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Attentional, anticipatory and spatial cognition fluctuate throughout the menstrual cycle: potential implications for female sport

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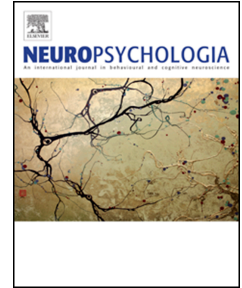
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cognition fluctuate throughout the menstrual cycle: potential implications for female sport

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Current research suggests that menstruating female athletes might be at greater risk of musculoskeletal injury in relation to hormonal changes throughout the menstrual cycle. A separate body of work suggests that spatial cognition might also fluctuate in a similar manner. Changes in spatial cognition could, in theory, be a contributing risk factor for injury, especially in fast-paced sports that require precise, millisecond accuracy in interactions with moving objects in the environment. However, existing theories surrounding causes for increased injury risk in menstruating females largely focus on biomechanical mechanisms, with little consideration of possible cognitive determinants of injury risk. Therefore, the aim of this proof-of-principle study was to explore whether menstruating females exhibit fluctuations in cognitive processes throughout their cycle on a novel sport-oriented cognitive test battery, designed to measure some of the mental processes putatively involved in these sporting situations.

A total of 394 participants completed an online cognitive battery, a mood scale and a symptom questionnaire twice, 14 days apart. After exclusions, 241 eligible participants were included in the analyses (mean: 28 ± 6 years) (male = 96, female(menstruating) = 105, female(contraception) = 47). Cycle phase for menstruating females was based on self-reported information. The cognitive battery was designed to measure reaction times, attention, visuospatial functions (including 3D mental rotation) and timing anticipation. Three composite scores were generated using factor analysis with varimax rotation (Errors, Reaction Time, Intra-Individual Variability). Mixed model ANOVAs and repeated measures ANOVAs were performed to test for between and within-subject effects.

There was no group difference in reaction times and accuracy between males and females (using contraception and not). However, within subject analyses revealed that regularly menstruating females performed better during menstruation compared to being in any other phase, with faster reaction times (10ms c.ca, $p < .01$), fewer errors ($p < .05$) and lower dispersion intra-individual variability ($p < .05$). In contrast they exhibited slower reaction times (10ms c.ca, $p < .01$) and poorer timing anticipation ($p < .01$) in the luteal phase, and more errors in the predicted ovulatory phase ($p < .01$). Self-reported mood, cognitive and physical symptoms were all worst during menstruation ($p < .01$), and a significant proportion of females felt that their symptoms were negatively affecting their cognitive performance during menstruation on testing day, which was incongruent with their actual performance.

These findings suggest that visuospatial and anticipatory processes may fluctuate throughout the menstrual cycle in the general population, with better performance during the menstrual phase and poorer performance during the luteal phase. If these extend to associations between phase-specific cognitive performance and injury incidence, they would support a cognitive theory of determinants of injury risk in cycling female athletes, opening an opportunity to develop mitigation strategies where appropriate.

Key words: injury, timing, anticipation, executive function, reaction time, eumenorrheic

1. Introduction

There has been an encouraging growth in female participation in team sports in recent years, reflected by a dramatic increase in media coverage in women's sport, such as football (soccer) (UEFA, 2022). However, a better understanding of female physiology is still needed for the provision of optimal athlete health support, as only 6% of the sport science literature so far focuses on all-female participant samples (Cowley et al., 2021). The possible impact of the menstrual cycle on athlete health has been of particular interest in recent literature, and still requires much investigation. Much research has investigated possible fluctuations in mood (Le et al., 2020), actual and perceived physical performance (Carmichael et al., 2021; McNulty et al., 2020), and injury risk (Martin et al., 2021) throughout the menstrual cycle, with suggestions that each of these aspects may be improved, or impaired, in specific phases, albeit with varied and sometimes contradicting results. A separate body of work, in the field of neuroscience, also reports changes in cognition and brain function throughout the menstrual cycle (Dubol et al., 2021). However, to date, there has not been an investigation of whether sport-related cognitive functions, specifically, fluctuate throughout the menstrual cycle. Therefore, this proof-of-principle study sought to investigate whether sport-related cognition fluctuates throughout the menstrual cycle, and whether sport participation and expertise might mediate such processes.

1.1. Sporting performance and injury risk in the menstrual cycle

The eumenorrheic menstrual cycle is characterised by a fluctuation in hormones over an average 28-day cycle (range 21-35 days), including oestrogen, progesterone, luteinising hormone (LH) and follicle stimulating hormone (FSH). The natural changes of hormone secretion, in particular oestrogen and progesterone, drive the main phases of the menstrual cycle. These include key phases such as: menstruation (days of bleed, low oestrogen and progesterone), the late follicular phase (oestrogen rises and peaks just before ovulation), ovulation (typically occurs mid-cycle) and finally the luteal phase (second half of the cycle), where the mid-luteal phase is characterised by high oestrogen and peak progesterone levels (McNulty et al., 2020).

Female athletes often perceive that their performance on physical tasks changes throughout their cycle, with perceived worse performance predominantly reported during the premenstrual and menstrual phases (Brown et al., 2021; Carmichael et al., 2021; Martin et al., 2018). Objective research in this field is still scant and provides variable outcomes, with trends that demonstrate worse or best performance in different phases according to the metric being measured (for example, strength and power might peak during ovulation Cook et al., 2017, while endurance and strength performance might be worse during menstruation McNulty et al., 2020), but no real conclusive evidence for a group-level detriment to performance in a single phase (Meignié et al., 2021), and a strong emphasis on inter and intra-individual differences, where some females are more strongly affected than others (McNulty et al., 2020; Colenso-Semple et al., 2023).

In addition, literature on injury prevalence in sport provides some objective evidence to support this where, in female team sports, multiple studies have found injury incidence to differ across the menstrual cycle (Martin et al., 2021). Considering that more than half of elite female athletes are naturally cycling (Martin et al., 2018; Barlow et al., 2023), the possible impact of the menstrual cycle on injury incidence and performance warrants further investigation. Although it is not clear that one specific phase may be associated with general injury incidence, evidence suggests a higher probability of certain types of injury in either the ovulatory or luteal phases compared to the menstruation and mid-follicular phase, with variable findings depending on the type and cause of injury (Martínez-Fortuny et al., 2023; Martin et al., 2021). For example, a particularly higher prevalence of non-contact muscle injuries (Barlow et al., 2023) and concussions (La Fontaine et al., 2019) have been reported in the luteal phase; while ACL injuries are reportedly least common in the luteal phase (Herzberg et al., 2017) and most common in the late follicular or ovulatory phases instead (Martin et al., 2021; Chidi-Ogbolu and Baar, 2019). Such effects have been reported in the general population (Balachandar et al., 2017), in mixed recreational and professional contact sports (Martínez-Fortuny et al., 2023) and in professional footballers specifically (Martin et al., 2021), suggesting that competitive level and sporting expertise might not be a protective, nor detrimental, factor. Together, these reviews suggest that hormonal changes in the

menstrual cycle and their associated effects might be linked to changes in injury risk in particular phases, with potentially differing mechanisms by phase and injury type.

In sport medicine literature, the most researched injury type in this context are anterior cruciate ligament (ACL) injuries. Some authors have hypothesised that changes in joint laxity and poorer neuromuscular control during ovulation could be driving the observed increase in ACL injuries in this phase (Chidi-Ogbolu and Baar, 2019; Balachandar et al., 2017; Herzberg et al., 2017). However, while knee joint laxity has been substantiated as a significant risk factor for ACL injuries in the general population (Zsidai et al., 2022; Sundemo et al., 2019) and in female athletes (Myer et al., 2008), a direct link between possible cycle-related changes in laxity and injury prevalence has not yet been established (Raj et al., 2023; Dos Santos et al., 2023; Martínez-Fortuny et al., 2023). In addition, joint laxity itself would not explain the reported prevalence of other forms of injury in other phases, nor the higher prevalence of events such as concussions and non-contact muscle injuries observed, for example, during the luteal phase (Barlow et al., 2023; La Fontaine et al., 2019). Furthermore, while changes in neuromuscular activation, strength and power throughout the menstrual cycle have also been reported, they too present several conflicting findings, with significant inter-individual variation, meaning that some females are more strongly affected than others (Carmichael et al., 2021; Cook et al., 2017; Blagrove et al., 2020). Overall, while these proposed cycle-specific and phase-specific mechanisms of injury risk and altered performance are all plausible, the experimental evidence for neuromuscular or biomechanical factors as predisposing for injury risk in the cycle is not conclusive (Dos Santos et al., 2023). It is therefore worth considering additional putative explanations to the reported changes in sporting performance and injury incidence in this context, particularly given the complex and multifactorial nature of sporting injuries. This paper, therefore, seeks to explore whether possible fluctuations in sport-related cognitive processes might also exist, providing a broader picture to such processes.

1.2. Sport-related cognition, expertise and the menstrual cycle

This study does not seek to discover the full possible mental changes that may or may not accompany the menstrual cycle: there are many excellent that provide this (e.g. Kowalczyk et

al., 2023; Le et al., 2020; Sundström Poromaa and Gingell, 2014). Instead, it aims to explore cognitive processes which might simultaneously (a) change with the menstrual cycle; (b) be relevant to sporting situations, especially those where injury might occur; and (c) be related to sporting expertise. Sporting expertise is included as an exploratory variable given its potential association with cognitive performance on sport-related cognitive processes.

To the authors' knowledge, there is no agreed set of cognitive processes that meet these three criteria. Therefore, this proof-of-principle study focused on selected cognitive measures that have been suggested, based on existing literature, to be relevant to sporting processes and which might also be sensitive to changes in the menstrual cycle. Practical experimental concerns have also been considered in their development, including the ability to administer the tests online, and to yield psychometrically suitable output within a target population of young healthy adults. A rationale for the selection of each cognitive subdomain is outlined below, while explanations pertaining the development of each task are included in the methods section.

1.2.1. Timing and processing speed

Existing research has only begun to explore the impact that the central nervous system may play in sport-related cognitive performance throughout the menstrual cycle. In particular, higher order cognitive processes such as executive function have largely not yet been considered. However, in separate bodies of literature, poorer executive functioning has been associated with increased injury incidence in both male and female athletes in prospective studies (Yamada and Matsumoto, 2009; Swanik et al., 2007), while sex hormones and the menstrual cycle have been sometimes associated with changes in inhibition and spatial cognition (Bernal and Paolieri, 2022; Pletzer et al., 2019; Sundström Poromaa and Gingell, 2014). It is therefore perhaps surprising that executive function has been given such little consideration as a possible mechanism of injury risk or altered performance during play. Furthermore, to the authors' knowledge, there has been no attempt so far to explore the possibility that sport-related cognitive processes might fluctuate throughout the menstrual cycle. If this is shown to be the case, and if such processes are indeed different in phases of the cycle that are typically related to sport-related injuries, then it's possible that these might

play a role as an underlying cause of injury risk in female athletes, or changes in performance during play.

It is worth emphasising that the sport science literature highlights a role for poorer cognition in increasing the incidence of sporting injuries. Concussion, for example, is a well-established determinant of injury risk, where athletes with a history of concussion are at least twice as likely to sustain a musculoskeletal injury (McPherson et al., 2019; Brooks et al., 2016), likely due to relative impairments in cognitive processes which are consequent to head impacts. Even more directly relevant to the current study, perhaps, is that slower processing speeds on computer-based assessments of executive function have been linked to a greater incidence of non-contact ACL injuries (Swanick et al., 2007) and lower limb sprains and strains (Wilkerson, 2012) in male and female athletes who had not suffered previous head injuries. Therefore, it is plausible that a relationship between slower cognitive processing speeds and increased injury exists, even in healthy athletes. This is likely due to the impact that an erroneous timing of movement, in the order of tens of milliseconds, can have on the outcome of a collision, as head impact accelerations in sport typically occur in 6–18 ms (Austin et al., 2023; Jones et al., 2023). Reaction times or motor speeds may intersect with processes involved in spatial anticipation (Loffing and Cañal-Bruland, 2017), defined as the mental processes involved in calculating the likely relative positions of two or more objects in relation to each other (e.g. when a player heads a ball crossed by another player into the goal).

Finally, it should also be considered that reaction times are typically reported to be faster in athletic populations compared to nonathletes (Hülsdünker et al., 2018), although a causal direction to this relationship has not yet been established. Nonetheless, a review on anticipation in sport by Williams and Jackson (2019) highlighted that experts typically exhibit better anticipatory processes through the integration of peripheral and foveal vision, and that perceptual training may improve such performances. Indeed, some of the likely cognitive functions involved in sports are directly relevant to complex time-critical dynamic decision-making (e.g. Lex et al., 2015; Holmes and Wright, 2017; Ottoboni; Yarrow, et al., 2009). Further, while deliberate practice might potentially play a role in the development of faster reaction times and improved task-specific performance (Ericsson, 2020; Ericsson et al., 1993), physical exercise, and the greater physical fitness associated with sport participation may also

play a role in such associations, as demonstrated by previous work from this group in both adults (Crum et al., 2022; 2024) and children (Watson et al., 2024).

Thus, tests of reaction time and processing speed, together with tests of spatial anticipation, might be particularly cogent to the current application, and were therefore included in the battery of tasks.

1.2.2. Attention and inhibition

Another cognitive construct which one might consider in terms of injury-proneness is that of sustained attention. Errors of commission and omission in sporting situations are often described as being secondary to a “lapse of attention”, and there is evidence for a link between “distraction attention” and sports injuries, albeit the direction of causation is unclear (Gray, 2015; Gkikopoulos et al., 2020). Attention has been somewhat researched in the context of the menstrual cycle, with conflicting results (Kowalczyk et al., 2023; Souza et al., 2012), with some studies reporting worse performance on attention tasks during the luteal phase, and others no significant difference (Souza et al., 2012). However, these studies adopted attention and executive function tasks such as the Stroop, the Brief Attention Task and the Mental Dice Task, whereas the task developed in this study (described below in the methods section) aimed to measure specifically the ability to maintain focused attention for a prolonged non-response period (Stay stimuli), followed by a rapid execution (Go stimuli), which might relate particularly more closely to the concept of ‘readiness’ in sport.

While reaction time speed, and the ability to maintain close attention to stimuli have immediate face validity when considering fast-moving dynamic situation such as in ball sports, inhibition also finds its relevance in restraining or controlling one’s behaviour in competitive situations. More specifically, motor inhibition is more directly involved in play, for instance in switching from execution to restraint in fast-paced situations (e.g. switching from attacking to defending in a confrontation, having to change direction in a team sport). A recent meta-analysis has found athletes to perform better in Go-NoGo tasks than non-athletes, although the direction of the causality remains to be determined (Albaladejo-Garcia et al., 2023). In relation to the menstrual cycle a meta-analysis by Kowalczyk et al. (2023) reported that the

global effect of the menstrual cycle on executive function was not significant, although inhibition was the domain that showed stronger effect trends ($\beta = .16$, 95%CI $-.07 - .39$), particularly for the luteal phase.

1.2.3. Spatial cognition

Turning to considerations of cognitive functions beyond information processing speed, sustained attention and inhibition, there is a body of literature separate to sport where there has been much interest on the effects of sex hormones, and the menstrual cycle, on the brain. Changes in cognition, regional brain connectivity, blood flow and even brain structure have been investigated, and the complexity of differential effects of hormonal interactions and individual differences on cognition have been largely emphasised in comprehensive reviews (Dubol et al., 2021; Beltz and Moser, 2019). Of these, there is some evidence to suggest hormonal or menstrual cycle related fluctuations in 3D spatial ability (Farage et al., 2008) and navigation strategy (Pletzer et al., 2019), but not in other forms of spatial tasks (Hausman et al., 2000; Phillips and Silverman, 1997). Mental rotation has gained the most attention in this field, yielding somewhat inconclusive results when the literature is considered collectively: most studies point towards better mental rotation performance in the early follicular phase, although an early meta-analysis deemed the pooled effect to be non-significant (Sundström Poromaa and Gingell, 2014) and it is currently less clear if there is a phase that exhibits worst performance (Bernal and Paolieri, 2022). Recent reviews have highlighted that inconsistencies between studies may be due to small sample sizes adopted in several papers, to methodological differences in both cognitive testing and menstrual cycle tracking, and to the high degree of individual variability in hormonal changes and their respective effects (Kowalczyk et al., 2023; Bernal and Paolieri, 2022). Of the early studies that do report significant effects, various elements of spatial cognition most consistently point towards better performance during the menstruation phase compared to either the ovulatory or mid-luteal phase (Maki et al., 2002; Hausman et al., 2000; McCormick and Teillon, 2000; Phillips and Silverman, 1997). In contrast, a more recent large-scale study by Shirazi et al. (2021) reported that 3D mental rotation accuracy was better when participants had higher progesterone levels (which is characteristic of the mid-luteal phase). In summary, while spatial cognition has received much attention in the menstrual cycle literature, there is no

clear consensus on whether a specific phase can be related to 'worse performance', but rather it is possible that strategic approaches might differ by phase instead.

In relation to sport and expertise, tasks such as 3D mental rotation likely capture certain aspects of cognition utilised in many sports (e.g. visualising the position of one's limb, the ball, other players and the goal, in a constantly changing environment), and have been shown to relate to a greater injury incidence in one prospective study (Yamada and Matsumoto, 2009). Various versions of the Vandenberg & Kuse (1978) 3D mental rotation task have been used to study the relationship between sporting participation or expertise, and mental rotation with mixed results. For example, both gymnasts and team sport athletes were reportedly faster but not more accurate than non-athletes on such tasks (Ozel et al., 2002), but when gymnasts were presented with human figures as stimuli instead of cubes, they were also more accurate than non-athletes (Jansen and Lehmann, 2013). In team sports, football players were only more accurate than non-athletes if they played at least 4 hours a week (Jansen et al., 2012; Jansen and Lehmann, 2013), and elite basketball players were more accurate than non-athletes, in a study where the stimuli presented consisted of basketball court schematics (Weigelt & Memmert, 2020). Therefore, athletes who engage in spatial processes as part of their expertise may indeed have better mental rotation performance than non-experts, and this appears to be specific to familiar stimuli. What is not clear, however, is the causal direction of the relationship between mental rotation performance and engaging in sporting endeavours that require it. Accordingly, the case for inclusion of a mental rotation task in the current study was made on the grounds of: a possible researched link with menstrual cycle fluctuations, its face validity for its application in sport-related cognitive processes and researched association with sporting expertise, and its possible association with greater injury incidence.

1.3. Study objectives

In summary, there is evidence, from separate bodies of work, to suggest that: female athletes might be at greater risk of sporting injuries during specific phases of their cycle depending on injury type; that poorer executive functioning might contribute to increased risk of injury in

athletes; and that there might be cyclical changes in executive function throughout the menstrual cycle. Together, these findings suggest that a cognitive determinant of injury risk might exist in cycling females, and warrants investigation. However, it has not yet been established whether sport-related cognitive domains do indeed change throughout the menstrual cycle, and whether sporting expertise might relate to such processes in this specific context.

To address this, a battery of tasks was developed here on the grounds of their prima facie construct relevance to: the cognitive processes involved in complex dynamic sporting play (especially with ball sports); to evidence of possible variation with the menstrual cycle; to previous evidence of alteration as a function of physical exercise; and to possible associations with sporting injuries. From the foregoing review, the candidate cognitive processes selected for this study included tests of attention and executive function, spatial cognition and spatial anticipation, especially ones that are speed- or timing-based.

Therefore, the aim of this proof-of-principle study was to identify whether cognitive control, spatial cognition and timing anticipation fluctuate throughout the menstrual cycle. Based on previous literature, we hypothesise that performance on these cognitive tests might be worse during the late follicular (peak oestrogen) or luteal (high oestrogen, peak progesterone) phases in naturally cycling female participants. A secondary exploratory analysis evaluated whether sporting expertise and experience related to performance on these tasks.

2. Methods

2.1. Participants

Participants were recruited via convenience and snowball sampling ($n = 84$), and the online Prolific research platform using stratified sampling targeting 18-35 year-old males and females ($n = 320$). Prolific participants were pre-screened for age, sex, contraception use and existing neurological conditions; they were UK-based and compensated at £10 per hour. In addition to the target sample of naturally cycling females (described below), males and females on hormonal contraception were recruited to provide control comparisons. Participants were excluded by age (≤ 18 and ≥ 35 years old), irregular menstruation,

amenorrhea (absence of menstruation), use of non-contraceptive hormones (e.g. hormone therapy), perimenopause, pregnancy or breastfeeding currently or in the last three months, or neurological complications. A power calculation was conducted to determine the total sample size required at $\alpha = .05$, power = .80 and effect size = 0.25 (based on effects sizes used in research on the effects of drug administration on cognitive function, Nevado-Holgado et al., 2016). A sample of 216 was required for between group comparison of 6 groups (4 phases, male, contraception groups), and a sample of 54 per cycle phase was required to conduct a two-way within-subjects ANOVAs. Ethical approval was granted by the UCL Research Ethics Committee (13985.007) in line with the declaration of Helsinki, and all participants provided informed consent prior to taking part in the study.

2.2. Study design

On their first test day (T1), participants completed an online questionnaire to provide demographic information, sporting participation and competitive level, use of hormonal medication or contraception and cycle characteristics (if female). Immediately after this, participants completed an online battery of cognitive tests, described below in the order in which they were administered, with respective justifications for their inclusion. These were followed by subjective reporting of mood and symptoms. Participants received an email 14 days later with a reminder to complete the cognitive battery, mood & symptoms questionnaire a second time (T2). It should be noted that not all participants completed the second testing phase exactly 14 days after the first one. Data was included in within-subject repeated measures analyses as long as participants responded within 21 days, and did not test in the same self-reported phase twice. The Gorilla Experiment Builder (www.gorilla.sc) was used to create and host the entire experiment.

2.3. Sporting participation information

Participants were asked about their participation in sport, and their competitive level, through an online demographic questionnaire. To quantify frequency of participation in different forms of physical activity, participants were asked how frequently they took part in sports of the following categories: endurance (such as running, rowing, cycle), power or strength sports (such as weight lifting, sprinting), ball sports (such as football, rugby tennis),

fight sports (such as boxing, karate), coordination sports (such as martial arts, gymnastics), fine skill sports (such as darts, snooker). Participants rated their frequency of participation for each as: 1 = never, 2 = less than once a month, 3 = 1-2 times a month, 4 = once a week, 5 = 2-4 times a week, 6 = 5-7 times a week, 7 = I am a highly trained athlete in this sport.

To quantify sporting expertise, participants were asked if they had ever regularly taken part in a sport; they specified which sport this was (e.g. rugby, cycling, football, etc...) and provided details on their participation or competitive level (recreational or competitive at club, regional or state level - there were no international competitors in this sample). Based on the assumed relevance of the cognitive test battery to ball sports in this study, participants were grouped by broad sport type (no sport, ball sports, other sports) and by competitive level (sedentary, recreational, competitive) for analysis.

2.4. Cycle regularity and phase categorisation

Participants were asked if they tracked their cycle using apps for at least the last three months prior to the study and thus were asked to provide their current predicted phase if known. In addition, naturally cycling females were asked to provide their current day of cycle (first day of bleed = 1) and the number of cycles they typically experience in a year, which was used to calculate their average cycle length. After excluding participants for polymenorrhea (>17 cycles per year), oligomenorrhea (<10 cycles per year), irregular cycles (self-reported) and spotting (>3 times a year), 73% of the remaining 105 naturally cycling participants reported using app-based cycle tracking, but only 67% reported a cycle phase provided by their app. For all remaining participants who did not report a predicted phase, menstrual cycle phase was estimated as follows. Early menstruation was determined as days 1-3 and referred to hereafter as the menstruation phase. The length of the follicular phase was calculated as $0.770(\text{mean length cycle}) - 7.685$ (Martin et al., 2021 McIntosh et al., 1980); all days from day 4 of the cycle to the end of the calculated follicular phase were labelled as follicular, except the last three days. The last three days of the calculated follicular phase were labelled as the predicted late follicular phase. The remaining time period after the predicted late follicular phase was labelled as the luteal phase. It should be emphasised that all phase categories used

throughout the paper have been largely estimated based on participant cycle tracking methods and not confirmed through hormonal testing.

2.5. Contraception information

Females using hormonal birth control were asked about contraception type (combined pill, progestin-only pill, implant type, etc) and phase (active, inactive pill). Participants were further grouped into two sub-categories according to exogenous hormonal concentrations. Of those who were included, 27 participants reported using combined hormonal contraception (18 using the 21-day monophasic combined pill, 4 using the 28-day monophasic combined pill, 2 using the 28-day multiphasic combined pill, 1 using the patch and 1 using the vaginal ring), and 20 reported using progesterone only contraception (8 using the progesterone only mini-pill, 5 using the hormonal intra-uterine device, 5 using the implant, 2 using the injection. Participants were only included repeated measures analyses if they tested while in an active hormonal contraception phase in both sessions due to the hormonal fluctuations that can occur during pill breaks (Curtis et al., 2006). It should be cautioned that within these sub-categories there is still much room for opposing effects of the exogenous hormones based on formulations and concentrations, and therefore any findings reported on this group should be considered with caution.

2.6. Cognitive tests

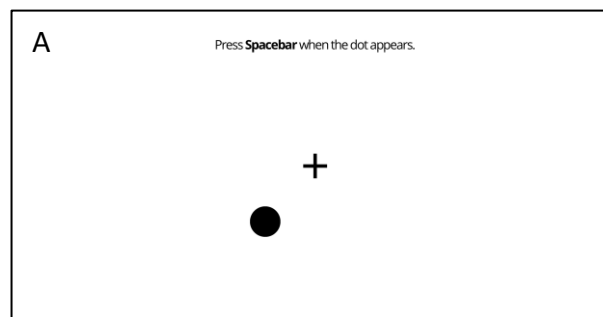
2.6.1. Spatial simple reaction time

The spatial simple reaction time task was a measure of simple response time which included a spatial element by varying the position of the stimulus on the screen (Figure 1). Participants were instructed to stare at a fixation cross throughout the task. A black dot would appear on the screen at fixed pseudorandomised inter-trial intervals (ITIs) and positions, at which point the participant would press the spacebar on their keyboard as quickly as possible. The dot stimuli appeared within a radius of 7 - 14.5 cm from the fixation cross, ensuring that the angle subtended between the eye (horizontal line), the fixation cross and the stimuli would be within 30 degrees of the central visual field (Spector, 1990), even when seated at a minimum of 0.4 m from a computer screen, in case a participant sat too close to the screen. Ten dot

variants were created to include all four angles of the screen (top, bottom, right and left) at both distances (far, close), and two on the horizontal line (close).

A total of 50 stimuli were presented in a fixed pseudorandomised order as follows. ITIs (milliseconds) were paired in such a way that each ITI always appeared once after each of the five used ITIs (e.g. 400-400, 400-600, 400-900, 400-1350, 400-2025). This resulted in 25 pairs. These pairs were then randomised within the first half of the task such that each pair appeared once over 25 stimuli, and that each ITI appeared five times. The pairs were randomised again in the second half of the task in a different order. Each of the ten dot variants were then paired with each ITI, such that each variant appeared once every 10 stimuli, with a different ITI every time across the 50 stimuli. Commission errors were defined as the number of times the spacebar was incorrectly pressed during the ITI. Mean reaction time was calculated as the mean of all correct responses after removing any response faster than 125 ms. Intra-individual variability was calculated as the standard deviation of all correct reaction times.

This spatial simple reaction time task was included in the battery in order to identify whether proposed fluctuations in spatial cognition described in the literature might be associated with attentional changes in monitoring a visual scene. This was contrasted by the following simple reaction time task which did not include spatial variation when stimuli appeared on the screen.



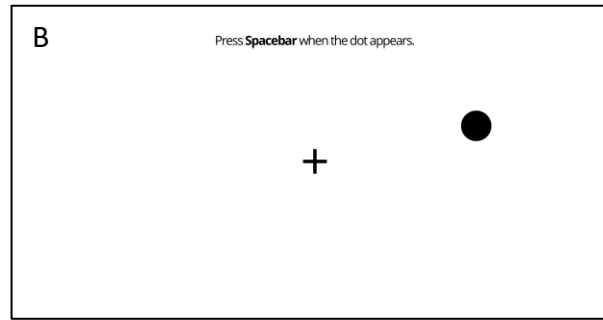


Figure 1 – Example stimuli of the spatial simple reaction time task. Examples of two of the ten stimuli variants are shown: A) close, bottom, left and B) far, top, right.

2.6.2. Smiley task battery

The simple reaction time (SRT) task, the sustained attention task (SA) and the inhibition task were developed as a triad of complementary tasks, as simplified versions of the battery used by Gilbert et al. (2006) and Volle et al. (2011) for functional neuroimaging and neuropsychological investigations of executive function. The stimuli adopted in this version used simplified “smiley face” ideograms in black and white in an attempt to reduce potential non-executive processing confounds related to individual differences in cultural, societal or age effects.

The order of the tasks was designed to maximise their construct validities, and were administered in the same order to all participants. This order was: Simple Reaction Time (SRT); Sustained Attention (SA); Inhibition (Figure 2). The SRT task established an indiscriminate motor response to any presented stimuli, the SA task established a learned response to press only at the “winky” face, and then the inhibition task required an inhibition of this learned response, i.e. not to press at the winky face. In essence, the SRT task is a contrast to the SA task, which is a contrast to the inhibition task. In practice, presenting the tasks in this fixed order means that the Sustained Attention (SA) task feels a little slow and boring after the Simple Reaction Time (SRT) task, thus tapping particularly strongly into the ability to maintain attention to task and to respond selectively to stimuli. Having both the SRT and SA tasks before the Inhibition tasks means that there is an established routine response to target stimuli which then has to be inhibited in the Inhibition condition, thereby increasing the inhibition demands of the final task.

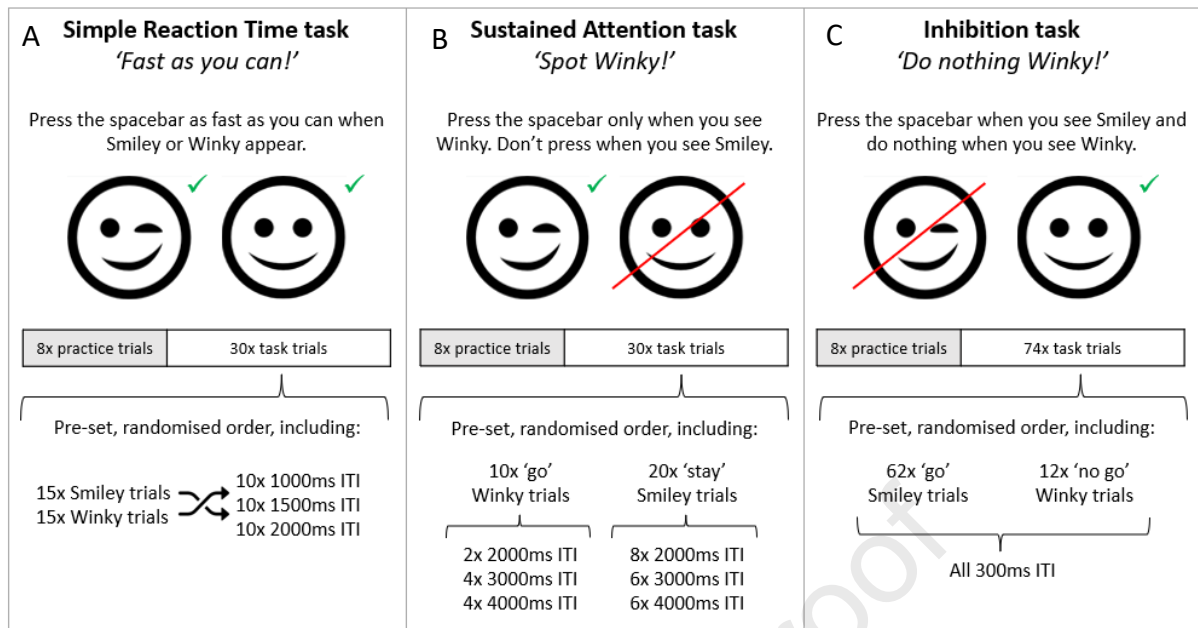


Figure 2 – Example visual of presented stimuli and order of stimulus presentation for the attentional tasks, including A) simple reaction time, B) sustained attention and C) inhibition. Tasks were always presented in this same order.

2.6.3. Simple reaction time

Of the smiley task battery, during the SRT task participants were instructed to press the spacebar as quickly as possible when a smiley or winky face appeared at the centre of their screen (Figure 2A). A total of 30 trials were presented (15 each) with ITIs of either 1000, 1500 or 2000 ms in a pseudorandomised order. Participants completed 8 practice trials before testing. Commission errors were defined as the number of times the spacebar was incorrectly pressed during the ITI. Mean reaction time was calculated as the mean of all correct responses after removing any response faster than 125 ms. Intra-individual variability was calculated as the standard deviation of all correct reaction times.

Simple reaction time was included in the battery in consideration of previous research that reports a relationship between reaction time and injury risk (Wilkerson, 2012; Swanick et al., 2007).

2.6.4. Sustained attention

The sustained attention task was always administered after the simple reaction time task, as explained above, and consisted in a Stay-Go task with extended intervals (Figure 2B).

Participants were instructed to press the spacebar when they saw a winky face, but not when they saw a smiley face. Ten Go trials, where the participant had to press the spacebar (a winky face) were interspersed within 20 Stay trials (or NoGo trials) where they were instructed not to press the spacebar (smiley face), with ITIs of 2000, 3000 or 4000 ms. Trial type and ITI were presented in a fixed but pseudorandomised order. Participants completed 8 practice trials before testing. Commission errors were defined as the number of times the spacebar was incorrectly pressed during a Stay trial (smiley face). Omission errors were defined as the number of times the participant failed to press the spacebar at a Go trial (winky face). Mean reaction time was calculated as the mean of all correct Go responses after removing any response faster than 125 ms.

2.6.5. Inhibition

The inhibition task consisted of a Go-NoGo task, which used the same stimuli as the sustained attention and simple reaction time tests, and was always administered after them, in the order described here (Figure 2C). In contrast to the sustained attention task, here participants were instructed to press the spacebar when they saw a smiley face, but not when they saw a winky face. Twelve NoGo (winky face) trials were interspersed within 62 Go (smiley face) trials, with ITIs of 300 ms; trial types were presented in a fixed but pseudorandomised order. Participants completed 8 practice trials before testing. NoGo errors were defined as the number of times the spacebar was incorrectly pressed during a NoGo trial (winky face). Mean reaction time was calculated as the mean of all correct Go responses after removing any response faster than 125 ms.

2.6.6. 3D Spatial perception

The design of this test was conceptually similar to the Warrington and James (1991) cube analysis test, which is a test typically used to measure visual perception in neurological patients. It involved 20 trials with one 3D object per stimulus. Each stimulus consisted of a drawing of 5-10 adjacent cubes (Figure 3A). Participants were instructed to count the number of cubes contained in the 3D object, providing their answers with arrow keys based on two options, as quickly as possible without making mistakes. Stimuli were presented as forced-choice responses in order to facilitate faster responses and enable the recording of

millisecond reaction times. Three practice trials were provided. The total number of errors was recorded, and mean reaction time was calculated for all correct and incorrect trials together. Most literature on cognition and the menstrual cycle focuses on 3D mental rotation (Bernal and Paolieri, 2022), but does not examine 3D visualisation. The cube analysis task was therefore included to measure the participants' awareness of a static 3D environment, therefore removing the variance attributed to mental rotation. This was included alongside the 3D mental rotation task to facilitate a contrast between the two.

2.6.7. 3D Mental rotation

The 3D mental rotation task was conceptually similar to the one developed by Vandenberg & Kuse (1978). It involved 24 trials with one 3D object as the primary stimulus (Figure 3B). Participants were presented with two other 3D objects, where one or neither represented a 3D rotation of the primary stimulus. Stimuli were presented as forced-choice responses in order to facilitate faster responses and enable the recording of millisecond reaction times. Five practice trials were provided. The total number of errors was recorded, and mean reaction time was calculated overall for all trials (correct and incorrect together).

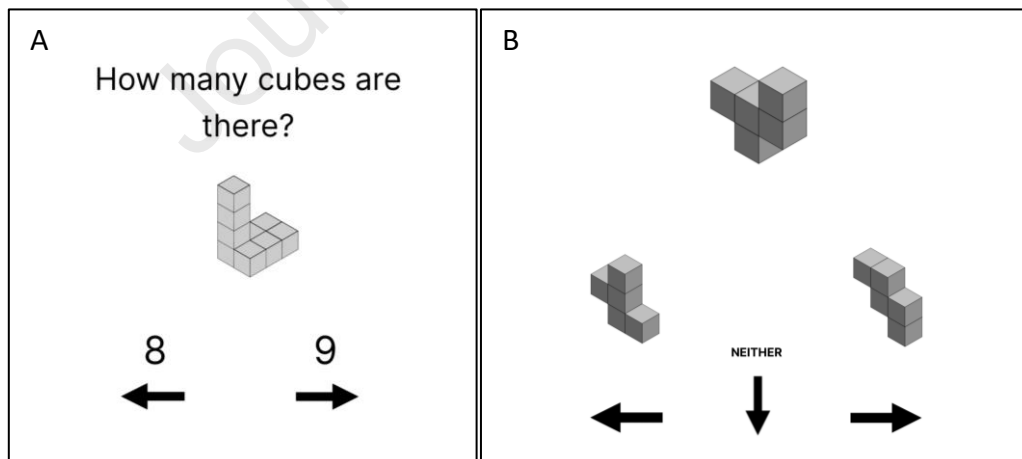


Figure 3 – Example of stimuli presented during the 3D spatial perception task (A) and the 3D mental rotation task (B). In A) participants were instructed to count the cubes that generate the object; in B) participants were instructed to identify which of the two provided answers at the bottom of the screen, if any, represented an identical but rotated object to the one shown at the top. In both tasks, participants were instructed to provide an answer using arrow keys on a computer keyboard as quickly as possible without making any mistakes.

2.6.8. Rhythmic timing anticipation

This was the first of two simple tasks which aimed to measure the cognitive processes underpinning timing anticipation perhaps relevant to sport. (For review of anticipation functions in sport see Williams and Jackson, 2019.)

In this task, a cat appeared in a frame-by-frame fashion at regular intervals in consecutive windows on a horizontal plane (as if walking through a house), either from right to left, or from left to right (Figure 4A). Intervals between frames were kept consistent at either 200ms, 300ms, 400ms, 500ms or 600ms within each trial in order to determine the speed of the cat's movement. Trials were pseudorandomised such that each speed appeared twice, once in each direction, in the first 10 trials and always appeared before or after a different speed. A total of 21 trials appeared in a mirrored order so that the 1st trial matched the 21st, with a place holder middle trial of 325ms ITIs. Participants were instructed to press the spacebar when they believed the image should appear at the final window, marked with a black circle. Three practice trials were provided prior to commencing the test. Timing error was calculated as the absolute difference between the moment participants pressed the spacebar and the moment the image should have appeared in the last window, whether too late or too early. Intra-individual variability was calculated as the standard deviation of all responses.

This task aimed to measure a participant's ability to predict when an unseen object, moving at regular intervals, would appear in a defined location. It should be noted that the frame-by-frame nature of the animation created a rhythmic element to the task which is likely to have influenced the participants' timing strategies. Timing anticipation tasks were included due to their relevance in sports where the accurate timing of moving objects is crucial to both optimal performance and avoidance of injury (Williams & Jackson, 2019). The two contrasting versions of timing anticipation were created in order to separate the participants' ability to time the movement of a single object through rhythm (assessed here) against their ability to anticipate the timing of two colliding objects (assessed in the next task).

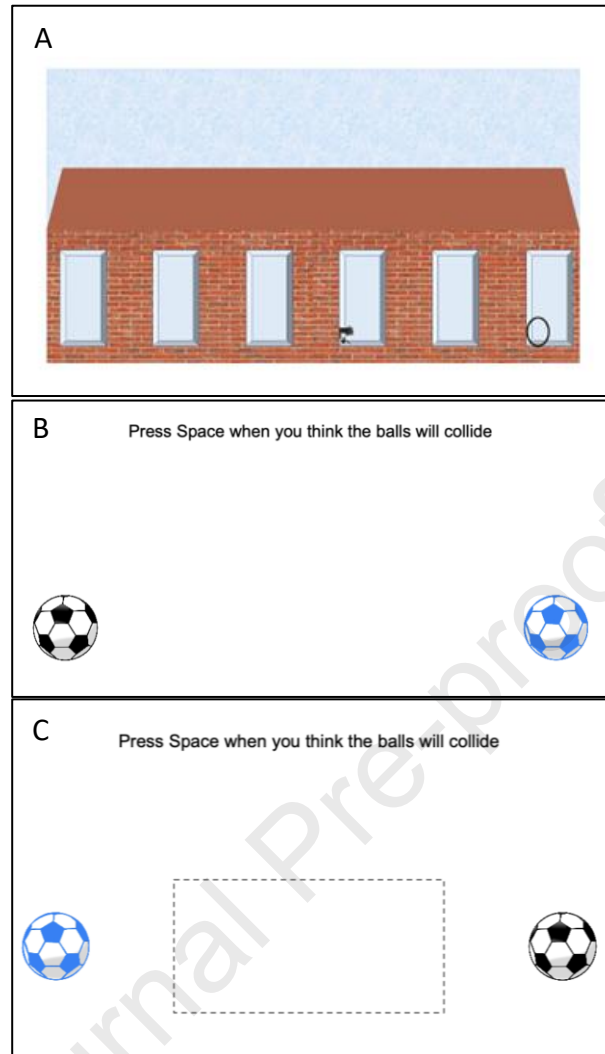


Figure 4 – Examples of the rhythmic timing anticipation (A) and the spatial timing anticipation (B,C) tasks. In A) the cat moved through the windows in a frame-by-frame fashion, participants were instructed to press the spacebar when they thought the cat would appear in the black circle; in B and C the two balls moved towards each other in a smooth animation, participants were asked to press the spacebar when they thought the balls would collide with each other. The balls were always visible in B, but disappeared behind the white box in C.

2.6.9. Spatial timing anticipation

The spatial timing anticipation task consisted of two footballs moving towards each other at a constant speed, on a horizontal line from opposite ends of the screen (Figure 4B). Speeds ranging from 6 to 18 $\text{m}\cdot\text{s}^{-1}$ were implemented, where the two objects moved sometimes at the same speed and sometimes at different speeds. The speeds were selected in line with object speeds commonly experienced in team sports, where the ball exit velocity following a header averages 10 $\text{m}\cdot\text{s}^{-1}$ (Schewchenko et al., 2005) and an instep ball kick from an amateur player can average 18 - 22 $\text{m}\cdot\text{s}^{-1}$ (Isokawa and Lees, 1988). After piloting and reliability testing,

the seven best trials were selected, where the two objects moved at the same speed in three trials (6, 9 and 18 $\text{m}\cdot\text{s}^{-1}$), and at two different speeds in four other trials (10 and 8 $\text{m}\cdot\text{s}^{-1}$, 12 and 6 $\text{m}\cdot\text{s}^{-1}$).

A further manipulation of the task consisted in having the objects remain visible for the entire trial or disappear behind a blank box prior to collision (Figure 4C). The resulting complete task consisted of 29 total trials, with seven visible trials appearing from slowest to fastest, followed by seven disappearing trials from slowest to fastest. The order of trials was then mirrored for the second half of the task, such that each trial occurred twice, once in the first half and once in the second half of the test. Participants were instructed to press the spacebar when they believed the two objects would collide with each other. Nine practice trials were provided prior to commencing the task. Feedback was provided to the participant at each practice trial by showing the ball position at space-bar press. Timing error was calculated as the absolute difference (ms) between the moment the participant pressed the spacebar and the moment the object actually collided on the screen. For the purposes of this study, all trials, visible and disappearing, were included in this calculation. Intra-individual variability was calculated as the standard deviation of all responses.

This task was included due to its relevance in determining the positioning of two moving objects in time and in space, with respect to each other, at different speeds, and not always being visible. The ability to anticipate the position of an object in space and time to millisecond accuracy, when the object is no longer within the visual field, is likely to be highly relevant to performance and injury occurrence (e.g. when timing the interaction of one's limb, a ball and other players; for further review of cognitive anticipation functions in sport, see Loffing and Cañal-Bruland, 2017).

2.7. Composite cognitive scores

For the purposes of clarity and brevity for this proof-of-principle study, three composite scores: Reaction Time, Errors and Variability, are presented here as a first-pass analysis, due to their advantage in ease of interpretability and to reduce the risk of a type I error. The composite scores should be considered the key outcome of the study, while the same

statistical tests on each individual cognitive test have been included for completion, for the reader's interest. Higher values on all scores indicate poorer performance.

To derive the Composite Reaction Time and Composite Error scores, a principal component factor analysis of the correlation matrix was performed, using Varimax rotation. Data from both time periods of testing was utilised on 15 test variables. The two-factor rotated solution yielded a first factor that explained 19% of the variance (eigenvalue 2.85) where all the variables that loaded in excess of 0.5 were reaction time (RT) variables, with the highest factor loadings being simple reaction time mean RT (loading 0.762) and Inhibition 'go' RT (0.759). The second orthogonal factor explained 16% of the variance (eigenvalue 2.41), and all the variables that loaded over 0.5 on this factor were error variables, excepting reaction time measures for the Cube Analysis and 3D mental rotation tasks, both of which showed second factor loadings for the timed variables which were in the opposite direction to the error variables, suggesting a form of speed-accuracy trade-off with these tasks. Together the two factors explained 35% of the variance across all 15 variables. Factor scores for each of these two factors were saved for each participant, and used in analyses. A subsequent factor analysis was performed to create the Composite Intra-Individual Variability factor, running a single-factor solution on the intra-individual variability scores (standard deviation of all correct reaction times) of spatial simple reaction time, non-spatial simple reaction time, sustained attention, inhibition, rhythmic timing anticipation and spatial timing anticipation. This yielded a factor that explained 20% of the variance (eigenvalue 2.17), where the variables that loaded in excess of 0.5 were the non-spatial simple reaction time (0.53), sustained attention (0.51), inhibition (0.59), and spatial timing anticipation (0.50).

2.8. Mood questionnaire

The Burgess Brief Mood Questionnaire was created to measure mood changes most often associated with physical exercise (Burgess and Ronca, in preparation). It consists of 10 mood statements such as "I feel sad" and "I feel alert", where the participant is asked to rate how they are feeling in relation to them on a 1-7 Likert scale. These statements aimed to measure the 5 mood state changes most commonly found from literature review when describing the effects of exercise, with each mood state (e.g. anxiety) assessed by both a negative (e.g. "I

feel nervous”) and a positive (“I feel calm”) statement, in order to try to reduce scale bias effects. When these 10 statements (5 positive; 5 negative) were subject to factor analysis (varimax rotation), two factor scores, Drive (feeling energetic and focused) and Serenity (feeling calm and content), were found (details in Supplement 1). Factor scores for each of the two factor scores were derived separately for the two testing occasions (T1 vs. T2), permitting a within-subject analyses that describes individuals’ relative changes within their peer and time group, rather than absolute mood differences between the two testing occasions. This has the potential advantage of reducing psychometric artefact at the individual level related to, for example, differences in response strategies and biases relating to scale use, making significant results, where discovered, more robust.

2.9. Symptom reporting

Naturally cycling participants were provided with a list of three negative moods (poor mood – such as stress, depression, anxiety; irritability; tiredness) and of symptoms typically experienced throughout the menstrual cycle (Davison et al., 2022). In addition to these, participants were provided with a longer list of cognitive-related symptoms (e.g. headaches, brain fog, irritability) and physical symptoms (e.g. stomach pain, back pain) based on Bruinvels et al. (2021). Participants were asked to select which of these symptoms they thought they typically experienced during menstruation, mid-cycle, and in the week before menstruation (yes/no answer).

After completing the cognitive tests, all participants (male and female) were presented with the same list of symptoms and asked to select any that they were experiencing at the time of testing. Participants were then asked if they believed that their symptoms on the day was negatively affecting their physical or cognitive performance.

2.10. Statistical analyses

All statistical analyses were conducted in R Studio (RStudio Team, 2022). Reliability testing of the cognitive tasks was performed using data from the first testing session of all participants who took part in this study. Internal consistency of the cognitive tasks was assessed using

Guttman bounds from split-half reliability testing (λ_3 and λ_4). Test-retest reliability was tested through intra-class correlations (ICC) on all participants who completed both sessions. For the analysis, all data were tested for normality using the Shapiro-Wilk test. Normalisation of cognitive test outcomes was attempted using Box-Cox transformations where possible. Spearman rank correlations were conducted between sporting level and cognitive performance. Between-subject analyses for all cognitive tests and mood were conducted using a mixed-model ANOVA between males, females using contraception and naturally cycling females, including time as a factor to check for test-retest effects. A further mixed model ANOVA was also conducted, dividing cycling females into groups by menstrual cycle phase. Further exploratory one-way between-group ANOVAs were conducted including primary sport and competitive level as factors to test for effects of sport involvement on cognitive performance. Within-subject analyses to test for phase effects were then conducted on the sample of naturally cycling females only, using a repeated measures ANOVA. Post-hoc pairwise comparisons were conducted with Holm correction to test for effects between groups and menstrual phases, adjusted p values were reported for these. Group differences in total number of reported physical and cognitive symptoms were tested using a Kruskal-Wallis test, with pairwise Wilcoxon comparisons with Holm correction as post-hoc. Assumed impact of symptoms on cognitive performance on the day of testing was assessed using a Chi-square test. Assumed prevalence of poor mood, and assumed impact of symptoms (yes/no response) on performance during each cycle phase (beginning/mid/end) within the menstruating group were analysed using a Chi-square test. A mediation analysis was attempted to test whether mood mediated the effect of cycle phase on cognitive scores. The alpha level was set to $p < .05$ for all analyses, adjusted p values are reported for post-hoc tests. Adjusted p values were reported for post-hoc tests.

3. Results

3.1. Reliability testing

Internal consistency was good to excellent on all tasks ($\lambda_3 = .64 - .98$, $\lambda_4 = .78 - .99$). Test-retest reliability was high to very high on all reaction time variables (ICC = $.67 - .86$) and less consistent on the error variables (ICC = $.28 - .76$). More detailed information is provided in Supplement 2.

3.2. Participants

A total of 394 participants completed the online battery of cognitive tasks at least once. Participants were excluded based on: being older than 35 ($n = 40$), having been pregnant or breastfeeding in the last 3 months ($n = 21$), experiencing menopause ($n = 9$), not regularly menstruating ($n = 6$), having tested in the inactive contraceptive pill phase ($n = 10$), or not completing both testing sessions ($n = 70$). A complete data set of two testing sessions from eligible participants was obtained from 241 participants (Table 1), of which 96 were male, 47 were females on active hormonal contraception in both timepoints (27 using combined contraception, 20 using progestin-only contraception), and 105 were regularly naturally cycling females with reportedly regular menstruation cycles (Table 2). For readability, naturally cycling females will be referred to as ‘menstruating’ throughout the results section.

Table 1 – Demographic information of the 241 participants who were included in the study analysis. Participants self-reported their sport participation level, classified as Sedentary (not taking part in any sport or regular physical activity), playing recreationally (regular participation in a sport but no competitive activity), or played competitively (at club or national level).

<i>Group</i>	<i>n</i>	<i>Age</i>	<i>Sedentary</i>	<i>Play recreational sport (ball, other)</i>	<i>Play competitive sport (ball, other)</i>
<i>Male</i>	96	29 ± 3	16 % *	43 (21, 22) %	42 (27, 15) %
<i>Contraception</i>	47	28 ± 4	28 %	37 (9, 28) %	38 (17, 19) %
<i>Menstruating</i>	105	28 ± 4	35 %	38 (11, 27) %	27 (18, 9) %

* Significantly lower prevalence of sedentary males compared to all other groups ($p = .02$)

Table 2 – Number of menstruating females who tested in each phase in T1 and T2.

	<i>Menstruation</i>	<i>Follicular</i>	<i>Late follicular</i>	<i>Luteal</i>
<i>Time 1</i>	23	31	16	35
<i>Time 2</i>	26	25	21	33

3.3. Between-subject effects on cognitive performance

For the Composite Reaction Time score, there was a significant time effect ($F(1,214) = 13.69$, $p < .001$) but no group effect and no group*time interaction, where participants were faster overall in the second test session, but there was no difference between males, females using contraception or menstruating females (

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Table 3). For Composite Errors, there was a group*time interaction ($F(2,212) = 4.4, p = .01$), where there were no significant differences between groups within timepoints, but the male ($p = .02$) and the menstruating group ($p = .001$) both committed significantly more errors in T2. There were no time or group effects in the Composite Intra-Individual Variability. These same analyses were conducted by splitting the contraception group into combined and progestin-only subgroups, the results did not differ (Supplement 3).

When analysing the individual cognitive tasks separately, the majority exhibited a time effect with improved reaction times across the entire cohort ($p < .05$), and only two tasks (sustained attention and 3D mental rotation) exhibited group differences in errors, where menstruating females performed worse (Supplement 3). Of note, there was a group effect for 3D mental rotation errors ($F(2,259) = 5.92, p = .003$), where only menstruating females committed more errors than males in both timepoints ($p = .002$). Further analysis revealed that the difference in 3D mental rotation errors males were only significant compared to females who tested in the luteal phase ($p = .03$) and the late follicular phase ($p = .048$). Further details of this analysis are included in Supplement 4.

Table 3 – Mean \pm s.d. for group-level comparisons of mood, symptoms and cognitive scores for all males and females who participated in the study. Only participants who completed both timepoint 1 (T1) and timepoint 2 (T2) are included in this analysis. A further analysis dividing contraception groups by hormonal combinations did not differ (included in Supplement 3). Detailed analysis on each individual task is included in Supplement 4 for completion.

		Male (n=96)	Contraception (n=47)	Menstruating (n=105)
Drive	T1	1.18 \pm 2.14	0.43 \pm 2.13[†]	0.41 \pm 2.32[†]
	T2	1.09 \pm 2.22	0.14 \pm 2.08[†]	0.37 \pm 2.25[†]
Serenity	T1	1.79 \pm 1.66	1.30 \pm 1.94	1.52 \pm 1.89[†]
	T2	1.85 \pm 1.83	1.13 \pm 2.02	1.01 \pm 2.11[†]
Cognitive symptoms (n)	T1	0.92 \pm 0.98	1.03 \pm 1.18	1.67 \pm 1.61^{††}
	T2	1.06 \pm 0.98	1.63 \pm 1.81	1.72 \pm 1.69^{††}
Physical symptoms (n)	T1	0.60 \pm 0.69	0.66 \pm 0.77	0.78 \pm 1.06[†]
	T2	0.44 \pm 0.77	0.47 \pm 0.68	1.06 \pm 1.17[†]
Composite Reaction Time	T1	0.03 \pm 0.95	-0.01 \pm 1.06	0.30 \pm 1.25
	T2	<i>-0.13 \pm 0.95^{^^^}</i>	<i>-0.24 \pm 0.77^{^^^}</i>	<i>-0.02 \pm 0.95^{^^^}</i>
Composite Errors	T1	-0.18 \pm 0.88	0.24 \pm 1.23	-0.11 \pm 1.07
	T2	0.07 \pm 0.99 [^]	0.03 \pm 0.76	<i>0.15 \pm 1.04^{^^}</i>
Composite Variability	T1	0.36 \pm 0.24	0.39 \pm 0.22	0.41 \pm 0.22
	T2	0.38 \pm 0.24	0.39 \pm 0.23	0.43 \pm 0.19

Significant within group time effect evidenced in *italics* [^] p < .05, ^{^^} p < .01, ^{^^^} p < .0001
Significantly different than the male group evidenced in **bold** [†] p < .05, ^{††} p < .01, ^{†††} p < .001

3.4. Sporting participation and cognitive performance

To explore whether sporting participation was associated with performance on the cognitive tasks, the relationship between sporting level and cognitive performance was tested through Spearman rank correlations in the full sample and by population group (male, contraception, menstruating). There were no significant relationships between cognitive composite scores and frequency of participation in endurance, power, ball, fight, coordination or fine skill sports.

The same correlations were also conducted on the individual cognitive tasks for each sport type. Only three significant correlations were found. Only males who reported higher frequency of participation in ball sports exhibited significantly slower reaction times on the three tasks that included a spatial element to them (spatial simple reaction time (Rho = .24, p = .02), 3D spatial perception (Rho = .30, p = .002) and 3D mental rotation (Rho = .21, p =

.03), with no difference in errors. Neither of the two female groups showed this relationship. No relationships were found between cognitive task scores and participation in endurance, power, fight, coordination or fine skill sports.

Between-subject ANOVAs were conducted between population groups (male, contraception, menstruating) by competitive level and sport type. There were no significant effects of competitive level or sport type on cognitive performance on any of the composite scores or individual tasks (Supplement 5).

Finally, frequency of participation, sport type and competitive level were included in an exploratory analysis as covariates in the menstrual cycle phase analysis (below) to test whether sport participation and expertise interacted with the effects of the menstrual cycle. No significant effects were found.

3.5. Within-subject effects of cycle phase on cognition

3.5.1. Cognitive composite scores

First, a mixed model ANOVA was run on the entire sample of naturally cycling females to test for phase effects. Since participants only tested twice, a subsequent within-subject repeated measures ANOVA was conducted to compare cognitive performance when testing in a specific phase compared to being in any other phase (Table 5). For this purpose, the data was split into four groups according to the phases in which participants had tested. For example, participants who tested once during the luteal phase, and once in any other phase, in randomised order, were included in the luteal phase analysis. The same was repeated for all other phases. Significant time effects were observed for all data sets as reported in the group level analysis, and therefore were not reported again here unless a phase*time interaction was present. Table 5 provides mean \pm SD scores for all cognitive tests across each cycle phase.

For Composite Reaction Time (suppl. 3), the mixed model ANOVA revealed time effects ($F(1,82) = 11.55, p = .001$) and a phase effect ($F(3,90) = 2.70, p = .05$), with slower reaction times in the luteal phase ($p = .048$) (Figure 5). When splitting the data to compare each of the four phases separately, the menstrual phase exhibited faster reaction times while the luteal

phase exhibited slower reaction times. There was a phase effect for menstruation ($F(1,35) = 3.12, p = .05$) and a phase*time interaction ($F(1,38) = 9.16, p = .004$), where participants were faster when they tested during the menstrual phase compared to any other phase in T1 only ($p = .007$). There were also phase effects for the luteal group ($F(1,54) = 6.80, p = .012$) and a phase*time interaction ($F(1,58) = 11.70, p = .001$), where participants were slower when they tested during the luteal phase in both T1 ($p < .001$) and T2 ($p = .05$). A time effect was present in all four groups ($p < .01$), where participants were faster on their second test session, but within-subject phase effects of the luteal phase remained significant despite this learning effect.

For Composite Errors, the mixed model ANOVA revealed time effects ($F(1,87) = 15.01, p < .001$) and phase effects ($F(3,98) = 7.00, p < .001$), with fixed effects for menstruation ($p = .05$), where participants committed less errors during menstruation compared to luteal ($p = .015$) and late follicular ($p = .002$), and more errors during the late follicular phase compared to menstruation (pairwise $p = .002$) and follicular ($p = .024$) (Figure 5). The within-subject analysis confirmed a phase effect for menstruation ($F(1,35) = 10.81, p = .002$) and for the follicular phase ($F(1,36) = 4.78, p = .035$), where participants committed less errors when they tested during these phases compared to being in any other phase. There were also phase effects for late follicular ($F(1,26) = 9.6, p = .005$) and for the luteal phase ($F(1,54) = 6.32, p = .014$) with more errors being committed during these phases. Therefore, phase effects on Composite Errors remained highly significant in all four phases despite the strong time effect.

For the Composite Intra-Individual Variability, the mixed model ANOVA revealed time ($F(1,91) = 4.26, p = .042$) and phase effects ($F(3,121) = 3.21, p = .025$) with fixed effects for menstruation ($p = .011$), where participants were less variable during menstruation compared to the luteal phase ($p = .026$) (Figure 5). The within subject analysis confirmed a phase effect for menstruation ($F(1,36) = 7.39, p = .010$) and a phase*time interaction ($F(1,39) = 9.45, p = .004$), where participants were less variable when testing during menstruation in T1 only ($p = .002$).

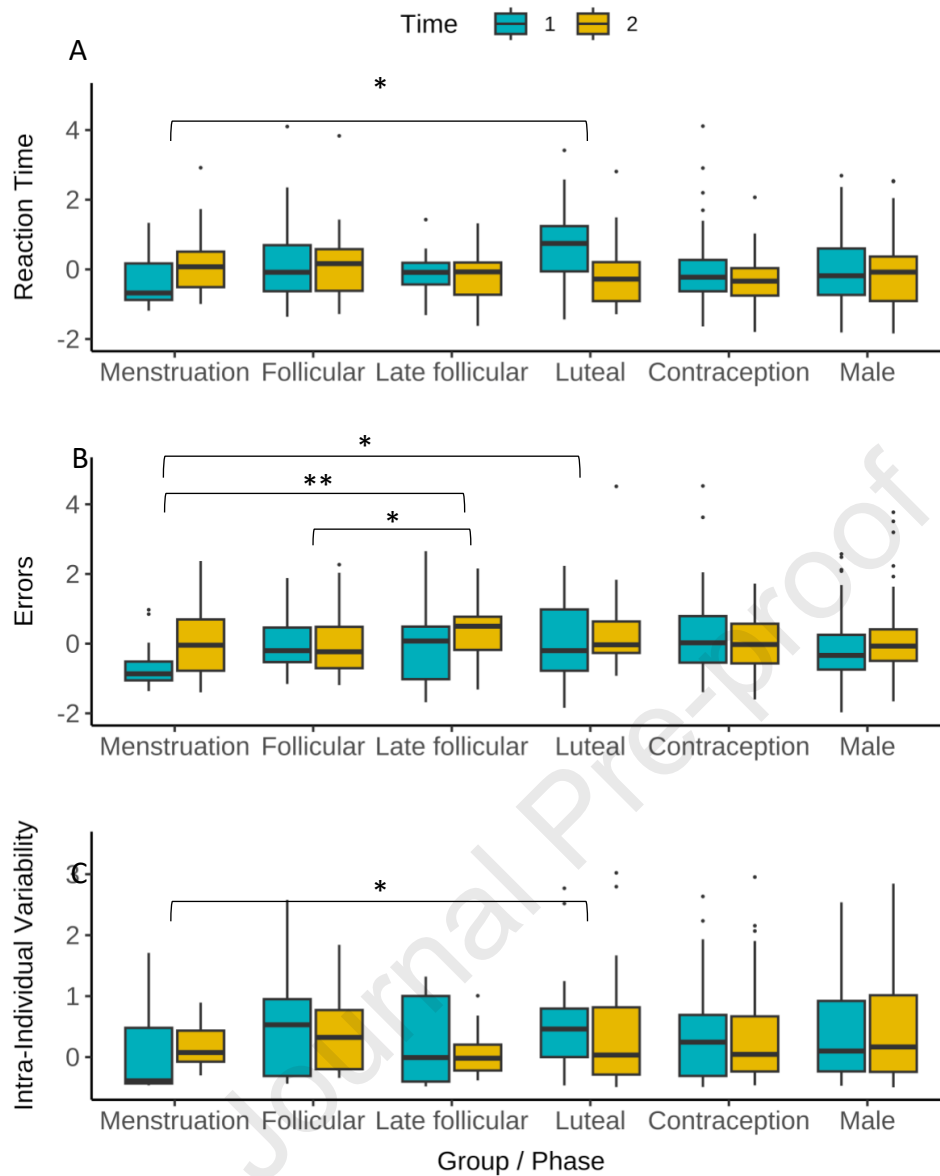


Figure 5 – Composite scores derived from the factor analysis conducted on the cognitive testing battery, A = Composite Reaction (relates to all reaction time variables calculated in ms), B = Errors (related to all commission errors, calculated as n errors), C = Intra-Individual Variability (relates to all variability scores calculated as the standard deviation of reaction times in each task). Lower scores indicate better performance on all scores. Two timepoints are provided for each participant, 14 days apart; naturally cycling females would have tested in separate phases and therefore appear in separate phases for T1 and T2. Results are shown from a mixed model ANOVA conducted on the entire study sample. Significance levels from Holm corrected between-group effects * ($p < .05$), ** ($p < .01$). Significant differences were observed between phases within the sample of menstruating females, but no significant differences were observed between the phase subgroups compared to females using contraception or males.

3.5.2. Attentional tasks

Overall performance on the non-spatial simple reaction time task was generally better during menstruation and worst during the luteal phase, although time effects were present throughout the sample. Within-subject analyses revealed that there was a menstruation phase effect ($F(1,32) = 4.45, p = .043$) and phase*time interaction ($F(1,33) = 6.58, p = .015$), with faster simple reaction times (SRT) during menstruation only in T1 ($p = .008$). There was also a luteal phase*time interaction ($F(1,57) = 5.36, p = .024$) where participants were slower during the luteal phase in T1 only ($p = .026$). SRT commission errors also displayed a menstruation phase effect ($F(1,37) = 12, p = .001$) with less errors being committed during this phase regardless of timepoint. There was a menstruation phase effect on SRT intra-individual variability ($F(1,37) = 10.27, p = .003$) and a phase*time interaction ($F(1,38) = 6.61, p = .014$) where participants were less variable during menstruation but only in T1 ($p = .002$). SRT intra-individual variability also revealed a luteal phase effect ($F(1,57) = 5.15, p = .027$) with more variability during the luteal phase. While the mean scores for the spatial SRT task displayed similar trends to the non-spatial SRT, these were not statistically significant.

Similarly to simple reaction time, reaction times on the sustained attention task were better during menstruation and worse during the luteal phase, but this difference was only present in T1. For SA reaction times there was a time*phase interaction for menstruation ($F(1,39) = 6.91, p = .012$) with faster reaction times during menstruation for T1 only ($p = .010$). There was a phase*time interaction for the luteal phase ($F(1,58) = 4.8, p = .03$), but pairwise comparisons did not reach significance after Bonferroni correction ($p = .07$). However, there was a phase effect for late follicular on SA commission errors ($F(1,54) = 5.27, p = .026$) and no time effect, with more errors committed during late follicular regardless of timepoint. There were no effects for SA omission errors.

A similar pattern was observed in the inhibition task, with generally better performance during menstruation and worse in the luteal phase. For inhibition reaction times there was a luteal time*phase interaction ($F(1,58) = 5.69, p = .02$) with slower reaction times in the luteal phase in T1 only ($p = .045$). For inhibition commission errors there was a phase effect for menstruation ($F(1,39) = 4.2, p = .047$) with less errors being committed during menstruation despite no difference in reaction times.

3.5.3. 3D spatial tasks

Performance on the 3D spatial awareness task was overall worse during the late follicular phase. There was a phase effect on both reaction time ($F(1,26) = 4.7, p = .039$) and errors ($F(1,27) = 7.74, p = .010$), with faster reaction times and more errors when testing during the late follicular phase. The 3D mental rotation task displayed a very different pattern, with slower reaction times during the menstrual phase (~ 50 ms average difference) compared to being in any other phase ($F(1,39) = 5.20, p = .028$), but no difference in mental rotation errors between any phases.

3.5.4. Timing anticipation

Performance on the spatial timing anticipation task was better during menstruation, but only in T1, and worse during the luteal phase regardless of timepoint. There was a phase*time interaction for timing error ($F(1,39) = 5.7, p = .020$) where participants were more accurate during menstruation in T1 only ($p = .028$), and a phase effect for the luteal phase ($F(1,57) = 4.08, p = .048$) with worst accuracy when testing in this phase compared to being in any other phase, regardless of timepoint. Similarly, there was a phase effect for menstruation on intra-individual variability ($F(1,39) = 5.07, p = .030$) and a phase*time interaction ($F(1,39) = 5.01, p = .031$) with less variability in the menstrual phase in T1 ($p = .020$) and a phase effect for luteal ($F(1,58) = 7.54, p = .008$) with greater variability when testing during the luteal phase regardless of timepoint. There were no significant phase effects on either the timing error or variability of the rhythmic timing anticipation task.

3.6. Mood and symptoms

3.6.1. Between-subject effects

Overall, naturally cycling females scored consistently lower than males on both mood factor scores, and reported a higher number of cognitive and physical symptoms than males.

There was a group effect for Drive ($F(2,258) = 4.89, p = .008$), where males reported significantly better Drive compared to both the menstruating ($p = .01$) and contraception groups ($p = .048$) (

Table 3). When the menstruating group were divided by phases ($F(5,339) = 4.04, p = .001$), females scored worse when in their menstruation phase compared to males ($p = .003$) and to females in the follicular phase ($p = .047$) (Figure 6). Similarly for Serenity, there were group effects ($F(1,260) = 3.65, p = .03$) where menstruating females reported significantly poorer serenity than males only ($p = .04$) and group-phase effects ($F(5,370) = 4.59, p < .001$) where females who were in their menstruation phase reported poorer Serenity than males ($p = .002$), and than females in both the follicular ($p = .01$) and late follicular phase ($p = .04$) (Figure 6).

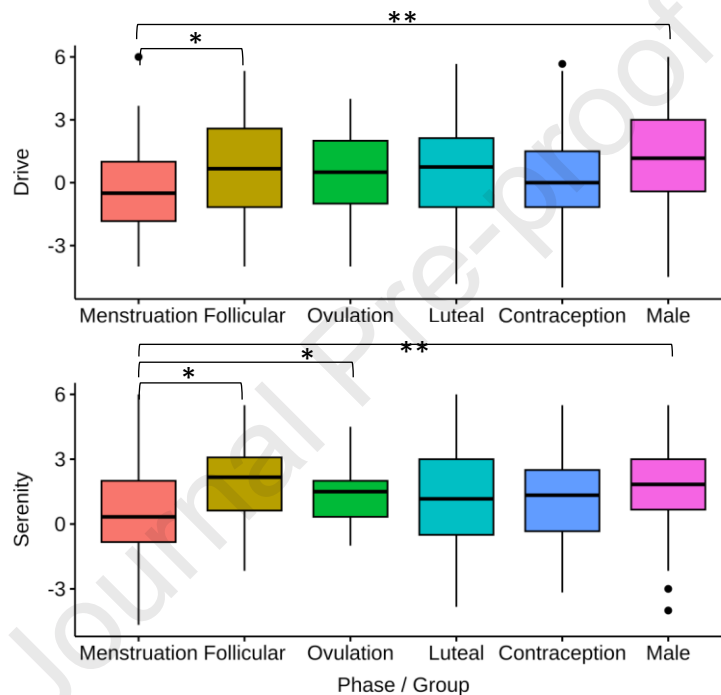


Figure 6 - Self-reported Drive and Serenity, derived as composite scores from the mood questionnaire, provided from all participants. Two timepoints (14 days apart) are provided for each participant, where the contraception and male groups include two reports per person and naturally cycling females reported mood twice, in two random phases. Significance levels from Holm corrected post-hoc analysis of the mixed model ANOVA: * ($p < .05$) ** ($p < .01$).

There was a significant difference by population group in the reporting of total number of cognitive symptoms on the day of cognitive testing ($H(2) = 9.46, p = .009$), where menstruating females reported more symptoms than males ($p = .009$). Further analysis revealed a phase and group difference ($H(5) = 20.54, p < .001$) where females in the menstruation phase reported more cognitive symptoms than males ($p < .001$), females using contraception ($p = .008$) and females in the follicular phase ($p = .005$). Similarly, there was a group difference in the reporting of physical symptoms ($H(2) = 7.61, p = .02$), where menstruating females reported more symptoms than males only ($p = .04$), and further analysis revealed a phase and

group difference ($H(5) = 16.92, p = .005$), where females in the menstruation phase reported more physical symptoms than males ($p = .007$) and than females using contraception ($p = .01$).

3.6.2. Within-subject effects of cycle phase

Menstruating females were asked if they thought their mood and symptoms normally changed throughout a typical cycle, by reporting the prevalence of each item (yes/no) for the period before menstruation, during menstruation or mid-cycle. When asked if they perceived that their feelings of poor mood (such as stress, anxiety and depression), irritability or tiredness changed throughout the cycle, a significant proportion of menstruating females reported that it did ($\chi^2(8, N=129) = 222.82, p < .001$), where 58% perceived that they typically experienced poorer mood in the week before their menstruation ($p < .001$ compared to mid-cycle), and 91%, 97% and 98% reported not normally feeling poor mood, irritability or tiredness mid-cycle ($p < .001$ compared to both during and before menstruation). They also perceived to typically experience more cognitive ($\chi^2(2, N=129) = 62.39, p < .001$) and physical ($\chi^2(2, N=129) = 70.95, p < .001$) symptoms both in the week before menstruation and during menstruation compared to mid-cycle.

In contrast, when asked about their mood on the day of testing, mood was reportedly poorer during menstruation (Figure 6). The mixed model ANOVA revealed a phase effect for Drive ($F(3,161) = 2.84, p = .04$) with fixed effects for being in the menstruation phase ($p = .02$). The repeated measures ANOVAs for within-subject data confirmed a phase effect for lower drive during menstruation compared to being in any other phase ($F(1,39) = 8.98, p = .005$). For Serenity, the mixed model ANOVA revealed a phase effect ($F(3,160) = 4.23, p = .007$), with fixed effects for menstruation ($p = .04$). Within subject comparisons confirmed a phase effect for menstruation, with poorer Serenity during this phase ($F(1,39) = 3.8, p = .05$), and a phase effect for late follicular ($F(1,27) = 9.63, p = .004$) with greater serenity during this phase compared to being in any other phase.

There was a significant difference in the number of cognitive symptoms presented by phase on the day of testing ($p = .01$), with more symptoms reported during the menstruation phase compared to the follicular phase ($p = .004$) but no significant difference with any other phase. The number of physical symptoms also differed by phase ($p = .03$) with more symptoms

reported during menstruation compared to late follicular ($p = .05$) and to the luteal phase ($p = .05$). A significant proportion of females who were in the menstrual phase on the day of testing perceived that their symptoms on the day impacted both their cognitive ability and their physical ability ($\chi^2(10, N=226) = 164.5, p < .001$) (Table 4).

A mediation analysis was attempted to test whether differences in Drive mediated differences in cognitive scores, but these were not significant (e.g. on Reaction Time: $b = .18, 95\% \text{ CI } [-1.61, 2.02], p = .5$). Cognitive symptoms did not mediate this relationship either ($b = .20, 95\% \text{ CI } [-1.23, 1.63], p = 0.4$).

Table 4 – Prevalence of participants who thought that their cognitive or physical ability was being negatively impacted on the day of testing. Both T1 and T2 are included, where menstruating females tested in a different phase each time.

	<i>Menstruation</i> (<i>n = 41</i>)	<i>Follicular</i> (<i>n = 40</i>)	<i>Late</i> <i>follicular</i> (<i>n = 29</i>)	<i>Luteal</i> (<i>n =</i> <i>60</i>)	<i>Contraception</i> (<i>n = 47</i>)	<i>Male</i> (<i>n =</i> <i>105</i>)
<i>Cognitive</i> <i>Impact</i>	39%***	14%	20%	19%	21%	7%
<i>Physical</i> <i>Impact</i>	36%***	11%	13%	22%	17%	8%

***Significant prevalence of 'Yes' responses ($p < .001$).

Table 5 – Within-subject analyses on all wellbeing and cognitive tasks. Participants were grouped according to the phases in which they tested in order to compare within-subject scores between being in a specific phase or in any other phase. E.g. under the Menstruation column, “Yes” refers to when the participant tested during the Menstruation phase, “No” indicates when the participant tested in any other phase. Cognitive tasks are presented in the order in which they were conducted in the test.

	Menstruation (n=41)		Follicular (n=40)		Late follicular (n=29)		Luteal (n=60)	
	Yes	No	Yes	No	Yes	No	Yes	No
<i>Drive</i>	-.05 ± 2.17**	1.13 ± 2.13	0.67 ± 2.20	0.17 ± 2.31	.33 ± 2.05	-.35 ± 2.16	.68 ± 2.39	.53 ± 2.20
<i>Serenity</i>	.59 ± 2.13*	1.48 ± 2.13	1.51 ± 1.99	.84 ± 2.09	1.40 ± 1.23**	.41 ± 1.84	1.05 ± 2.25	1.36 ± 2.13
<i>Cognitive Symptoms (count)</i>	2.00 ± 1.65**	1.00 ± 1.39	0.96 ± 0.88**	2.29 ± 2.03	1.50 ± 1.32	1.95 ± 1.47	1.72 ± 2.00	1.47 ± 1.44
<i>Physical Symptoms (count)</i>	1.30 ± 1.33	.97 ± .75	.78 ± .95	.86 ± 1.08	.63 ± .81	.95 ± .97	.68 ± .73	.81 ± 1.26
<i>Composite Reaction Time</i>	-.03 ± .90††	.34 ± 1.31	.12 ± 1.32	.16 ± 1.40	-.17 ± .74	-.25 ± .68	.37 ± 1.32**	.13 ± 1.05
<i>Composite Errors</i>	-.18 ± 1.03*	.16 ± 1.19	.01 ± 1.01*	.29 ± 1.20	.21 ± 1.15**	-.18 ± .89	.12 ± 1.17*	-.09 ± 1.03
<i>Composite Intra-individual variability</i>	.37 ± .22††	.44 ± .22	.44 ± .19	.42 ± .23	.39 ± .19	.41 ± .20	.45 ± .21	.41 ± .19
<i>Spatial simple reaction time</i>								
<i>Reaction time (ms)</i>	320 ± 39	329 ± 50	335 ± 63	329 ± 79	320 ± 32	313 ± 34	329 ± 66	330 ± 46
<i>Commission errors</i>	1.17 ± 2.05	.76 ± 1.04	1.02 ± 1.61	.95 ± 2.02	.41 ± .87	.59 ± .73	.72 ± .98	.98 ± 1.52
<i>Intra-individual variability (sd)</i>	65 ± 33	75 ± 52	79 ± 48	80 ± 65	63 ± 35	55 ± 20	71 ± 55	67 ± 30
<i>Simple reaction time</i>								
<i>Reaction time (ms)</i>	303 ± 31††	329 ± 68	311 ± 73	311 ± 78	311 ± 49	301 ± 31	321 ± 73††	309 ± 55
<i>Commission errors</i>	.51 ± .93***	1.00 ± 1.20	.78 ± .97	.63 ± .85	.62 ± .82	.57 ± 1.07	.76 ± 1.21	.55 ± .96
<i>Intra-individual variability (sd)</i>	61 ± 32†	86 ± 46	74 ± 49	68 ± 38	70 ± 31	73 ± 43	76 ± 43*	62 ± 34
<i>Sustained attention</i>								
<i>Reaction time (ms)</i>	539 ± 110††	550 ± 120	545 ± 121	553 ± 124	537 ± 95	527 ± 97	555 ± 119	546 ± 108
<i>Commission errors</i>	.44 ± .55	.46 ± .81	.38 ± .59	.69 ± .83	.76 ± 1.12*	.24 ± .44	.39 ± .56	.42 ± .59
<i>Intra-individual variability (sd)</i>	105 ± 66	125 ± 139	115 ± 59	121 ± 100	125 ± 123	105 ± 63	124 ± 116	116 ± 82
<i>Inhibition</i>								
<i>Reaction time (ms)</i>	419 ± 89	418 ± 97	421 ± 95	430 ± 105	381 ± 54	401 ± 83	438 ± 100†	423 ± 81
<i>NoGo errors</i>	1.73 ± 1.87*	2.15 ± 1.89	1.48 ± 1.45	1.40 ± 1.58	2.03 ± 1.55	2.07 ± 1.46	1.87 ± 1.75	1.62 ± 1.65
<i>Intra-individual variability (sd)</i>	106 ± 73	103 ± 77	118 ± 88	118 ± 87	84 ± 37	112 ± 90	117 ± 80	105 ± 59
<i>3D spatial awareness</i>								
<i>Reaction time (ms)</i>	2832 ± 878	2873 ± 942	2794 ± 848	2566 ± 1017	2604 ± 838*	2848 ± 1103	2849 ± 1118	2859 ± 834
<i>Incorrect responses</i>	1.51 ± 1.85	1.80 ± 2.40	1.88 ± 1.99	2.75 ± 2.97	2.00 ± 2.31**	1.07 ± 1.33	1.83 ± 2.67	1.49 ± 1.76
<i>3D mental rotation</i>								

	Journal Pre-proof					2269 ± 663	2297 ± 847	2309 ± 742
<i>Reaction time (ms)</i>								
<i>Incorrect responses</i>	4.15 ± 2.66	4.44 ± 2.97	4.53 ± 4.00	4.92 ± 3.72	4.18 ± 1.93	3.69 ± 2.14	4.85 ± 3.55	4.63 ± 3.52
<i>Rhythmic timing anticipation</i>								
<i>Timing Error (ms)</i>	339 ± 120	343 ± 123	351 ± 119	349 ± 112	343 ± 113	336 ± 93	318 ± 81	319 ± 96
<i>Intra-individual variability (sd)</i>	204 ± 99	220 ± 110	233 ± 157	205 ± 100	214 ± 104	197 ± 54	198 ± 77	214 ± 136
<i>Spatial timing anticipation</i>								
<i>Timing Error (ms)</i>	82 ± 67†	93 ± 65	74 ± 49	85 ± 62	70 ± 32	66 ± 25	88 ± 51*	76 ± 44
<i>Intra-individual variability (sd)</i>	112 ± 132*	127 ± 112	99 ± 86	137 ± 146	97 ± 80	89 ± 50	132 ± 100**	100 ± 76

Significance levels following a two-way ANOVA with post-hoc Holm correction:

Significant phase effect compared to not being in the stated phase * p < .05, ** p < .01, *** p < .001

Significant phase effect compared to not being in the stated phase, but only significant in test session 1 †p < .05, †† p < .01, ††† p < .001

This proof-of-principle study sought to test whether sport-related cognitive processes fluctuate throughout the menstrual cycle. In this general population sample, naturally cycling females exhibited better overall cognitive scores during menstruation, and worse during the luteal phase, compared to other phases. The convergence of faster reaction times, reduced variability and fewer errors within the same phase provides a strong indication that a cognitive advantage may indeed be present during menstruation. Participants exhibited better overall cognitive scores during menstruation, even though they reported poorer mood and symptoms during this phase, and perceived that their symptoms were negatively affecting their cognitive performance. This incongruence between female participants' subjective perceptions of their performance and their objective cognitive scores provides a positive outlook on the female menstrual cycle which could aid in shaping attitudes towards sporting behaviours. In contrast, less optimal performance on cognitive tasks was observed in the late follicular and luteal phases, with differential effects between cognitive subdomains, and worse overall performance in the luteal phase, particularly in the novel sport-related task of spatial timing anticipation.

4.1. Group-level analysis

Group analyses including males and females using contraception were used to provide control comparisons for the menstruating participants. There were no significant differences between population groups on the composite scores. This suggests that, at population level, there are few differences in the forms of cognition measured here, if any, between males, females using contraception and menstruating females.

However, it should be noted that when analysing each task individually, a specific difference in 3D mental rotation errors (but not on any other tasks) was found between males and menstruating females, but not females using contraception (Supplement 3, 4). Specifically, menstruating females committed more errors than males when they tested during their predicted late follicular or luteal phases but not when they tested in the menstrual or follicular phases, which is consistent with previous findings (McCormick and Teillon, 2000; Moody, 1997). In addition, mood and symptoms experienced on the day of testing were all reported to be worse for menstruating females compared to men, but only for those females who were

cognition throughout the cycle.

An additional exploratory analysis was conducted on the relationship between sport participation, competitive level and cognitive performance in the same, divided by population group. Neither sport type, competitive level or frequency of participation in sport sub-types had any significant effects on the composite scores of cognitive performances. One weak correlation was observed between males who participated in ball sports and reaction times in the spatial tasks (discussed below). While this contradicts some literature that suggests that athletes often have faster reaction times than the general population (Hülsdünker et al., 2018), it should be noted that such effects are typically found when the athletes tested have a high competitive level, and the cognitive tasks implemented involve processes and stimuli that are relevant to the specific sport of the athlete (Weigelt & Memmert, 2020; Jansen and Lehmann, 2013). It is possible that effects were not found here given the variability in sport types performed by the sample included in this study, and lack of elite athletes, suggesting that sport-related cognitive advantages may be sport-specific, and might require a higher degree of expertise than the regional competitive level reported in this sample.

4.2. Timing anticipation

Composite Reaction Time, Composite Errors, simple reaction time and spatial timing anticipation were all significantly worse during the luteal phase, regardless of test-retest learning effects. Spatial timing anticipation, but not rhythmic timing anticipation, portrayed the most compelling and relevant phase differences, with poorer performance during the luteal phase. To the authors' knowledge, no other study to date has investigated changes in timing anticipation throughout the cycle, making this is a novel finding which contributes to a cognitive theory of determinants of injury risk in female athletes.

The majority of the luteal phase is characterised by high levels of progesterone and elevated oestrogen, although lower than in the late follicular phase. Oestrogen is known to have excitatory effects on the cerebral cortex (Smith et al., 2000; Shaywitz et al., 1999), whereas progesterone has been found to inhibit motor evoked potentials, with effects in similar magnitude to benzodiazepine drugs, by acting on the GABA_a receptor complex (Smith et al.,

study.

Early studies have reported improved fine motor skill in the luteal phase compared to the menstrual phase (Hampson, 1990; Hampson and Kimura, 1988), relating it to progesterone's stabilising effect on motor control (Zimmerman & Parlee, 1973). This points towards a plausibly complex relationship between neurocognitive and motor processes where slower reaction times induced by lower cortical excitability might increase risk of injury in the luteal phase due to inaccurate spatial-temporal precision, in spite of improved fine motor control. The spatial timing anticipation task was created to illustrate this combination, mimicking the sport-related skill of timing movements against moving objects which may, or may not, be visible at the time of impact (e.g. heading a ball, making a tackle). The greater intraindividual variability and poorer precision on this task in the luteal phase, concomitant with slower composite processing speeds, suggests that this phase might be more likely to exhibit slower motor processes, and therefore poorer timing of movements in fast-paced and changing settings, explaining the increased incidence of injuries observed during the luteal phase in team sports (Barlow et al., 2023; La Fontaine et al., 2019).

4.3. Attentional control

Simple reaction time (with and without a spatial component), sustained attention and inhibition (Go/No-Go) tasks were implemented in this study to test the possible effects of hormonal changes on attentional (or cognitive) control. Participants generally exhibited faster reaction times and fewer errors during menstruation, contrasting with more errors on the sustained attention task during the late follicular phase, and slower reaction times in both simple reaction time and inhibition during the luteal phase.

The poorer attentional control observed here in the late follicular phase reflects related findings from the literature. While there is a dearth of literature pertaining to the broader concept of attentional or cognitive control in the menstrual cycle, Colzato et al. (2010) have reported less efficient inhibition in the ovulatory phase measured via a stop-signal task, which further correlated with oestradiol levels and not progesterone. This is congruent with the cortical excitatory effect of oestrogen, observed principally during the late follicular phase

parietal lobes (Smith et al., 2002; Shaywitz et al., 1999), which are respectively responsible for executive function (including attentional and cognitive control) and spatial cognition, among their many functions. In contrast, the slower reaction times in attentional tasks during the luteal phase, in absence of any difference in errors, is consistent with a theory that the inhibitory effects of progesterone on cortical activation (Section 3.2) might be driving slower responses during this phase.

Of note, while the non-spatial (i.e. conventional) simple reaction time task exhibited significant differences between phases, the spatial simple reaction time task did not. The two versions of task were included to investigate whether potential fluctuations in reaction time throughout the menstrual cycle might be related to the spatial attention component of a task, or purely to changes in reaction time speed. These findings might suggest that fluctuations in executive function across the cycle might be more closely related to simple attentional control and processing speeds, and not to more complex changes in visuo-perceptual awareness.

4.4. 3D Spatial cognition

Visuo-perceptual and visuo-spatial cognition demonstrated differing results. Naturally cycling females scored worse than males on 3D mental rotation if they were tested during their late follicular or luteal phase, but there were no menstrual phase differences within the sample of menstruating participants when analysed for within subject effects. However, performance on the 3D spatial perception task (cube counting) showed the opposite pattern; males and females did not differ overall, but menstruating females scored worse during the late follicular phase compared to other phases in the within-subjects analysis. These results might suggest poorer 3D spatial cognition during the late follicular phase, however the effect was small, and therefore must be interpreted with caution. This aligns with the high variability of results reported elsewhere (Sundström Poromaa and Gingell, 2014).

It has been proposed that males outperform females in 3D mental rotation tasks (Halari et al., 2005; Nyborg, 1983), although there are several studies that challenge these findings. For example, Hausmann et al. (2009) reported that males only outperformed females when participants had been primed to believe in such gender stereotypes. In studies on athletes,

players (Jahnsen and Lehman, 2013), and gender disparities were diminished when human figures were adopted as stimuli instead of cubes (Jahnsen and Lehman, 2013). Other authors reported that females only scored lower than males when they were tested in the luteal phase, but not during menstruation (McCormick and Teillon, 2000; Moody, 1997), which is consistent with the findings reported here. Some authors hypothesise that this might be due to the more stable hormonal profile during menstruation, where oestrogen and progesterone are lower than in the luteal phase. Therefore, while a gender difference in 3D mental rotation might exist, the literature does not present conclusive results, and these might be influenced by hormonal differences, by psychological factors or by familiarity with given stimuli related to sporting expertise. While this study did not find a relationship between cognitive performance and sport-type or expertise, it is possible that such differences may only occur in elite athletes (who were not present in this sample) and that such 'expertise' also means that athletes may be more likely to outperform novices when presented with stimuli that are highly familiar to their sporting environment, such as human figures (Jansen and Lehmann, 2013) or basketball courts (Weigelt & Memmert, 2020). If this is the case, then further research might want to investigate if the reverse effect is also true, for example whether training the mind to rotate familiar environments and objects improve performance on the field. And if the changes in spatial cognition throughout the menstrual cycle happen to be linked to potential increases in injury risk as reported by Yamada and Matsumoto (2009), future interventions could perhaps investigate whether improving this ability might reduce the risk of sporting injuries.

It has been long hypothesised that sex hormone concentrations might drive male advantages in spatial ability (Hausmann et al., 2000; Nyborg, 1983), although testosterone levels have not always been associated with improved performance on spatial tasks (Hausman et al., 2009; Yang et al., 2007, Halari et al., 2005). The findings reported here further discount the theoretical impact of testosterone on spatial cognition in menstruating females, considering that testosterone peaks during ovulation (Rothman et al., 2011), where this study found poorer scores. Furthermore, oral contraception use has been associated with lower testosterone levels (Liening et al., 2010), but females using contraception in this sample, and in the literature, did not score differently to males. Finally, oestrogen, but not progesterone, has been suggested to negatively impact 3D mental rotation in naturally cycling females (Maki et al., 2002; Hausman et al., 2000) which might explain why menstruating females scored

suggest further research is needed to better understand how hormonal changes influence 3D spatial cognition.

The faster speeds and increased errors observed in the 3D spatial perception task during the late follicular phase may also indicate a change in strategy aligned with the more impulsive behaviour observed in the sustained attention task, rather than an isolated detriment to 3D spatial cognition (Sundström Poromaa and Gingell, 2014). Changes in navigation strategies, not outcomes, have been observed between phases (Scheuringer and Pletzer, 2017; Hussain et al., 2016), which proposes that menstrual cycle-dependent changes in cognition may not necessarily be tied to a change in performance outcome, but rather in how that outcome is achieved (Pletzer et al., 2019).

Of note, the two 3D spatial tasks, and the spatial simple reaction time task, were the only tasks that exhibited a significant, but weak, relationship with participation in ball sports, although the direction of the relationship was unexpected: males who participated more frequently in ball sports exhibited slower reaction times on these three tasks (but no difference in errors). This finding somewhat contradicts previous studies reporting that footballers who participate frequently in their sport tend to be more accurate on 3D mental rotation tasks than non-athletes (Jansen et al., 2012; Jansen and Lehmann, 2013). As mentioned above in relation to the late follicular phase in women, it's possible that that the slower reaction times, with no difference in errors, might reflect a difference in strategy rather than in performance. Further research is needed to understand the true nature of the relationship between participation in ball sports and spatial cognition in males, and how this might relate to strategic approaches during play.

4.5. Mood and perceived performance

Finally, the relationship between mood and cognition was investigated in this study, to identify whether females' perceptions matched their performance outcomes. Before completing any cognitive tests, naturally cycling participants in this study were asked about their perceptions of their own changes in mood, symptoms and performance throughout a typical cycle. Participants reported that, in a typical cycle, they normally experience a higher number of

menstruation, not during. These perceptions contrast the results of the cognitive tests: on the day of testing, even though menstruating females reported poorest mood and symptoms if testing during menstruation, and 39% believed that their cognition was being negatively impacted by their mood during this phase, they performed best across the cognitive battery on that same day. Interestingly, mood or symptoms did not mediate the effect of phase on cognition throughout the cycle in this sample. This suggests that the processes that impact mood and cognition in the context of the menstrual cycle might differ. Indeed, it is worth noting that the three areas of reporting (cognition, mood and symptoms) have different representations within the brain, even though they may interact with the same single phenomenon (in this case hormonal changes throughout the menstrual cycle). For instance, mood is often associated with orbitofrontal and ventromedial aspects of the prefrontal cortex (Kringelbach, 2005), inhibition is more strongly characterised by lateral aspects of the prefrontal cortex (Snow, 2016), and spatial cognition is largely related to parietal lobe activity (Marshall and Fink, 2001).

This incongruence between perceptions and objective results is possibly rooted in erroneous popular beliefs, including the assumption that all females suffer from poorer mood pre-menstruation. A systematic review by Romans et al. (2012) discounted this belief, concluding that there is no clear scientific evidence to support the notion that mood deteriorates cyclically in the pre-menstrual phase. The literature, in fact, reports that negative mood is typically worst during actual days of bleeding (Martin et al., 2018; Romans et al., 2013), which is consistent with the findings reported here. It should be emphasised that there is a high individual variability in how females experience, and perceive, any potential changes throughout the menstrual cycle, and therefore group-level findings may not be applicable to all. Nonetheless, the disconnection between symptoms, mood and cognitive performance observed in this sample can provide the basis for positive conversations between coaches and athletes, emphasising that symptoms do not necessarily impact performance.

According to a narrative review by Carmichael et al. (2021), 50-71% of female athletes perceive their performance to be impaired during the late luteal (pre-menstruation) and menstruation phases. Some studies have indeed reported phase effects on specific aspects of performance where, for example, meta-analysis by McNulty et al., (2020) reported that endurance and strength performance were likely reduced during the early follicular phase,

aligns with the cognitive findings reported here, where menstruating females performed generally worse during the luteal phase, but the high variability in the data warrants caution in interpreting this as a generalisable effect. Indeed, a recent review by Le et al. (2020) on changes in cognition and mood throughout the cycle emphasises the impact that individual differences have on such effects, as some studies have found stronger cognitive effects in women who suffer from premenstrual syndrome. Therefore, it is important to acknowledge that there is significant intra- and inter-individual variation in cycle patterns and associated symptoms, and the individual nature of each female's unique cycle means that symptoms and experiences will not be universal.

Therefore, based on the data reported here, there appears to be an incongruence between females' perceptions of how the cycle impacts their mood, and their actual reporting of mood and symptoms, and an even stronger conflict between females' perception of their cognitive performance and their actual outcomes. These results are aligned with the literature (Martin et al., 2018; Romans et al., 2013, 2012) and contribute to the argument that pre-menstrual mood changes might be an erroneous belief worth challenging, but, more importantly, that cognitive performance appears to peak when females believe they are at their worst. The data presented here points to an existing complex puzzle in moods and in cognitive sub-domains, but it would be precarious to conclude that the menstrual cycle has determining effects on cognition in all females and in every cycle. An understanding of a potential interaction between mood, cognition and the menstrual cycle should be explored through further research, as it could prove highly impactful for those most affected.

In the context of sport participation, this contrast between poorer mood and better cognitive performance during menstruation provides a positive outlook on the menstrual cycle which could help reshape perceptions of performance in naturally cycling females, and aid coaches in supporting female athletes in their approaches to training and competition.

To the authors' knowledge, this is the first study to implement a sport-related cognitive battery to investigate cognitive changes across the menstrual cycle. A key strength is the inclusion of males, females using contraception and naturally cycling females, enabling better comparison to reference groups. The novel cognitive battery implemented here was largely based on existing validated tasks, except for two novel tasks of timing anticipation, and presented good to excellent internal consistency and reliability. To reduce type I error, composite scores from the cognitive battery were derived and used as the primary analysis of the paper. It should be cautioned that, as a model of the information processing components of these tasks, the factor solution is oversimplified and there is much detail that is not included. As a follow up to this primary analysis, and merely to provide a full picture, the additional analyses conducted on individual tasks are presented to allow for in-depth examination of a broad number of cognitive domains that underpin the findings observed in the composite scores.

The use of an online platform to deliver the tasks enabled the researchers to reach a wider and more diverse population than what is normally possible in university settings. However, this also meant that the conditions under which tests were conducted was unknown and not controlled, and task outcomes are also subject to reliable broadband connections on participants' laptops.

There was a high sensitivity to novelty in the cognitive tasks, which was evidenced by the strong time effects observed in the test-retest analyses. This learning effect weakened the within-subject analysis, and might explain why phase differences in reaction time variables were often only present in the first testing session. Further to this, it is well established that the construct validity of executive function tasks changes with repetition, where the first administration of a task measures what it was intended to, but subsequent performances are subject to changes in strategy. This is a perennial and unavoidable methodological challenge for researchers of executive functions which should be accounted for in the interpretation of repeated tests (Burgess, 1997). Error variables provided more consistent results even after controlling for test-retest effects. This reflects previous research, where multiple authors (Mordecai et al., 2008; Maki et al., 2002; Hampson, 1990), reported significant learning effects on 3D spatial tasks with some sex and cycle phase differences evidenced in the first testing

removal of several reaction time effects in the second testing session might suggest that, where hormonal effects do exist, they might perhaps be removed through practice, providing a promising avenue to potentially reducing injury risk in female athletes through pre-game drills.

The study was well powered for analyses between broad population groups, which provides some confidence that, at broad population level, cognitive performance on these tasks does not differ significantly between males, females on contraception and naturally cycling females. The within-subject phase analysis was slightly underpowered for some phases, but was well powered for the luteal phase analysis.

It should also be noted that the sample studied here included young (18-35 year-old) healthy males and females who are at the height of their cognitive development and therefore may potentially introduce a ceiling effect with the tests presented. From a certain perspective, this is a strength of the study, since if significant findings are reported in a young healthy group, they might indeed be attributed to the variables studied. Nonetheless, this also creates a limitation for generalisation outside this demographic. Studies pertinent to hormonal effects on cognitive function have shown stronger effects in peri- and post-menopausal women where oestrogen deficiency is sustained for prolonged period of time, rather than a few days, indicating that hormonal changes do indeed impact brain function, but this effect might be minimised in healthy young cycling females (Sundström Poromaa and Gingell, 2014).

The inclusion of a hormonal contraception group enabled to test for within and between-gender effects, comparing menstruating females with females that exhibit an assumed specific hormonal profile. Nonetheless, it should be noted that the participants included in the contraception group made use of different forms of contraception with differing hormonal concentrations and combinations, therefore the hormonal profile of this group cannot be considered homogenous and caution is warranted with extrapolating these results to use of any form of hormonal contraception. Considering the effects found in this study in menstruating females, further research might want to explore how different concentrations and combinations of hormonal contraception might influence cognitive function in pre-menopausal females.

Much attention had been given to the luteal phase in the literature, with little focus on cognition in the ovulatory phase (Bernal and Paolieri, 2022), largely due to the difficulty in identifying this phase without the use of biomarkers. App-based tracking methods have enabled females to gain more control and understanding over their cycles in recent years and, while these still present their limitations in accuracy (Broad et al., 2022), they provide a useful general indication of cycle phase for females with regular cycles (Elliot-Sale et al., 2021). It should, however, be cautioned that all phases reported in this paper were grossly estimated based on participant app tracking (of at least 3 months prior to the study). Ovulation was not confirmed and the luteal phase was not broken down into its three key sub-stages, as both of these could only be done accurately through the use of biomarkers. Considering that there are strong differences in hormonal levels and their fluctuations between individuals (Cook et al., 2017) which are further subject to circadian changes (Liening et al., 2010), future research should consider measuring hormonal levels at the time of testing, and accounting for circadian changes, to yield more robust results and to determine whether hormonal concentrations are the driving factors of cognitive changes throughout the cycle. As previously discussed, it should also be emphasised that there are high intra- and inter- individual differences in females' experiences of, and reactions to, their cycles. This is reflected in the noticeable variability in the data and cautions against the generalisability of the results in such studies, as some females may be affected more strongly than others, and perhaps others not at all. Therefore, there is a need for larger sample sizes to better explore individual differences in links between menstrual cycle phase and cognitive performance. This could prove particularly beneficial for those most affected.

4.7. Conclusion

The findings from this proof-of-principle study suggest that visuospatial and anticipatory processes, which might be involved in some sporting activities, fluctuate throughout the menstrual cycle. Performance on the cognitive tasks, most notably for spatial timing anticipation, was better during the menstrual phase and worse during the luteal phase. Females reported lower wellbeing scores during menstruation, where they also perceived their cognitive performance was negatively affected. In contrast to this, performance on the cognitive tasks, most notably for spatial timing anticipation, was better during the menstrual phase and worse during the luteal phase. This pattern therefore provides prima facie evidence

for a hypothesis that there could be cognitive determinants to injury risk in some female

against any generalisability of the findings, and further research is required to corroborate these results. Further research with accurate cycle tracking, and in relation to objective measures of sport performance or injury risk, is required to corroborate these findings and determine their usefulness and application in athletic populations. If this theory holds true through further testing in female athletes, future research should consider the development of effective mitigation strategies where appropriate.

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