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Original Article

Comparison of neuromuscular performance, perceived effort, and well-being across menstrual cycle phases in female youth soccer players

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Abstract:
The nature of the human being, especially that of women, is complex (de Jonge et al., 2019). Various interacting variables, including physiological, biomechanical, physical, psychological, and emotional factors, constitute the nature of women. In this context, across the menstrual cycle (MC) there are fluctuations in the levels of sex hormones such as progesterone, estrogen, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), which is crucial to regulate ovulation and delimiting phases (Romero-Moraleda et al. 2019). This study aimed to compare neuromuscular performance, perceived exertion, and well-being across menstrual cycle (MC), specifically follicular phase (FPh) and luteal phase (LPh) in under-17 players from national soccer team. Thirteen players (age = 15.69 ± 0.48 years) were assessed for mean propulsive velocity (MPV) in the back squat, countermovement jump (CMJ), 10-meter time (T10). Additionally, subjective measures were collected using a well-being questionnaire (WQ) administered before training, wich evaluated muscular pain, mood, stress, sleep, fatigue and overall well-being (the sum of these five factors), as the rating of perceived exertion (RPE) scale after training. Just T10 exhibited only trivial to small differences between phases (p = .040; ES = 0.13), favoring LPh. Both MPV30 and CMJ showed trivial differences between phases. The remaining variables demonstrated unclear differences (p > .05) across the MC phases. Based on the results, it can be inferred that the MC phases had a discernible impact only on T10 in this sample, where participants exhibited superior performance in LPh, albeit with trivial to small effects that may lack clinical significance. Conversely, subjective measures obtained from the WQ and RPE scale indicated unclear differences between phases.
Key Words: **woman, athletic, fatigue, physiological, ovulation.**

Introduction

According to data published by the International Federation of Association Football (FIFA), the development of women's football has grown in recent years. In fact, 73% of federations have an active women's national team, surpassing the 55% reported in 2015 (FIFA, 2019). Globally, there are currently 13 million women participating in organized football, but only 4 million are officially registered with federations. Of these, 945 thousand are over 18 years old, and 3 million are under 18 years old (FIFA, 2019). The nature of the human being, especially that of women, is complex (de Jonge et al., 2019). Various interacting variables, including physiological, biomechanical, physical, psychological, and emotional factors, constitute the nature of women. In this context, across the menstrual cycle (MC) there are fluctuations in the levels of sex hormones such as progesterone, estrogen, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), which is crucial to regulate ovulation and delimiting phases (Romero-Moraleda et al. 2019). The MC is traditionally divided into two phases: follicular phase (FPh) and luteal phase (LPh), or three phases, which includes the ovulatory phase, based on the ovarian function (Janse De Jonge, 2003).

The FPh begins on the first day of menstruation, lasts an average of 9-14 days, where follicles grow under the influence of FSH and estrogen levels secreted by the surrounding cells increase slowly. This is followed by pituitary gland' LH secretion. As estrogen levels increase, an LH surge occurs, leading to ovulation approximately 1 day later. This marks the beginning of the ovulatory phase, which lasts about 5 days (Julian et

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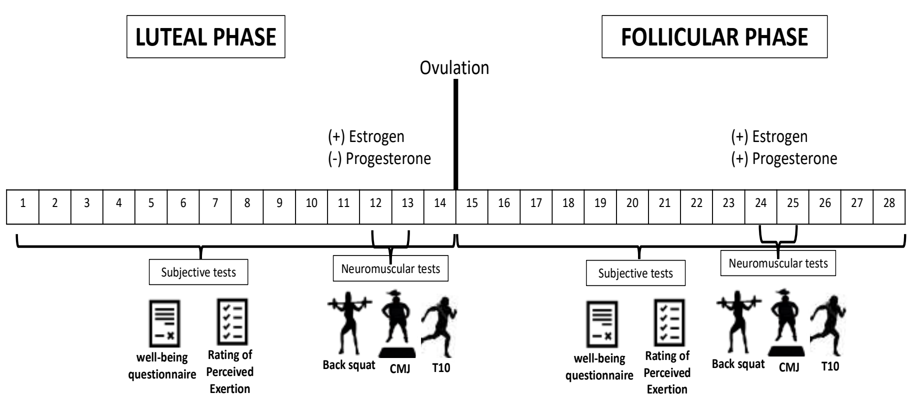
al. 2020). During these phases, the endometrial thickness increases to receive the embryo. A few days after the follicle releases the egg, it transforms into the progesterone-secreting corpus luteum, giving rise to the LPh, which typically lasts 14 days. Towards the end of the Lph, progesterone secretion ceases, the endometrium is no longer sustained, and menstrual bleeding occurs. As estrogen levels decrease during these days, FSH secretion increases, restarting the cycle. The three phases of the cycle differ from each other in the proportions of estrogen and progesterone levels: (1) in the early FPh, there are low levels of both estrogen and progesterone; (2) in the pre-ovulatory phase, estrogen levels are high, and progesterone levels are low; and (3) in the LPh, both estrogen and progesterone levels are high (Constantini et al. 2005).

The MC has historically been the focus of myths and misinformation, leading to ideas that limit women's activities (García-Pinillos et al., 2021). Circulating estrogen and progesterone cause variations in the levels of some cardiovascular, respiratory, and metabolic parameters, with subsequent consequences for strength and aerobic-anaerobic performance (Constantini et al. 2005). The evolution of women's football has prompted scientific and sports professionals to increase research on the MC and its influence on athletes' performance (Sánchez et al. 2022). However, studies have yielded contradictory results. For example, Romero-Moraleda et al. (2019) investigated the influence of the MC on strength and power performance, finding no differences among the three phases (LPh vs. early FPh vs. late FPh). On the other hand, Julian et al. (2020), examined differences in match load in professional adult female players in Germany, using a global positioning system, and found differences in speed band 17-20 km/h (FPh vs. FPh). The study by García-Pinillos et al. (2021), analyzed a battery of jumping tests and sprint performance in trained adult women, showing differences only in squat jump between the ovulatory phase and the early FPh, reaching the same conclusion as previous studies regarding these physical tests. Another study on young football players found no differences in sprint, change of direction, and vertical jump performance, but did find differences in the overall well-being obtained by self-reported questionnaires between the menstruation phase and LPh vs. FPh (Sánchez et al. 2022). Additionally, the existence of menstrual symptoms in the LPh and premenstrual symptoms in the FPh, such as fluid retention (causing swelling, congestion, and discomfort), weight gain, mood changes (irritability, depression, loss of motivation), and dysmenorrhea, could lead to a deterioration of athletes' well-being (Villa del Bosque, 2013). Similarly, psychological and physiological stress or mood changes during the premenstrual and menstrual phases are associated with decreased energy levels and/or impaired cognitive function due to the MC (Konovalova, 2013). Such changes could be reflected in common measures of training load quantification, such as subjective rate of perceived exertion (RPE) scale and fatigue perception, such as well-being obtained by self-reported questionnaires (Merino-Muñoz, Pérez-Contreras, et al. 2021; Villaseca-Vicuña et al. 2020).

Therefore, it seems that the information is contradictory, and there is no consensus on the effect of the MC on female athletes (García-Pinillos et al. 2021; Romero-Moraleda et al. 2019; Sánchez et al. 2022). Hence, the objective of this study was to compare neuromuscular performance, perceived exertion, and well-being during the phases of the MC in under-17 female soccer players.



Material & methods

Design

This study employed a quantitative, cross-sectional, descriptive, and comparative design. It focused on comparing neuromuscular performance, perceived exertion, and well-being during the phases of the MC in under-17 female soccer players.

Participants

A non-randomized convenience sampling method was utilized. The participants consisted of 13 under-17 female soccer players (age = 15.69 ± 0.48 years; body mass = 56.6 ± 7.3 kg; height = 161.72 ± 6.32 cm) from one national soccer team. They participated in official competitions proposed by the South American Football Confederation (CONMEBOL), specifically in the South American Championship in Uruguay in March 2022 and the FIFA World Cup in India in 2022. Inclusion criteria were: i) completion of training during the study period, ii) a minimum experience of at least 4 years in a federated soccer practice, iii) regularity in MC during the evaluation period (27 ± 2 days, range = 24-31 days), iv) no injury during the assessment period, v) no menstrual disorders (e.g., dysmenorrhea, amenorrhea, or intense symptoms associated with premenstrual syndrome), and vi) no use of oral contraceptive methods. Ten players with irregular menstrual cycles were excluded from the study. All players were informed about the study and its objectives.

Ethical considerations

This intervention did not disrupt the normal soccer training and did not involve motor actions different from regular training or common physical assessments conducted by the team. Participants provided signed informed consent before data collection and were free to withdraw from the study at any time. Additionally, signed consent was obtained from parents or guardians. Before the soccer season began, all players underwent a comprehensive medical examination. The study was conducted in accordance with the Declaration of Helsinki (World Medical Association, 2013), which outlines ethical principles for medical research involving human subjects. Additionally, the study received approval from the Institutional Ethics Committee of the Virgen Macarena and Virgen del Rocío University Hospitals in Seville, Spain (C.P. RENFEFUTCHILE—C.I. 2355-N - 20, June 28, 2021). This ensured that the participants were actively engaged in official competitions, contributing to the study's relevance.

Procedure

The MC was divided into two phases for estimation. The FPh was defined from the first day of menstruation until the day of ovulation. The first assessment was conducted between days 12 and 13, characterized by low progesterone levels and high estrogen concentrations. On the other hand, LPh was established after ovulation, concluding just before the onset of the next MC. The second measurement was taken between days 24 and 25, coinciding with the highest peaks of estrogen and progesterone. The phases were determined by My Calendar application. (Gonçalves et al. 2021). Each player downloaded the application and recorded her MC. According to the calendar filled out by the participants, neuromuscular performance was assessed using the following neuromuscular tests in the specified order: mean propulsive velocity (MPV) in back squat, countermovement jump (CMJ), and 10-meter sprint time (T10). Additionally, subjective tests were recorded throughout the MC using the well-being questionnaire (WQ) (McLean et al. 2010). These evaluations occurred 30 minutes before and after training, utilizing the RPE scale (Impellizzeri et al. 2004) (Figure 1). The assessment period began in January and concluded in February 2022, just before the start of the Under-17 South American Championship, which served as a qualifier for the 2022 World Cup in India.

The players were already familiar with these evaluations as the physical conditioning area of the national team utilized these assessment protocols with both the senior and junior teams (Villaseca-Vicuña, Jesam-Sarquis et al. 2021). To ensure consistency, a standardized warm-up routine was established, involving slow running, multidirectional movements, and dynamic stretching, followed by a specific warm-up for each test lasting 15-20 minutes. The tests were conducted individually at the training center, maintaining consistent spatial conditions to minimize potential influences on the results. All assessments were conducted at the headquarters of the team Soccer Federation at 10:00 AM, with a temperature of 15 °C and a relative humidity of 54%.

Variables and instruments

Menstrual cycle phases (MC)

The phases of the MC were determined using the MY CALENDAR® mobile application for Apple and Google. This app allows players to record their menstrual period, facilitating the estimation of the FPh and LPh for each player. The application has demonstrated utility in determining menstrual phases and is regularly used among women participating in sports (Gonçalves et al. 2021).

Neuromuscular Performance

Mean Propulsive Velocity in Back Squat (MPV)

This test has been validated to assess levels of muscular strength in the lower limbs of soccer players (Pareja-Blanco et al. 2020; Villaseca-Vicuña, Otero-Saborido et al. 2021). Prior to measurement, each player performed a specific warm-up consisting of 3 sets and 3 repetitions with a load of 20 kg. During the evaluation, each player performed 2 sets (1 set per load) of 3 repetitions each, with loads of 20 and 30 kg, respectively, with a recovery time of 3 minutes between sets. The concentric phase of the exercise was to be performed at the maximum possible velocity. Two variables were recorded: (a) the estimated one-repetition maximum (RM), determined from the mean propulsive velocity (MPV) of the last load of the test (30 kg), calculated using the equation proposed by Pareja-Blanco et al. (2020) for a female athlete population and (b) relative strength (RS), obtained from the ratio RM/body mass. A linear velocity transducer (Chronojump®, Barcelona, Spain) was used for measuring MPV. The equation for calculating 1RM in the squat based on the MPV in the last load is as follows: $\%1RM = (-42.196\ MPV2 - 31.018\ MPV + 112.937)$.

Countermovement Jump (CMJ)

This test is regularly conducted to measure the lower limb power of female soccer players (Bishop et al. 2018; Villaseca-Vicuña, Molina-Sotomayor, et al. 2021). Prior to the test, each player performed a specific warm-up consisting of 5 jumps to a jump box with a target surface 30 cm above the ground. During the test, participants executed 3 attempts. The starting position was upright with hands on hips throughout the test to eliminate any influence from arm swinging. From this position, the participant rapidly flexed the knees to approximately 90 degrees and then immediately propelled themselves to jump vertically as high as possible, landing on both feet simultaneously with extended knees (approximately 180 degrees) (Kammoun et al. 2020). If the evaluator observed an execution error, the jump was invalidated, and the attempt was repeated. This test was assessed using the mobile application My Jump 2 app® (Madrid, Spain), with 3 repetitions and a 3-minute rest between each trial. Jump height (cm) was recorded, and the average of the 3 attempts was selected for statistical analysis. Recently, Balsalobre-Fernández et al. (Balsalobre-Fernández et al. 2015), validated this application using a high-speed camera of 120 Hz mounted on an iPhone 5s (Apple®, California, USA). For convenience, in the present investigation, the application was installed on an iPhone XR (Apple®, California, USA), which included the same functions as the iPhone 5s. To standardize video recording analysis, the tablet was placed vertically on the ground facing the participants' feet at a distance of 1.5 m and a recording speed of 240 Hz.

10-Meter Sprint Time (T10)

This test is commonly used to assess acceleration in soccer players (Pardos- Mainer et al. 2019; Villaseca-Vicuña, Molina-Sotomayor, et al. 2021). Prior to the assessment, each player performed a specific warm-up consisting of 5 progressive 10-meter sprints. There were 3 attempts at the 10-meter sprint, with a 3-minute recovery time between repetitions. The evaluation took place on a natural grass soccer field at 10:00 AM, with a temperature of 15 °C and a relative humidity of 54%. The starting position was standing, with the front foot placed just behind a line located 0.5 m from the first photoelectric cell at a height of 1 m, to prevent the laser from being interrupted by the head or arms at the start of the sprint. Two photoelectric cells (Microgate®, Bolzano, Italy) were positioned at the start and at the 10-meter mark. The average of the 3 attempts in the 10-meter sprint (T10) was used for analysis.

Subjetive tests

Well-being Questionnaire (WQ)

During the study period, players completed the WQ 30 minutes before each training session, using a cloud-based Google Drive® spreadsheet (Google Drive®, California, USA). The approach proposed by (McLean et al., 2010). Fatigue (FAT), sleep quality (SQ), muscle soreness (MS), stress level (SL), and mood (MD) were assessed using the WQ on a scale of 1 to 5 points, with 5 indicating maximum well-being (very, very good). Total well-being (TB) was then determined by summing the five scores (McLean et al. 2010). There was no presence of peers during the response to avoid biases in responses (Minett et al. 2021)

Rate Perception of Effort (RPE)

To quantify the intensity of each training session, the RPE scale was used 30 minutes after each training session (Borg, 1990). Each player rated their perceived exertion using a scale from 0 to 10 on a Google Drive® cloud-based spreadsheet (Google Drive®, California, USA). There was no presence of peers during the response to prevent biases in responses (Minett et al. 2021).

Statical Analysis

The distribution of variables was examined using the Shapiro-Wilk test and histograms. Mean (M) and standard deviation (SD) are reported for normal variables, while median and 25th and 75th percentiles are presented for non-normal variables. The paired Student's t-test was conducted for normal variables; if normality assumptions were not met, the Wilcoxon test was employed.

The magnitude of differences for normal variables was assessed using the Cohen's d, with the average standardizer being the mean of SD [(SD1 + SD2) / 2] (Lakens, 2013). Results were categorized based on the following thresholds: <0.2: trivial; 0.21–0.6: small; 0.61–1.2: moderate; 1.21–2.0: large; and >2.0: very large (Hopkins et al., 2009). For non-normal variables, the Wilcoxon's r was used as the effect size ($Z/\sqrt{n}$) (Fritz et al. 2012). Categorization followed these thresholds: <0.1: trivial; 0.11–0.3: small; 0.31–0.5: moderate; and >0.50: large. Effects surpassing trivial levels were considered unclear (Hopkins et al. 2009). A 95% confidence interval was calculated for both effect sizes. An alpha level of 0.05 was established. The analysis was conducted using SPSS software for Microsoft Windows (SPSS IBM®, version 24, New York, USA).

Results

Table 1 displays the results for variables with a normal distribution. Only T10 exhibited trivial to small differences between phases (p = .040; ES = 0.13), while the remaining variables MPV30 and CMJ showed trivial differences between phases (p > .05).

Table 1. Comparison between menstrual cycle phases in the performance of normal variables.

	FPh		LPh		
Variables	**M**	**±SD**	**M**	**±SD**	***p***
CMJ (cm)	28.5	2.59	28.9	3.75	.662
MPV30 (m/s)	1.05	0.08	1.05	0.11	.848
T10 (s)	1.86*	0.07	1.91	0.10	.040

Note. *: Difference $p < .05$; M: mean; SD: standard deviation; CMJ: countermovement jump; MPV30: mean propulsive velocity 30 kg; T10: 10-Meter sprint time.

In Figure 2, the magnitudes of effect size in the performance of normal variables can be observed.

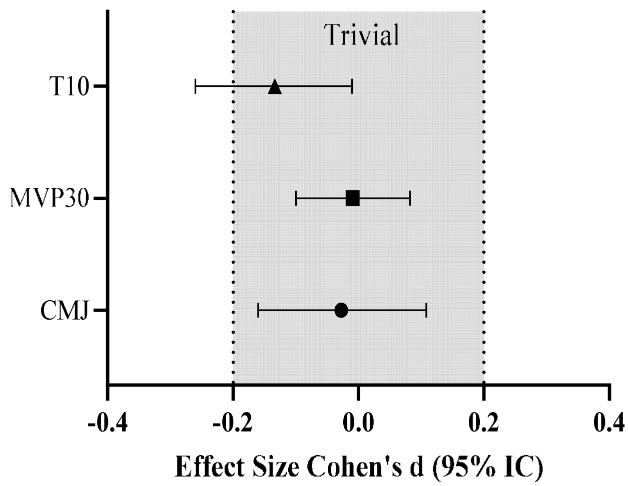


Figure 2. Effect Sizes (ES) Cohen's d with 95% Confidence Interval.

In Table 2, the results of non-normal variables are presented. All variables showed unclear differences ($p > .05$).

Table 2. Comparison between menstrual cycle phases in the performance and subjective tests of non-normal variables.

	FPh			LPh			
Variables	**Md**	**P25**	**P75**	**Md**	**P25**	**P75**	***p***
MPV20	1.16	1.12	1.25	1.18	1.10	1.24	.528
RM (kg)	88.6	69.5	103	91.9	71.4	119	.583
RS (RM/kg)	1.43	1.37	1.87	1.58	1.43	1.89	.480
Fatigue (UA)	3.40	3.00	4.00	3.50	3.00	4.25	.506
Sleep quality (UA)	4.00	3.60	4.00	3.67	3.25	4.00	.092
Muscle Soreness (UA)	3.29	3.11	4.00	3.80	3.00	4.25	.222
Stress level (UA)	3.29	3.00	4.00	3.50	3.00	4.00	.917
Mood (UA)	4.00	4.00	4.70	4.00	4.00	5.00	.866
Total Well-being (UA)	17.5	17.0	20.2	17.3	16.0	20.8	.727
RPE (UA)	6.00	4.00	6.50	5.50	3.75	6.20	.346

Note. Md: Median; P25: 25th Percentile; P75: 75th Percentile; MPV20: Mean Propulsive Velocity 20 kg; RM: Maximum Repetition in squat test; RS: Relative Strength; UA: Arbitrary Units.

In Figure 3, the magnitudes of effect size in the performance of non-normal variables are observed.

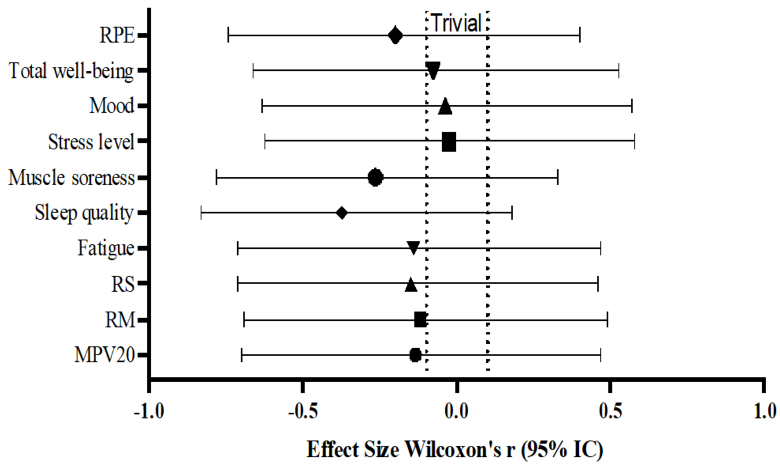


Figure 3. Effect Sizes (ES) r Wilcoxon with 95% Confidence Interval.

Dicussion

The objective of this study was to compare neuromuscular performance, perceived exertion, and well-being during the phases of the MC in under-17 female soccer players. The main findings of the present investigation were that only T10 showed trivial to small differences between phases, demonstrating better performance in the follicular phase (FPh). The remaining variables exhibited trivial and unclear effects. From a physiological perspective, the potential differences could be explained by the fact that in the FPh athletes have elevated estrogen levels, which may enhance muscular strength, speed, and endurance (Constantini et al. 2005; Janse De Jonge, 2003), and thus explain that they were faster in the follicular phase (FF) compared to the luteal phase (FL). However, these results are not in agreement with those found by Sánchez et al. (2022) who did not find differences between MC phases in female soccer players during the 40-meter sprint test. Although they divided the MC into three phases, no differences were evident in any of these. On the other hand, the 10-meter and 40-meter tests may assess different capacities, such as acceleration and maximum speed, respectively. Strong associations have been found between these capacities, but not as strong in female soccer players (r = .77 to .88) (Merino-Muñoz, Vidal-Maturana et al. 2021; Villaseca-Vicuña, Otero-Saborido et al. 2021), which suggested that hormonal changes do not lead to performance differences in sprinting (Sánchez et al. 2022). Additionally, it has been reported that isolated natural concentrations of 17b-estradiol with low progesterone levels do not have an effect on sprint performance (Tsampoukos et al. 2010). Similar findings were reported by García-Pinillos et al. (García-Pinillos et al. 2021) in endurance athletes, where they found trivial differences in T10 ($p > .05$). These studies highlight contradictions with the results obtained in relation to the sprint test. Although these effects seem to be not clinically significant, a 1% difference is mentioned to make a significant impact during soccer matches (Haugen & Buchheit 2016). In this regard, a similar pattern is observed in the vertical jump test, where Sánchez et al. (Sánchez et al. 2022) noted trivial to small differences in squat jump ($p > .05$), and García-Pinillos et al. (García-Pinillos et al. 2021) reported small differences in SJ ($p < .05$) but not in CMJ ($p > .05$). Both studies concluded that MC phases do not significantly affect neuromuscular performance, possibly because the effects are trivial to small. In fact, a recent narrative review mentioned that studies analyzing neuromuscular performance report unclear and inconsistent effects on the impact of the MC. However, this review also states that studies examining perceived performance consistently demonstrate that female athletes identify their performance as relatively worse during the early follicular and late luteal phases (Carmichael et al. 2021), which could be reflected in a higher perception of effort and poorer well-being, associated with a greater perception of motor performance fatigue (Behrens et al. 2022).

On the other hand, the results regarding RPE, and well-being state showed no differences between phases of the CM (Comparative Model) ($p > .05$). These findings are contradictory to those reported by Sánchez et al. (Sánchez et al. 2022) in adolescent female soccer players were the results revealed a significantly poorer overall well-being state ($p < .01$) during LPh to the FPh. The observed variations may be attributed to several factors. Firstly, it should be noted that the study conducted by Sánchez et al. (Sánchez et al., 2022) characterized the MC in three phases. Additionally, the well-being questionnaire employed in the study did not encompass the mood item. Furthermore, one should take into account the potential influence of athletic proficiency, as higher levels of experience in sports might mitigate fluctuations in well-being states, as suggested by previous research (Esteves et al. 2020; Villaseca-Vicuña, Pérez-Contreras et al. 2021).

While this study contributes to the existing research on MC in young female soccer players, it is not without limitations. Firstly, no direct hormonal assessment was performed to define MC phases. In fact, an analysis of hormone concentrations related to performance in each MC phase could contribute to understanding the reasons for the absence of performance changes across the MC. Secondly, it is crucial to acknowledge that soccer performance is multifactorial; therefore, it would

have been important to evaluate other performance variables during MC phases, such as body composition, aerobic endurance, or the physical and technical demands during competition. Future research should examine the individual effects of MC phases, considering the potential inter-subject variability in the effects of MC phases. Analyzing individual inferences through single-subject (n=1) or magnitude-based inferences could reveal different effects among players (Barry, 1996; Buchheit, 2016, 2017)

Practical applications

While the literature is inconclusive regarding how the MC influences neuromuscular performance, this research provides relevant information on MC and measures of neuromuscular performance, as well as subjective perceptions of effort and well-being. This information could benefit the scientific community and sports coaches in establishing both objective (physical tests) and subjective (questionnaires) monitoring strategies on an individual basis. This, in turn, allows for the development of protocols tailored to the needs of each athlete, enabling the application of transdisciplinary strategies. Ultimately, controlling performance variables is a crucial element in sports and applied sciences. Therefore, the use of low-cost applications and questionnaires could significantly contribute to the sports community, as they are economically accessible to any staff member (López, 2017).

Conclusions

Based on the results, it can be concluded that CM phases only affected T10 in this sample, where there was a higher performance in FPh, but with trivial to small effects that may not be substantial in practice. On the other hand, subjective measures from the well-being questionnaire and RPE showed unclear differences between phases. Further studies with a larger number of subjects (statistical power) are needed to validate the results of this study.

Acknowledgments

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Conflicts of interest

No potential conflict of interest was reported by the author(s)

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