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Cycles and Strains:

A Systematic Review and Meta-Analysis of the Influence of the Menstrual Cycle on Muscle Injuries Supplementary materials: https://osf.io/mr6y8 For correspondence: ross.julian@uni-muenster.de

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Summary Box

What is already known:

- Hormonal fluctuations across the menstrual cycle can affect musculoskeletal properties, potentially influencing the injury risk for female athletes.
- Previous research on the association between menstrual cycle phases and injuries, particularly ACL injuries, has produced inconsistent findings.

What are the new findings:

- No statistically significant association was found between menstrual cycle phases and muscle injury occurrence in female team sport athletes.
- Inconsistent menstrual phase classifications and reliance on self-reported data limit the robustness of current findings, highlighting the need for standardised, hormone-verified methodologies in future research.

Keywords: Female Athlete, Follicular Phase, Injury Occurrence, Luteal Phase, Oestrogen, Team Sport

All authors have read and approved this version of the manuscript.

ABSTRACT

Objective: This systematic review aimed to explore the association between menstrual cycle phases and the occurrence of muscle injuries in female team sport athletes.

Design: The systematic review and meta-analysis followed PRISMA 2020 guidelines, supplemented by PERSiST guidance.

Data Sources: Three databases (PubMed, Scopus, and SPORTDiscus) were searched from inception to mid-January 2024. The search terms included keywords related to muscle injuries and menstrual cycle phases.

Eligibility Criteria for Selecting Studies: Included studies involved female team sport athletes of reproductive age with regular menstrual cycles and needed to compare muscle injury events across at least two menstrual cycle phases. Exclusion criteria included the use of hormonal contraceptives or medications that affect the menstrual cycle or the musculoskeletal system, as well as menstrual dysfunction.

Results: Three studies met the inclusion criteria, encompassing injury data from 318 participants. Meta-analysis revealed a pooled Risk Ratio (RR) of 1.18 (95% CI: 0.75 to 1.86, p = 0.46) for muscle injury occurrence between luteal and follicular phases, indicating no significant association. Certainty of cumulative evidence was assessed to be 'very low' due to methodological limitations

Conclusion: This review found no statistically significant relationship between menstrual cycle phases and muscle injuries. However, the robustness of the findings is limited by methodological issues, such as inconsistent phase classifications and reliance on inaccurate methods for identifying menstrual cycle phases. Future research employing standardised, physiologically accurate methods for classifying and detecting menstrual cycle phases is necessary to understand better the potential links between hormonal fluctuations and injury risk.

1 INTRODUCTION

Over the past few decades, women's sport has seen rapid growth in professionalism and commercialisation due to investments, structured development, and strategic planning [1–4]. This evolution has inherently led to an increase in the physical demands on athletes [5] as well as a rise in the frequency, intensity, and competitiveness of training and competitions [6], potentially elevating the risk of injury [7]. Given the significant health and performance-related

impacts of injuries [8–12], there is a pressing need to design and investigate effective injurymitigation strategies.

Despite the significant underrepresentation of female participants in sport medicine research [13, 14], numerous studies have highlighted notable differences in the predominant injury types, incidence, and burden between male and female athletes [15–18]. These disparities have prompted calls for research on female-specific injury prevention programmes, including the effects of sex-specific biological factors [1, 17, 19].

Arguably, one of the most prominent factors in eumenorrheic female athletes is the menstrual cycle, which has been shown to influence physiological functions and systems [20–22]. These cyclical changes in ovarian hormone concentrations, which can be observed in Figure 1, lead to distinct hormonal profiles that can be used to identify and differentiate menstrual phases [23, 24]. Based on an idealised 28-day cycle, the menstrual cycle can be divided into the early follicular phase (days 1-5), mid follicular phase (days 6-8), late follicular phase (days 9-13), ovulation (day 14), early luteal phase (days 15-20), mid-luteal phase (days 21-24), and the late luteal phase (days 25-28) [21].

Figure 1



Schematic Hormonal Course of an Idealised 28-day Cycle

Note. Solid line: Oestrogen. Dashed line: Progesterone. Adapted from McNulty et al. [22].

Systematic reviews and meta-analyses examining potential menstrual cycle-related injury risks seem to have primarily focused on the anterior cruciate ligament (ACL) [25–28]. This focus is likely due to previously demonstrated effects of ovarian hormones and the menstrual cycle on collagen formation [29, 30], the structural integrity of the ACL [31, 32], and knee laxity [31, 32]. Though rare, ACL injuries often sideline athletes for several months [33, 34], and jeopardise careers [34].

In contrast, muscle injuries are generally less severe but occur significantly more frequently. For example, in elite-level women's football, muscle injuries in the thigh alone have been observed to occur approximately 16 times more often than ACL injuries [34], with 15-20% of cases sidelining athletes for longer than a month [34]. Muscle strains are also the most common type of injury in both male and female athletes across various sport disciplines [34– 37]. Given evidence suggesting that menstrual cycle hormones may affect soft tissue compliance [38–40] and neuromuscular ability [41–43], it seems plausible to assume a possible connection between the menstrual cycle and muscle injury risk.

To the best of the authors' knowledge, this is the first attempt to provide a systematic and comprehensive overview of the influence of the menstrual cycle on muscle injury occurrence. The insights from this review may be valuable for athletes, coaches, practitioners, researchers, and other stakeholders working to understand and prevent muscle injuries.

2 METHODS

This review was pre-registered on the Open Science Framework (OSF), adheres to the PRISMA 2020 statement guidelines [46] and covers all the items of the PRISMA checklist (https://osf.io/mr6y8). Further, the Prisma in Exercise, Rehabilitation, Sport medicine and SporTs science (PERSIST) guidance was followed [47].

2.1 Study Inclusion and Exclusion Criteria

The PICOS framework (consisting of Population, Intervention, Comparator, Outcomes, and Study Design) guided the process of determining this review's relevant parameters.

2.1.1 Population

Participants included female team sport athletes who fulfilled the following criteria: (a) were of reproductive age (post-menarche and premenopausal), (b) had regular menstruations and ovulation cycles, (c) were non-users of any hormonal contraceptives or medications that affect the menstrual cycle or musculoskeletal system, and (d) were free from any menstrual dysfunctions (such as amenorrhea or anovulatory cycles) or other conditions that can influence their menstrual cycle and hormone profile or musculoskeletal system (such as pregnancy and relative energy deficiency syndrome). No restrictions were imposed regarding the athletes' competition level.

2.1.2 Intervention

No particular intervention was investigated, but participants had to meet the population criteria above. Studies had to verify the participants' menstrual cycle phases through established means, and the classification used for the menstrual cycle phases had to be consistent with the existing literature.

2.1.3 Comparator

To understand the potential association between the menstrual cycle and the occurrence of muscle injuries, included studies were required to compare an outcome measure (i.e., injury number or injury rate incidence) at a minimum of two menstrual cycle phases. Injuries were defined as an occurrence that prevents an athlete from participating in training or match-play for a minimum of a day.

2.1.4 Outcomes

The primary outcome was the occurrence of non-contact muscle injuries. Further, related outcomes such as injury incidence rate and muscular injuries with unclear differentiation between contact and non-contact origin were considered.

2.1.5 Study Design

Studies were considered for inclusion if they met the following criteria: (a) fully published in a peer-reviewed journal, (b) written in English or translated and published in English, and (c) had the primary or secondary objective of assessing the incidence of muscle injuries within the

phases of the menstrual cycle. Any reviews, case reports, editorials, conference abstracts, clinical commentaries, dissertations, and unpublished studies were excluded. No restrictions were placed on the date of publication.

2.2 Search Strategy

A systematic electronic literature search was conducted independently in mid-January 2024 by two reviewers (YG and DS), each using the three databases PubMed (including MEDLINE), Scopus and SPORTDiscus to identify all relevant articles. The following search string was used: ("muscle*" OR "muscular") AND (injur* OR strain* OR tear* OR rupture* OR "injury incidence") AND ("menstrual cycle" OR "menstrual phase" OR "menstrual" OR "menstruation" OR "follicular phase" OR "luteal phase" OR "ovulation" OR "ovulatory" OR "sex hormone*"). Databases were searched from inception onwards. The reference lists of relevant articles obtained were hand-searched to identify further potential studies that could be added manually.

2.3 Study Selection, Data Extraction and Quality Assessment

2.3.1 Screening and Selection of Eligible Studies

All search results were saved and managed in the systematic review software 'Rayyan' [48]. This tool was utilised to sort through, screen, and include qualified records. Any duplicates were automatically identified by comparing title, year, volume, and authorship. Afterwards, two reviewers (YG and DS) independently verified the accuracy of duplicates before removing them from consideration.

All the remaining articles underwent an independent two-phase screening strategy by two reviewers (YG and DS). In phase 1, the titles and abstracts were examined against the predetermined eligibility criteria. If neither the title nor the abstract of an article showed indications of meeting the inclusion criteria or met at least one of the exclusion criteria, the article was excluded in phase 1.

The full-text versions of the remaining articles were then read as phase 2 to confirm eligibility. In cases where studies were reported in multiple publications, all reports were collated. In case of disagreement on eligibility between the two reviewers, the most experienced reviewer (RJ) was consulted, and his decision was deemed final.

2.3.2 Data Extraction

Two reviewers (YG and DS) independently extracted the data using a standardised template. Any discrepancies were identified and addressed through a consensus-based discussion and reviewing the original article. The matter was referred to the most experienced researcher (RJ) for consultation in unresolved disagreements. A comprehensive list of the extracted data items is provided on OSF (<u>https://osf.io/mr6y8</u>).

2.3.3 Quality Assessment

The quality assessment was conducted independently by two reviewers (YG and DS) following the Grading of Recommendations Assessment Development and Evaluation (GRADE) system of rating quality of evidence [49]. This approach evaluates the certainty in the cumulative evidence based on five domains: risk of bias, indirectness, inconsistency, imprecision, or evidence of publication bias. Each study was individually assessed for risk of bias with the QUIPS (Quality In Prognosis Studies) appraisal tool [50]. Based on the QUIPS tool results, each study was assigned an adequate a priori quality rating of either 'high', 'moderate', 'low' or 'very low'.

Following McNulty et al. [22] and based on the recommendations of De Jonge et al. [51], the initial rating was either maintained or downgraded based on two questions considered vital to assessing the indirectness of the research studies:

(*Q1*) Was the menstrual cycle phase confirmed using blood samples or urinary ovulation detection *kits*? The initial rating was maintained if the study confirmed the menstrual cycle phase using these methods. If not, the study was downgraded by one level. For example, a study initially rated as 'high' would be downgraded to 'moderate' if it did not use blood samples or urinary detection kits for confirmation.

(*Q2*) Was the injury medically diagnosed by qualified experts or means? The rating was downgraded by one level if the study did not report that qualified medical personnel recorded and diagnosed injuries. If medical staff were involved in diagnosing injuries, the Q1 rating was maintained.

Consistency was ascertained through meta-analysis, visual inspection of effect size estimates, and overlap of confidence intervals, supplemented by statistical tests for heterogeneity. Precision was judged by closely examining the number of data points supporting the relevant outcome (with outcomes based on < 5 data points being downgraded) and visual analysis of the width of the confidence intervals. The assessment of publication bias included visual examination of result patterns (funnel plot), Egger's test and the recommendations of the GRADE workgroup [52].

These procedures collectively led to a final certainty rating for the cumulative evidence as 'high', 'moderate', 'low', or 'very low' [53]. Disagreements were resolved through discussion. If no consensus was reached, a third reviewer (RJ) made the final decision. Based on this appraisal strategy, no studies were excluded.

2.4 Data Synthesis

To facilitate a consistent analysis across studies, each study's menstrual cycle phases were aligned according to a predefined two-phase classification scheme commonly employed by prior menstrual cycle research [51]. The follicular phase was defined as extending from the onset of menstruation up to ovulation, and the luteal phase was considered to encompass ovulation and the remaining days until the start of the next menstrual period. The extracted data was utilised to align the phases of each eligible study to the predetermined classification for statistical analysis. This alignment was carried out based on the days of a 28-day idealised cycle length or the contextual information provided by the study in cases where the precise duration of each classified phase was ambiguous. Ovulation was deemed to start on day 13 of a 28-day idealised menstrual cycle in line with McNulty et al. [22]. Since none of the eligible studies focused exclusively on muscle injuries, the absolute number of muscle injuries was selected as the primary outcome for statistical analysis, as it was the only consistent and comparable metric across all studies.

A frequentist approach was employed for this meta-analysis, using the Risk Ratio (RR) as the primary effect size. This choice allowed for a direct comparison of the injury occurrence between the follicular and luteal phases, the latter of which acted as the 'control condition' across the selected studies. For each study, the RR was calculated as:

$RR = \frac{Number \ of \ Injuries \ in \ Luteal \ Phase}{Number \ of \ Injuries \ in \ Follicular \ Phase}$

The log-transformed Risk Ratio (logRR) and its associated standard error (SE) were computed to standardise the effect sizes across studies, facilitating pooling across studies. The metaanalysis was conducted using both fixed-effect (common-effect) and random-effects models to explore the impact of potential heterogeneity among studies. The fixed-effect model assumes that all studies estimate a common underlying effect size, attributing any observed differences solely to within-study variation. In contrast, the random-effects model accounts for both within and between-study heterogeneity, assuming that the true effect size may vary across studies. In both models, weights were assigned to each study based on the inverse of the logRR's variance. The random-effects model further adjusted the weights to account for between-study variability.

Heterogeneity across the studies was assessed using the I² statistic, which quantifies the proportion of total variation due to between-study heterogeneity. An I² value of 100% indicates maximal inconsistency.

Further, tau² was calculated using the Restricted Maximum Likelihood (REML) method to estimate the between-study variance, providing a refined measure of variability beyond what is captured by I² alone. Cochran's Q statistic was used to test the null hypothesis that all studies evaluate the same effect. A leave-one-out sensitivity analysis was conducted to ensure the robustness of the findings.

The results were visually summarised using a forest plot, which displays each study's individual RR and 95% confidence intervals (CIs) and the pooled effect sizes from both models. The forest plot also illustrates the weight assigned to each study, emphasising each contribution to the overall effect estimate. All analyses were conducted in the statistical software R Version 4.2.2 [54], including the use of the R package meta [55].

3 RESULTS

3.1 Literature Search

Figure 2 illustrates the search and selection of studies in a flow chart.

Figure 2

PRISMA Flow Diagram of the Literature Search



3.2 Study Characteristics

The final analysis included data from three studies [56–58]. The combined total number of participants observed in two [56, 58] of the three studies was 205, while the total number of participants observed in the third study was not ascertainable. However, all relevant injury data in this study were obtained from 113 participants [57]. Two studies were conducted in

professional football and one in professional futsal. All studies assessed the menstrual data via self-reporting of the players through either a mobile menstrual tracking application [58], a combination of self-reported menstrual cycle length and a regression equation [57], or a mixture of calendar-based counting and different mobile applications [56]. Details of the study characteristics of the included studies can be found in Table 1.

3.3 Study Findings

Muscle injury occurrence across the studies showed variation between menstrual cycle phases, with inconsistent results reported. One study [57] observed a considerable increase in muscular injuries during the late follicular phase, as defined in their research. In contrast, another study reported the highest injury rates during the luteal phase, particularly in the premenstrual window [58]. A third study [56] indicated a tendency toward higher injury frequencies in the follicular phase compared to the other phases they defined, although these differences were not statistically significant. Detailed findings from each study are presented in Table 1.

3.4 Quality Assessment of Included Studies

Three studies (100%) were considered to be at high risk of bias after the risk of bias assessment of the individual study level obtained from the QUIPS tool and additional questions regarding the menstrual cycle phase and injury detection and verification. All studies were allocated an a priori rating of 'moderate' but downgraded based on the additional questions (Q1) and (Q2) regarding the method of menstrual phase verification and injury diagnosis and recording. Details of the domain-based risk of bias assessment from the QUIPS tool and (Q1) and (Q2) can be found on OSF (https://osf.io/mr6y8).

No asymmetry was observed following a visual examination of the funnel plot. Egger's test also indicated no detectable publication bias (p = 0.92). However, because all included papers are observational studies, the recommendations of the GRADE workgroup were followed, and publication bias was considered to be inherently substantial [52]. Based on the GRADE approach, the certainty of the cumulative evidence of this review was assessed to be 'very low' [53].

Table 1

Study	Sample	Menstrual Cycle Classification	Method of MC Phase Verification	Method of Injury Diagnosis	Findings	
Lago- Fuentes et al. [56]	N = 179 Players of the Spanish First and Second National Futsal League	Follicular Phase (Day 1-12) <i>Ovulatory Phase</i> (Day 13-15) <i>Luteal Phase</i> (Day 16-28)	Self-report via calendar- based counting and different apps	No Information	No statistical differences in the injury occurrence between the MC phases. Tendencies of higher frequencies in the <i>Follicular Phase</i> .	
Martin et al. [57]	N = eight playing squads over four years, comprised of 3,947 individual player camp attendances (Injury data was obtained from n = 113) Players selected for the England National Football Team Under 15s – Senior Level	Follicular Phase (Time between the first day of the menses and the late Follicular Phase) Late Follicular Phase (Day of luteinising hormone peak and the two preceding days) Luteal Phase (Any time point following the Late Follicular Phase)	Self-report via typical cycle length and regression equation	Recorded by each team's medical support staff and classified using the Orchard Sports Injury Classification System by a medical professional	Muscle injuries were approximately twice as common in the <i>Late</i> <i>Follicular Phase</i> compared to the <i>Follicular Phase</i> and <i>Luteal Phase</i> per 1,000 person-days	
Barlow et al. [58]	N = 26 24.1 ± 4.6 years of age Players of a professional Women's Super League Football Club	 Phase 1 (Menstruation) Phase 2 (Remainder of the predicted follicular phase) Phase 3 (Majority of the luteal phase) Phase 4 (Premenstrual window, defined as the five days before the onset of menstruation) 	Self-report via a mobile tracking application that recorded menstruation days and intensity of flow	Recorded by the football club's medical support staff and classified using the Orchard Sports Injury Classification System	Muscle injuries occurred more commonly in <i>Phase 3</i> and <i>Phase 4</i> than in <i>Phase 1</i> and 2. Compared to <i>Phase 1</i> , muscle injuries occurred three times more likely in <i>Phase 2</i> , five times more likely in <i>Phase 3</i> , and over six times more likely in <i>Phase 4</i> per 1,000 person-days	

Study Characteristics of Included Studies

3.5 Meta-Analysis Results

The common-effect model meta-analysis revealed a pooled Risk Ratio of 1.18 (95% CI: 0.86 to 1.60, z = 1.03, p = 0.30), suggesting no statistically significant difference in injury occurrence between the luteal and follicular phases. The random-effects model, which accounts for between-study heterogeneity, produced a similar pooled RR of 1.18 (95% CI: 0.75 to 1.86, z = 0.73, p = 0.46), further indicating the absence of a significant association between the menstrual cycle phase and muscle injury occurrence. The results of the meta-analysis are visualised in Figure 3.

The heterogeneity across studies was moderate, with an I² of 49.5% (95% CI: 0.0% to 85.3%) and a tau² of 0.08 (95% CI: 0.00 to 7.21). Cochran's Q test for heterogeneity yielded a Q value of 3.96 (d.f. = 2, p = 0.14), indicating that the observed variation across studies could be due to chance rather than true heterogeneity.

The leave-one-out sensitivity analysis to examine each study's influence on the overall results elicited varying RRs from 1.01 to 1.40, with corresponding 95% CIs that consistently overlapped and included the null value (RR = 1.0), indicating no significant effect. The pooled RR increased slightly to 1.22 (95% CI: 0.50 to 2.98, z = 0.43, p = 0.67) and the highest heterogeneity was observed (I² = 74.6%, Q = 3.93, d.f. = 1, p = 0.05) with the exclusion of the Lago-Fuentes et al. paper [56], suggesting a significant impact on the overall results. In contrast, the lowest heterogeneity was observed with an I² of 3.6% (Q = 1.04, d.f. = 1, p = 0.31) with the exclusion of the Barlow et al. study [58], resulting in a pooled RR of 1.01 (95% CI: 0.71 to 1.45, z = 0.07, p = 0.94).

Figure 3



Study	logRR	SE(logRR)	F	Risk R	atio	RR	95%-CI	Weight (common)	Weight (random)
Lago-Fuentes et al. [56] Martin et al. [57]	0.1366	0.2137		*	<u> </u>	1.15	[0.75; 1.74] [0.41; 1.46]	53.8% 23.6%	42.6% 29.0%
Barlow et al. [58] Common effect model Random effects model	0.6568	0.3293	r	- W		- 1.93 1.18 1.18	[1.01; 3.68] [0.86; 1.60] [0.75; 1.86]	22.6% 100.0%	28.4% 100.0%
Heterogeneity: $I^2 = 50\%$, τ^2	² = 0.0798	, p = 0.14	0.5 Greater Rit Follicular Ph	1 sk ase	2 Greater Risk Luteal Phase				

4 DISCUSSION

This systematic review and meta-analysis sought to elucidate whether the menstrual cycle and its phases have a significant impact on the occurrence of muscle injuries in eumenorrheic female team sport athletes. The premise that there might be variability in muscle injuries across the menstrual cycle is grounded in previous findings on the effects of the inherent hormonal fluctuations on musculoskeletal function introduced at the beginning of this review, including altered soft tissue plasticity [38–40], impacted collagen metabolism [29, 30], and potentially distorted neuromuscular control [41–43] and proprioception [45].

Despite the biological and theoretical plausibility, the current meta-analysis did not find a statistically significant difference in the distribution of muscle injuries between the follicular and luteal phases of the menstrual cycle. The pooled Risk Ratio of 1.18, derived from both fixed-effect and random-effects models, indicates a non-significant association between the menstrual cycle phase and the instance of muscular injuries. This suggests that while hormonal fluctuations may influence musculoskeletal properties, these changes may not translate into a measurable difference in injury risk across the menstrual cycle. Notably, this conflicts with the athletes' perception, as over half of female athletes report a changed perceived risk of injury throughout the menstrual cycle [59]. When examining the RRs and conclusions from the individual studies, discordant and conflicting results become apparent, mirroring previous research predominantly centred on ACL injuries with similarly inconsistent findings [27, 28]. While these findings provide valuable information and appear to correspond loosely with existing evidence from similar research, they should be approached cautiously due to several significant limitations in the current literature and this review.

The two paramount limitations are (a) the inconsistency and dissimilarity in the classification of the menstrual cycle phases across scientific literature and (b) the methods used by the included studies to detect and verify the menstrual cycle phases.

The inconsistent classification of menstrual phases across studies poses significant challenges for comparing and interpreting findings. There is considerable variation in how the menstrual cycle is divided, with differences in the number of phases, terminology, duration, and specific time points assigned to each phase. This variety is visualised in Figure 4. As a result of this, events occurring at the same specific time point in the menstrual cycle might be interpreted

and reported in completely unrelated and conflicting phases across the scientific literature. This issue is exacerbated when attempting to synthesise evidence and data across studies, as the heterogeneity introduced by different phase definitions complicates meta-analytical approaches, increasing the risk of biased, erroneous, or misleading findings.

Figure 4





Note. The first three studies were included in the meta-analysis. *Classification scheme for the statistical analysis of this review. **Classification scheme of McNulty et al. [22].

In order to statistically synthesise and analyse the data of the included studies for this review, all respective phase classifications were aligned to a two-phase system, creating a follicular phase and a luteal phase. While this allowed for statistical analysis across the findings of the included studies and aligns with the majority of previous menstrual cycle research, which typically distinguishes and compares these two phases [51], it is vital to note that it does not accurately reflect the complex dynamic of the typical menstrual cycle, involving multiple fluctuations in oestrogen and progesterone levels at various stages.

Given the non-linear relationship between circulating hormones and their physiological effects, these distinct changes in the hormonal milieu and specific hormonal peaks may have substantial impacts on key biological factors related to injury risk, including muscle proteostasis and tissue stiffness [60]. This may be further magnified by evidence that mRNA and protein levels of oestrogen and progesterone receptors vary across the course of the menstrual cycle [61]. Thus, reducing the menstrual cycle to unsuitably broad and generalised phases, as was possibly done for the analysis, may overlook critical hormonal fluctuations and their potential effects.

Furthermore, the identification and verification of the menstrual cycle phases in the included studies were substantially based on self-reported data and calendar-based counting methods, which are inherently prone to inaccuracies. These methods, while convenient and considerably more cost-effective compared to hormonal assays, rely on temporal estimates and are susceptible to miscalculations and fallacious reflections of an individual's menstrual cycle due to a wide range of factors, including inter- and intra-individual variability in the cycle length and ovulation timing, as well as further unrecognised variability in the cycle induced through stress, exercise, illness, and changes in body weight [62–64]. Additionally, these methods typically define menstrual cycle phases based on self-reported data regarding the onset of menstruation. However, it is essential to recognise that the occurrence of regular menstrual bleeding does not necessarily indicate an ovulatory cycle with the corresponding typical hormonal course [51, 65]. As a result, the sole use of calendar-based methods for phase identification is considered insufficiently accurate for reliably determining the menstrual cycle phases [51].

Several confounding factors may have obscured potential associations between menstrual cycle phases and muscle injuries. These possible confounders include, but are not limited to, variations in training load, cumulative training and game exposure, psychophysiological influences such as stress and fatigue, and, most notably, the athletes' previous injury history, given that previous muscle injury is widely recognised as the most significant risk factor for future muscle injury [66]. This complexity is exemplified by Lago-Fuentes et al. [57], who observed a higher incidence of injuries during the season's first quarter, attributing this to increased training load during that period. Further, they reported that over 60% of injuries occurred in the later stages of training sessions, highlighting the possible role of fatigue.

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Moreover, the Lago-Fuentes et al. study [56] collected data over two full seasons but only excluded and controlled for hormonal contraceptives in the second season. The relatively low percentage (5%) of excluded hormonal contraceptive users in the second season might suggest a limited impact on the data from the first season and ultimately led to the study's inclusion after consensus-based discussion. However, it is important to acknowledge that a small, albeit unknown, proportion of data from participants using hormonal contraceptives was included in this review's final analysis.

Similarly, the authors cannot guarantee that all injury data were derived from non-contact events. While muscle injuries, particularly strains and tears, are typically attributed to intrinsic factors, the injury mechanism, stemming from excessive tensile or shear forces leading to the failure of muscle fibres and their surrounding connective tissue, can also result from external forces such as traumatic contact [66]. Any muscle injuries caused by external contact included in this review might have skewed the results.

The findings and limitations of this review highlight the need for more robust and welldesigned research to explore the relationship between the menstrual cycle and muscle injury risk. Future research should prioritise the use of hormonal assays, such as serum measurements of oestrogen and progesterone, as well as urinary ovulation detection kits, to accurately verify menstrual cycle phases. Adopting the methodological recommendations, guidelines, and protocols established by DeJonge et al. [51] to strengthen quality across the scientific body. In addition, the classification scheme of the menstrual cycle needs to be standardised to simplify evidence synthesis and mitigate the loss of intricate data details through necessary phase alignment. The authors recommend using the classification system previously used by McNulty et al. [22] (visualised in Figure 4), given that it includes the three most distinctive hormonal profiles of the menstrual cycle, namely, the early follicular phase with low oestrogen and low progesterone, the late follicular phase with high oestrogen and low progesterone, and the mid-luteal phase with high oestrogen and high progesterone, as reported by DeJonge and colleagues [51].

This approach may uncover more subtle associations between hormonal fluctuations and injury risk that were not detectable in the current analysis. It could also clarify the extent to which hormonal effects, such as progesterone's commonly associated stabilising influence on collagen [67, 68] and oestrogen's regulatory role in tissue metabolism [60, 69], contribute to

injury susceptibility in this context. By elucidating these relationships, this approach could serve as a foundation for investigating potential causative links, rather than mere associations, between the hormonal fluctuations of the menstrual cycle and muscular injuries. The authors acknowledge that these recommendations for future research pose substantial challenges, including the invasiveness of procedures, associated costs, and the need for specialised facilities and personnel, which may seem discouraging. However, research in this area is critically needed, and finding a compromise is essential for advancing the field. Based on DeJonge and colleagues' recommendations [51], this review suggests that studies unable to incorporate direct hormone measurements may still achieve sufficient accuracy by employing large sample sizes and using urinary detection kits to measure luteinising hormone.

It is important to recognise that categorising injury events into specific menstrual cycle phases will inevitably involve some degree of approximation, estimation, and retrospective day-counting. However, the absence of direct physiological measurements significantly amplifies the risk of inaccuracies, misrepresentations, and misclassifications, thereby undermining the ability to draw robust conclusions about the influence of menstrual cycle phases on injury occurrence. Ultimately, the accuracy of scientific findings must be solid and reliable, ensuring that their applicability to real-world settings is not compromised by a foundation built on uncertainty.

5 CONCLUSION

This meta-analysis did not reveal a statistically significant association between menstrual cycle phases and muscle injury incidence in female team sport athletes. However, the divergence between this finding and athletes' reports of perceived cyclical vulnerability, along with the limitations of the included studies, suggest that the true relationship may be more complex than currently understood. Although the theoretical basis for hormonal influences on tissue properties is compelling, methodological limitations, most notably inconsistent phase classification and the use of imprecise, self-reported measures for menstrual phase detection, may have masked subtle but clinically relevant effects. Future research in this field is needed and should be obliged to adopt standardised, physiologically accurate methods for detecting and classifying menstrual phases to enhance the comparability of studies and ultimately lead to more effective injury prevention strategies tailored to female athletes.

Contributors YG and RJ designed the research. YG and DS conducted the systematic literature search and screening of the search results, including the two-phase screening process. YG and DS extracted the data. YG performed all the statistical analyses. All authors had full access to all the data in this review and take full responsibility as guarantors for the integrity of the data and the accuracy of the data analyses. YG drafted the manuscript with critical input from RJ and DS. All authors contributed to data interpretation and critically revised the manuscript. The corresponding author (RJ) attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Registration The study was registered at Open Science Framework (<u>https://osf.io/mr6y8</u>)

Availability of Data and Material Please contact the corresponding author (RJ) for data requests.

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Conflict of Interest Yannik Guthardt, Ross Julian, and Debby Sargent declare that they have no potential conflicts of interest with the content of this review.

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