertion (RPE) method. The CR10 Borg scale (Borg, 1998) was employed, with participants rating their perception of effort on a scale ranging from 0 to 10, where 0 indicated "no effort at all" and 10 represented "maximum effort." Participants were instructed to provide their individual responses after each session. Prior to the study, all participants were familiarized with the scale to ensure accurate self-assessment. Additionally, participants were allowed to report mid-range values (e.g., 4.5, 6.5) for more precise measurements. The final RPE score (A.U.) was used to monitor and control the intensity of the training sessions and games.

Menstrual cycle

The menstrual cycle phases considered in this study were the early follicular phase (days 2 to 5) and the mid-luteal phase (days 20 to 23) (Reed and Carr, 2000). The identification of these phases was based on each participant's self-reported data, including the first day of menstruation and the last day of their cycle, as documented specifically for the study. To track the menstrual cycle, participants used the Clue mobile app, which provided detailed monitoring of the cycle length and phase. Researchers had full access to the data entered by participants, ensuring tracking of the early follicular and mid-luteal phases.

Statistical procedures

Sample size estimation was performed a priori using G*Power (v3.1.9.7). Based on a desired statistical power of 0.8, an effect size of 0.2 (considered a small effect size), and the design involving one group with 16 repeated measurements (format, menstrual cycle, and moments of evaluation), the analysis recommended a sample size of 16 participants to achieve adequate power for detecting meaningful effects.

In this study, a three-way repeated measures analysis of variance (ANOVA) was conducted to examine the effects of play format (1v1 and 5v5), hormonal phase (early follicular and mid-luteal), and time point (rest, post-exercise, 24 hours, and 48 hours) on players' responses. The analysis was performed using the SPSS (v28.0; IBM, 2021). The repeated measures nature of the design accounts for the fact that the same participants were assessed across the different conditions. The assumptions of the three-way repeated measures ANOVA were checked by inspecting normality of the residuals (shapiro-wilk $p > 0.05$), Mauchly's test of sphericity, and the absence of extreme outliers. In cases where Mauchly's test indicated a violation of sphericity, the Greenhouse-Geisser correction was applied to adjust the degrees of freedom. Effect sizes were calculated using partial eta squared (ηp^2), with thresholds for small, medium, and large effects set at 0.01, 0.06, and 0.14, respectively. A p-value of <0.05 was considered statistically significant. Post-hoc analyses using Bonferroni corrections were performed to further explore significant interactions, when necessary, to identify pairwise differences between the levels of the factors and time points.

Results

Table 1 presents the mean and standard deviation values for muscle inflammation, function, and soreness across both formats during the two menstrual cycle phases. Significant interactions were found between format, menstrual cycle phase, and time points of assessment for RSI ($p = 0.040$; $\eta p^2 = 0.154$, a moderate-to-large effect size) and IL-6 ($p < 0.001$; $\eta p^2 = 0.773$, a very large effect size), but not for DOMS ($p = 0.121$; $\eta p^2 = 0.283$, a moderate effect size, despite its non-significant p-value). Cohen's d values highlight the practical magnitude of these changes. RSI decreased substantially post-exercise in both phases (early-follicular: 1v1 d = 3.92; 5v5 d = 1.69; mid-luteal: 1v1 d = 4.89; 5v5 d = 2.85), with partial recovery by 48h. IL-6 showed large increases post-exercise (early-follicular: 1v1 d = –8.62; 5v5 d = -11.47; mid-luteal: 1v1 d = -22.36; 5v5 d = -6.91), decreasing thereafter. DOMS peaked at 24h (up to d = -2.94), reflecting delayed soreness. These values underscore the substantial physiological and perceptual effects of match play across formats and menstrual phases.

Figure 3 shows the RSI at 1v1 and 5v5 conditions at four different assessment time points in the early follicular and mid-luteal phases. During the early follicular phase, no significant differences between formats were observed at rest ($p = 0.908$). However, RSI values were significantly lower in the 1v1 format compared to 5v5 at post-session ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$). Similarly, in the mid-luteal phase, no significant differences were found at rest ($p = 0.460$), but RSI was again significantly lower in the 1v1 format at post-session ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$) compared to 5v5.

Table 1. Mean and standard deviation values for muscle inflammation, function, and soreness in both formats during the two menstrual cycle phases.

	1v1 rest	1v1 post	1v1 24h	1v1 48h	5v5 rest	5v5 post	5v5 24h	5v5 48h
				Early-follicular				
RSI (m/s)	2.36 ± 0.13	1.85 ± 0.13	1.87 ± 0.09	2.17 ± 0.08	2.36 ± 0.13	2.14 ± 0.12	2.23 ± 0.12	2.31 ± 0.12
IL-6 (pg/mL)	0.47 ± 0.10	5.46 ± 0.71	1.88 ± 0.25	0.46 ± 0.04	0.41 ± 0.07	4.07 ± 0.30	2.41 ± 0.18	0.45 ± 0.06
DOMS (au)	0.15 ± 0.37	0.80 ± 0.70	2.60 ± 1.05	1.60 ± 0.88	0.30 ± 0.47	0.60 ± 0.60	1.50 ± 0.69	0.55 ± 0.61
				Mid-luteal				
RSI (m/s)	2.28 ± 0.11	1.80 ± 0.09	2.03 ± 0.08	2.03 ± 0.08	2.28 ± 0.10	2.02 ± 0.07	2.12 ± 0.07	2.21 ± 0.07
IL-6 (pg/mL)	1.23 ± 0.12	7.56 ± 0.32	3.07 ± 0.30	1.63 ± 0.37	1.25 ± 0.12	4.00 ± 0.55	3.05 ± 0.24	1.58 ± 0.20
DOMS (au)	0.40 ± 0.50	0.85 ± 0.67	3.60 ± 1.35	2.20 ± 0.95	0.45 ± 0.51	0.95 ± 0.61	1.85 ± 0.99	0.70 ± 0.66

RSI: reactive strength index; IL-6: Interleukin-6; DOMS: delayed onset muscle soreness

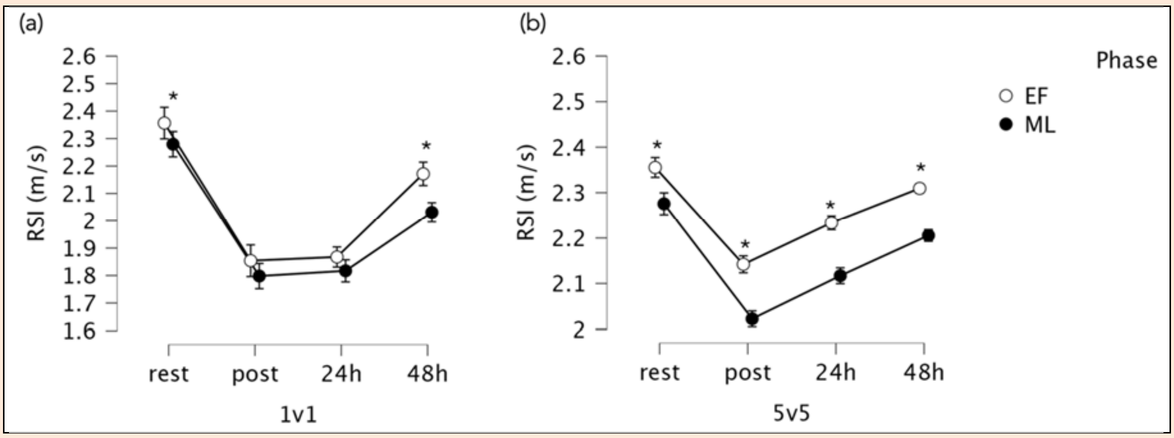

Figure 3. Reactive Strength Index (RSI) at 1v1 (a) and 5v5 (b) conditions at four different assessment time points in the early follicular (EF) and mid-luteal (ML) phases. * Indicates significant differences between the early follicular and mid-luteal phases.

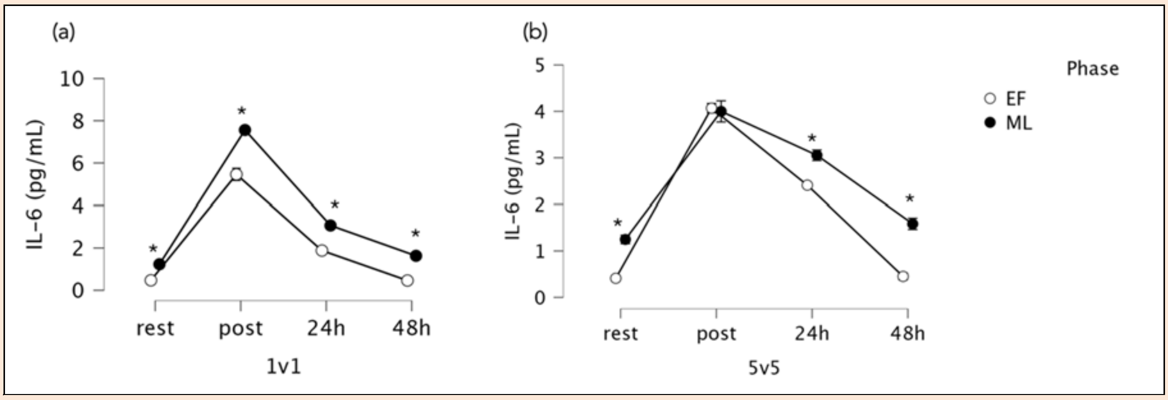

Figure 4. Interleukin-6 (IL-6) at 1v1 (a) and 5v5 (b) conditions at four different assessment time points in the early follicular (EF) and mid-luteal (ML) phases. * Indicates significant differences between the early follicular and mid-luteal phases.

In the 1v1 format, no significant differences between menstrual cycle phases were observed at post-session ($p = 0.188$) and 24 hours post ($p = 0.081$). However, RSI was significantly higher during the early follicular phase at rest ($p < 0.001$) and 48 hours post ($p < 0.001$). In the 5v5 format, RSI was consistently and significantly higher in the early follicular phase compared to the mid-luteal phase at rest ($p < 0.001$), post-session ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$).

In the 1v1 format during the early follicular phase, RSI was significantly higher at rest compared to post-session ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$). Additionally, RSI at 48 hours post was significantly greater than at post-session ($p < 0.001$) and 24 hours post ($p < 0.001$), while no significant difference was found between post-session and 24 hours post ($p > 0.999$). Similarly, at 1v1 during mid-luteal phase, RSI was significantly higher at rest compared to post-session ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$). Additionally, RSI at 48 hours post was significantly greater than at post-session ($p < 0.001$) and 24 hours post ($p < 0.001$), while no significant difference was found between post-session and 24 hours post ($p > 0.999$).

In the 5v5 format during the early follicular phase, RSI was significantly higher at rest compared to post-session ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$). Additionally, RSI at 48 hours post was significantly greater than at post-session ($p < 0.001$) and 24 hours post ($p < 0.001$). Similarly, in the 5v5 format during the mid-luteal phase, RSI was significantly higher at rest compared to post-session ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$). RSI at 48 hours post was also significantly greater than at post-session ($p < 0.001$) and 24 hours post ($p < 0.001$).

Figure 4 shows IL-6 levels in the 1v1 and 5v5 conditions at four different assessment time points during the early follicular and mid-luteal phases. In the early follicular phase, IL-6 was significantly higher in the 1v1 condition than in 5v5 at rest ($p = 0.002$) and immediately post-session ($p < 0.001$). Conversely, at 24 hours post, IL-6 was significantly higher in the 5v5 condition ($p < 0.001$), with no significant differences observed at 48 hours post ($p = 0.539$). During the mid-luteal phase, no significant differences between formats were found at rest ($p = 0.107$), 24 hours post ($p = 0.905$), or 48 hours post ($p = 0.651$). However, IL-6 levels were significantly higher in the 1v1 format immediately post-session ($p < 0.001$).

In the 1v1 format, IL-6 levels were significantly higher during the mid-luteal phase at all time points: rest ($p < 0.001$), post-session ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$). In the 5v5 format, IL-6 was significantly greater in the mid-luteal phase at rest ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$), but not immediately post-session ($p = 0.631$).

In the 1v1 format during the early follicular phase, IL-6 levels were significantly higher immediately post-session compared to rest ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$). Additionally, IL-6 at 24 hours post was significantly greater than at rest ($p < 0.001$) and 48 hours post ($p < 0.001$), while no significant difference was found between rest and 48 hours post ($p > 0.999$). During the mid-luteal phase in the 1v1 format, IL-6 was significantly higher post-session than at rest ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$). IL-6 at 24 hours post was also significantly greater than at rest ($p < 0.001$) and 48 hours post ($p < 0.001$). Moreover, IL-6 at 48 hours post was significantly higher than at rest ($p = 0.002$).

In the 5v5 format during the early follicular phase, IL-6 levels were significantly higher immediately post-session compared to rest ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$). Additionally, IL-6 at 24 hours post was significantly greater than at rest ($p < 0.001$) and 48 hours post ($p < 0.001$), while no significant difference was found between rest and 48 hours post ($p = 0.096$). During the mid-luteal phase in the 5v5 format, IL-6 was significantly higher post-session than at rest ($p < 0.001$), 24 hours post ($p < 0.001$), and 48 hours post ($p < 0.001$). IL-6 at 24 hours post was also significantly greater than at rest ($p < 0.001$) and 48 hours post ($p < 0.001$). Moreover, IL-6 at 48 hours post was significantly higher than at rest ($p < 0.001$).

Figure 5 shows DOMS in the 1v1 and 5v5 conditions at four different assessment time points during the early follicular and mid-luteal phases. No significant interactions were found between format and menstrual cycle phase ($p = 0.384$; $\eta p^2 = 0.040$), or between menstrual cycle phase and time point of assessment ($p = 0.123$; $\eta p^2 = 0.282$) for DOMS. However, a significant interaction was observed between format and time ($p = 0.001$; $\eta p^2 = 0.805$).

Specifically, no differences were observed between the 1v1 and 5v5 formats at rest ($p = 0.385$) and immediately post-session ($p = 0.741$). However, DOMS was significantly greater in the 1v1 format at 24 hours post ($p < 0.001$) and 48 hours post ($p < 0.001$).

Figure 6 shows the RPE scores for both formats of play during the early follicular and mid-luteal phases. No significant interaction was observed between menstrual cycle phase and format of play ($p = 0.314$).

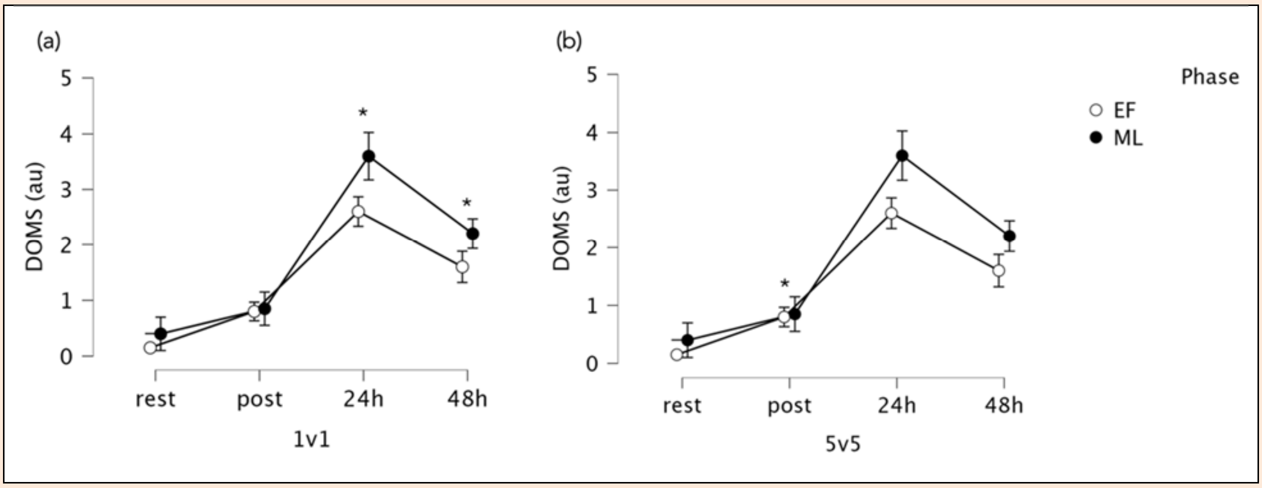

Figure 5. Delayed onset muscle soreness (DOMS) at 1v1 (a) and 5v5 (b) conditions at four different assessment time points in the early follicular (EF) and mid-luteal (ML) phases. * Indicates significant differences between the early follicular and mid-luteal phases.

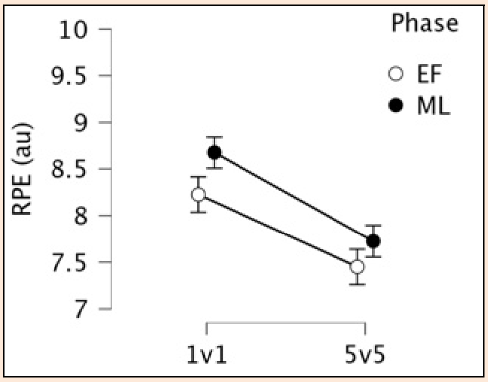

Figure 6. Rating of perceived exertion (RPE) at 1v1 and 5v5 conditions in the early follicular (EF) and mid-luteal (ML) phases.

Discussion

The aim of this study was twofold: (i) to compare potential variations in reactive strength index (RSI), interleukin-6 (IL-6), and delayed onset muscle soreness (DOMS) between the early follicular and mid-luteal phases in response to small-sided games (SSGs); and (ii) to analyse potential interactions in the magnitude of responses to different SSG formats, specifically 1v1 and 5v5. The findings of this study show significant effects of menstrual cycle phase on muscle function and inflammation following exercise. Across both 1v1 and 5v5 formats, the early follicular phase was consistently associated with higher RSI values at rest and during recovery compared to the mid-luteal phase, indicating better muscle function during this phase. Furthermore, IL-6 levels were markedly elevated during the mid-luteal phase at nearly all time points, suggesting a heightened inflammatory response in this phase regardless of exercise format. Although delayed DOMS did not differ significantly between menstrual phases, the differences in RSI and IL-6 emphasize that menstrual cycle fluctuations may influence muscle recovery and inflammation after SSGs.

The RSI was consistently higher during the early follicular phase compared to the mid-luteal phase, both at rest and during recovery periods, particularly in the 5v5 format. This finding aligns with evidence suggesting that neuromuscular performance varies across the menstrual cycle, with hormonal fluctuations influencing fatigue, motor unit behavior, and injury risk. While some studies report that the follicular phase (characterized by low concentrations of estrogen and progesterone) is associated with decreased muscular performance and strength (Weidauer et al., 2020), others indicate improved neuromuscular efficiency and faster recovery during this phase (Tenan et al., 2016). Estrogen has been described as exerting protective effects on skeletal muscle, which may partly explain the more efficient recovery observed in the follicular phase (McNulty et al., 2020). Moreover, neuromuscular changes occurring in later phases of the cycle may contribute to increased vulnerability. For instance, during ovulation and the luteal phase - when estrogen and progesterone levels rise - motor unit firing rates decrease and potentials become more complex (Piasecki et al., 2023).

In both formats, RSI decreased significantly immediately following the training session, with partial recovery observed within 48 hours. However, the magnitude of this reduction was greater in the 1v1 format, likely due to higher-intensity stimuli combined with shorter rest intervals, resulting in greater immediate neuromuscular fatigue. This is consistent with prior research indicating that higher-intensity efforts with limited recovery time led to more pronounced performance impairments. Fiorenza et al. (2019) found that multiple long-duration sprints induced larger declines in performance and greater peripheral fatigue compared to work-matched short-duration sprints. Similarly, Froyd et al. (Froyd et al., 2013) observed that most reductions in muscle function occur within the first 40% of high-intensity exercise, although substantial recovery can take place within 1 - 2 minutes post-exercise. Borji et al. (2013) reported greater force decline and neural activation failure in individuals with intellectual disabilities following high-intensity intermittent exercise. Conversely, McClean et al. (Mcclean et al., 2023) demonstrated that shorter high-intensity cycling intervals (1-minute work/rest) led to less neuromuscular fatigue and perceived exertion compared to longer intervals (3-minute work/rest) at equivalent workloads. These findings underscore the complex relationship between exercise intensity, duration, and recovery, which may also depend on the specific exercise modality and participant characteristics. Notably, in the 5v5 format, RSI remained significantly higher at all evaluation points during the follicular phase, potentially reflecting greater muscular resilience during this phase, even under more prolonged or cumulative neuromuscular stress. However, the literature presents mixed findings regarding neuromuscular performance across menstrual phases. These inconsistencies may be influenced by key moderators such as training status, baseline fitness, and inter-individual hormonal variability, including differences in estrogen and progesterone sensitivity. Furthermore, methodological differences across studies (e.g., timing of testing relative to ovulation, exercise protocols, and hormonal confirmation methods) can substantially affect outcome interpretations. Future research should better control or stratify for these factors to clarify phase-dependent effects on neuromuscular function.

IL-6 responses varied significantly across menstrual cycle phases, with levels notably higher during the mid-luteal phase at most time points and formats, indicating an enhanced inflammatory response. This aligns with evidence that progesterone, which predominates in the luteal phase, plays a key role in modulating inflammatory cytokines (Bruinvels et al., 2017). Specifically, progesterone has been shown to increase the synthesis and secretion of pro-inflammatory cytokines, including IL-6, as part of its role in preparing the body for potential pregnancy. This is thought to be a physiological adaptation that supports the implantation process. During the luteal phase, progesterone can promote the release of inflammatory mediators from immune cells, potentially leading to elevated systemic inflammation (Walusimbi and Pate, 2013). In the context of physical exercise, this heightened inflammatory environment may amplify the body's response to metabolic stress induced by exercise, which in turn could lead to higher IL-6 levels (Pedersen and Fischer, 2007). Exercise-induced muscle damage, particularly from intense or prolonged efforts, stimulates cytokine release, including IL-6, as part of the muscle repair and recovery process (Fischer, 2002). However, during the luteal phase, the increased levels of progesterone could further augment this response, resulting in greater IL-6 release in comparison to other phases of the cycle (Konecna et al., 2000). In our study, IL-6 was elevated immediately post-exercise in both 1v1 and 5v5 formats, particularly during the mid-luteal phase, suggesting a synergistic effect between the hormonal environment and exercise-induced inflammation.

Unlike RSI and IL-6, DOMS did not show significant differences between menstrual cycle phases, although it was higher in the 1v1 format at 24- and 48-hours post-exercise. These findings suggest that the subjective perception of muscle soreness is more closely related to the nature of the training load than to hormonal status, with the 1v1 format likely inducing more muscle microlesions, as also evidenced by higher IL-6 levels immediately post-exercise. Furthermore, no significant differences were observed in RPE between cycle phases, indicating that acute perception of exercise intensity may not fully reflect the physiological changes seen in inflammatory responses or neuromuscular recovery. One possible explanation for the lack of phase-related differences in DOMS is the hormonal modulation of pain perception. Estrogen, in particular, has been shown to exert analgesic effects, potentially blunting the sensation of soreness during phases with higher estrogen concentrations (Stening et al., 2007). Conversely, progesterone may have varying effects on central pain processing (Frye and Duncan, 1994). These hormonal influences could mask or dampen subjective reports of soreness, even when objective markers such as IL-6 or RSI indicate physiological stress. This aligns with research suggesting that subjective measures of exertion and pain are more closely associated with training load than with objective physiological markers. Borresen and Lambert (2008) found that session-RPE correlates strongly with heart rate-based methods of quantifying training load. Hollander et al. (2008) demonstrated that muscle loading, rather than contraction type, predominantly influences perceived exertion and pain during resistance exercise. Similarly, Lenka et al. (2015) reported a linear relationship between subjective exertion and objective load up to the anaerobic threshold. A systematic review by Saw et al. (2016) further confirmed that subjective measures are more sensitive and consistent indicators of both acute and chronic training loads compared to objective measures, noting that subjective well-being typically decreases with increased training load and improves as load decreases. Therefore, the use of subjective tools, such as RPE scales, appears to be a reliable method for monitoring athletes' training responses, both independently and in conjunction with objective assessments.

These results have direct implications for the training and recovery of female athletes. Firstly, the improved muscle recovery and reduced inflammatory response during the follicular phase suggest that this period may be more suitable for higher-intensity sessions, such as more demanding small-sided games. Conversely, the luteal phase, associated with increased inflammation and lower RSI, may require specific recovery interventions and load management. Coaches might consider reducing training intensity or volume during the mid-luteal phase, incorporating more technical or tactical work with lower neuromuscular demand. Additionally, extending recovery intervals, using active recovery strategies, and scheduling lighter sessions (e.g., mobility, low-intensity aerobic work, or stretching) can help mitigate the elevated inflammatory and fatigue responses observed during this phase. Furthermore, monitoring the menstrual cycle should be integrated into individualized training planning, with particular attention to the type of stimulus applied (1v1 vs. 5v5), as different formats elicit distinct physiological responses.

Despite its relevant findings, this study has some limitations. The sample size and the lack of direct hormonal monitoring (e.g., estrogen and progesterone assays) limit the accuracy in identifying menstrual cycle phases. Additionally, factors such as diet, sleep, and stress, which may influence inflammatory and recovery responses, were not controlled. Future studies should consider individualized hormonal assessments, include different types of exercise, and explore other physiological and perceptual variables, such as cortisol, TNF-α, and psychological state. In addition, comparing hormonal contraceptive users with non-users during similar small-sided game formats could help isolate the specific effects of endogenous versus exogenous hormonal profiles on recovery, inflammation, and neuromuscular performance.

Conclusion

In conclusion, this study demonstrates that the menstrual cycle phase significantly affects muscle function and inflammatory responses following exercise, with the early follicular phase associated with enhanced neuromuscular performance (higher RSI) and a reduced inflammatory response (lower IL-6 levels) compared to the mid-luteal phase. These differences were consistent across both 1v1 and 5v5 small-sided game formats, although the 1v1 format elicited greater acute neuromuscular fatigue and muscle soreness (DOMS), regardless of menstrual phase. The absence of significant differences in DOMS and RPE between phases suggests that subjective perceptions of effort and soreness are more closely related to the nature and intensity of the training load than to hormonal status. These findings underscore the importance of integrating menstrual cycle monitoring into individualized training periodization for female athletes, allowing for the adjustment of exercise intensity and format according to hormonal fluctuations to optimize performance and recovery. Practically, this may involve scheduling high-intensity or high-load training sessions during the early follicular phase, when recovery capacity appears enhanced, while emphasizing recovery strategies or lower-intensity work during the mid-luteal phase. Additionally, IL-6 could serve as a potential biomarker for monitoring inflammation and guiding recovery needs across the cycle. Additionally, these finding also highlight the value of combining subjective and objective measures for effective training load management.

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Key points

- Menstrual cycle phase affects neuromuscular performance and inflammation: The early follicular phase was associated with higher reactive strength index (RSI) and lower interleukin-6 (IL-6) levels compared to the mid-luteal phase, suggesting better muscle function and reduced inflammatory response.
- SSG format influences fatigue and recovery: The 1v1 format induced greater immediate neuromuscular fatigue and higher DOMS values than the 5v5 format, likely due to higher intensity and reduced recovery time.
- Perceived exertion is independent of menstrual phase: Ratings of perceived exertion (RPE) and DOMS did not differ significantly between menstrual phases, indicating that subjective responses may be more influenced by training load than hormonal fluctuations.
- Implications for training periodization: Aligning high-intensity sessions with the early follicular phase and managing load during the mid-luteal phase may optimize performance and recovery in female athletes.
- Relevance of individualized monitoring: The study reinforces the importance of integrating menstrual cycle tracking and combining subjective and objective metrics for tailored training and recovery strategies.

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