PHYSICAL PROFILING OF THE FEMALE FOOTBALLER:

Special reference to the menstrual cycle

By

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CONTENTS

ABSTRACT

There has been an exponential rise in the development and professionalism of female football, which has led to an increase in scientific research. However, there still remains a large disparity to male football and alarmingly, much of the current research has been replicated from male football ignoring the inherent physical and physiological differences between genders. One such physiological phenomena is the menstrual cycle. The menstrual cycle is a common and regular occurrence for females, whereby, monthly fluctuating hormones work in synergy and cause alterations in physiology and potentially physical performance. Though these physiological and physical changes have been well documented, there remains a paucity of research investigating the effects of the menstrual cycle on physical performance in team sports and particularly football. Accordingly, the aims of this thesis were three-fold 1) to investigate football-specific physical and physiological characteristics of the female footballer, 2) to determine the potential effects of the menstrual cycle on isolated measures of football-specific performance, and 3) to observe whether the menstrual cycle modulates changes in physical performance during competitive match-play.

To facilitate aim one, 36 sub-elite female footballers provided blood samples and completed a battery of isolated physical tests of football-specific performance (countermovement jump, 3x30m sprint, 4x5m change of direction and the Yo-Yo intermittent endurance test level 2) at three timepoints throughout the season. To fulfil aim two, nine sub-elite females completed the same series of physical tests as aim one (except the Yo-Yo intermittent endurance test level 1 was used) during two specifically chosen phases of the menstrual cycle (follicular phase, days 5-7; luteal phase, days 21-22). Finally, to accomplish aim three, 15 elite female footballers were observed during a 4-month period throughout a total of 76 match observations, during the main phases of the menstrual cycle (36 in follicular phase and 40 in luteal phase).

There was an observed change in physical performance across the season. Peak performance was observed following the preseason period, however, reductions in physical performance were observed as the season continued. Inversely, blood-borne parameters (e.g. creatine kinase) were elevated when performance was at its highest level, whilst decreasing as the season continued. The results of study two suggested there was an observed reduction in maximal endurance performance during the luteal phase, with a 14% reduction in meters covered during

the Yo-Yo intermittent endurance test level 1 compared to the follicular phase. However, no changes were observed in sprinting, agility, or countermovement jump performance. The findings of study three indicated that during competitive match-play there was an observed difference in high intensity distance covered across the menstrual cycle; with 12% greater distance covered during the luteal phase. However, this difference was smaller than the match-to-match variation (coefficient of variation = 39.5%). There were no observed differences between phases across all other competitive physical performance metrics. On the individual level, differences between phases were only systematically observable in one player.

The findings of this thesis provide further insight into the physical profile of the female footballer and is the first to consider female specific physiology as a factor that could potentially alter physical performance. The current studies indicate that isolated sport-specific physical performance fluctuates throughout the season, nevertheless, when assessing or monitoring endurance performance, it would appear advantageous to keep the phase of the menstrual cycle (when possible) consistent or at least documented. However, these observed effects were not so prominent during competition. Therefore, the menstrual cycle, does not seem do play a major contributing factor to variations in physical performance during matches and interventions are not particularly necessary. The current studies provide implications for the design and micro management of training, monitoring and the assessment of the female footballer, whilst affirming that the menstrual cycle should not be perceived as a mitigating factor for changes in physical performance. Finally, this thesis has also provided further evidence of the complexity when assessing the menstrual cycle alongside aspects of physical activity and overall performance.

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LIST OF ABBREVIATIONS

Adenosine triphosphate	ATP
Anaerobic speed reserve	ASR
Average heart rate	$HR_{average}$
Basal body temperature	BBT
Beats per minute	bpm
Blood lactate	Bla
Bone mineral density	BMD
Central defender	CB
Central midfielder	CM
Change of direction	COD
Coefficient of variation	CV
Confidence intervals	CI
Countermovement jump	CMJ
Creatine kinase	CK
Defenders	DEF
Drop jump	DJ
Early follicular phase	EFP
Effect size	ES
Fédération Internationale de Football Association	FIFA
Five-jump test	5JT
Follicular phase	FP
Follicular stimulating hormone	FSH
Forwards	FWD
Global positioning systems	GPS

Goalkeeper	GK
Heart rate	HR
High intensity	HI
High speed running	HSR
Individualised	IND
Intrauterine devices	IUD
Judo specific fitness test	JSFT
Lactate threshold speed	LTS
Late follicular phase	LFP
Late luteal phase	LLP
Low intensity	LI
Luteal phase	LP
Luteal phase deficiency	LPD
Luteinizing hormone	LH
Magnitude-based differences	MBD
Maximal aerobic speed	MAS
Maximal oxygen consumption	$\dot{V}O_{2max}$
Maximal sprinting speed	MSS
Maximum heart rate	HR_{max}
Menstrual cycle phases	MCP
Meters per minute	$m \cdot min^{-1}$
Mid follicular phase	MFP
Mid luteal phase	MLP
Midfielders	MID
Moderate intensity	MI

Oral contraceptives	OCP
Peak heart rate	HR_{peak}
Peak oxygen consumption	$\dot{V}O_{2peak}$
Phosphocreatine	PCr
Premenstrual syndrome	PMS
Record of perceived exertion	RPE
Repeated sprint shuttle ability	RSSA
Squat jump	SJ
Standard deviation	SD
Total distance	TD
Union of European Football Associations	UEFA
Vertical jump	VJ
Very high intensity	VHI
Very high-speed running	VHSR
Wide defender	FB
Wide midfielder	WM
Yo-Yo intermittent endurance test	YYIET
Yo-Yo intermittent endurance test level 1	YYIET1
Yo-Yo intermittent endurance test level 2	YYIET2
Yo-Yo intermittent recovery test level 1	YYIRT1
Yo-Yo intermittent recovery test level 2	YYIRT2
Yo-Yo intermittent test	YYIT

1.0

INTRODUCTION

1.0 INTRODUCTION

Association football (often referred to as Soccer), is a multifaceted sport, requiring players to have high levels of physical, technical and tactical abilities [1, 2]. Even though technical and tactical abilities are of grave importance, enhanced physical capacities are often identified as an important determinant of football performance [3-7]. The physical profile of a football match is regularly characterised as a self-modulated, multi-directional, and intermittent based activity; whereby, multiple high intensity (HI) bouts (running and sprinting) are interspersed with, periods of low intensity (walking, jogging), active, and passive recovery [8-11]. During a 90-minute match, previous time motion analysis and global positioning systems (GPS) have been used to record a total distance (TD) of approximately 10-12km, with the majority of this distance covered at low (LI) and moderate (MI) intensities, irrespective of gender [3, 4, 7, 11-19]. It could therefore be suggested that football is predominantly an aerobic sport in nature with anaerobic contributions in the form of HI actions interspersing these low intensity and passive periods [2, 4, 20, 21]. These periods of HI activity have been attributed to the most decisive actions during competitive football matches [2, 17, 22], thus suggesting that successful performance in football is largely dependent on a players ability to reproduce bouts of HI exercise and reduce the onset of fatigue [2, 20, 21, 23, 24]. However, the ability to do so may be influenced by other factors, such as gender [7], competitive standard [12] or level of fitness [4]. The knowledge of the activity profile and gross demands of the activity profile provide some overview of the demands of the sport; however, this needs to be considered in relation to the physical responses and the sporadic and contextual specific nature of match-play.

The all-inclusive understanding of the physical and technical demands of football provides the basis for effective scientific support [10, 19, 25], informing practitioners of physical development requirements [2, 19, 25, 26] and preparing players for 'worst case' scenarios; whereby, optimal strategies can be implemented to maintain peak performance [14, 25, 27]. As seen in the ergonomic model for the analysis of football from Reilly, [25] (Figure 1), the demands of match-play are integral prior to optimising a players physical profile. At present, the vast majority of this research has been conducted in male footballers [7, 28]. Unlike other sports (e.g. Tennis), male and female football is played under the same rule constraints and with the same equipment. Thus, there is often a belief that the physical response, requirements, and demands are the same for both genders. Research has suggested that the relative physiological loading is similar and that the energy systems are also similarly taxed in both genders [2, 7, 28]. However, it has been suggested that female players cover considerably less HI distance than male footballers [2, 7, 28]. Although this information could be considered to provide information regarding the physical demands of female match-play, there is a lack of specificity in the data collection and in the considerations of the female footballer, potentially underestimating the 'true' demands and physical requirements.

Although there is a plethora of information about the physical capacities and match demands of male football, there is a clear disparity in the amount of research that exists for the female footballer [7]. Over the last decade, the interest, funding, participation, and professionalism of the female game has exponentially increased [28, 29]. This can be observed through an increase of 34 % of females participating in football rising to 29 million from 2000 to 2011 [29]. Moreover, the World Cup in Canada (2015) resulted in a new total attendance record for a FIFA competition. Additionally, there was an increase of over 33% in viewership of the Women's World Cup in Canada, than the 2011 FIFA Women's World Cup held in Germany [30]. Furthermore, as of 2020, UEFA will increase funding for all its 55-member associations by 50% [31]. Collectively, this has driven the professionalism within the female game, with players at the highest level now becoming employed on a professional or semi-professional basis [28, 31]. Consequently, elite players are now facing an increase in match exposures, alongside, a concurrent increase in training loads and volumes [28]. These players are therefore exposed to increasingly greater and ever-changing physical demands and, as such, comprehensive descriptions of the physical capacities and match demands of the female footballer is needed to enable coaches and sports science support staff to identify the current demands placed on players during match-play, and apply this data to training and testing procedures.

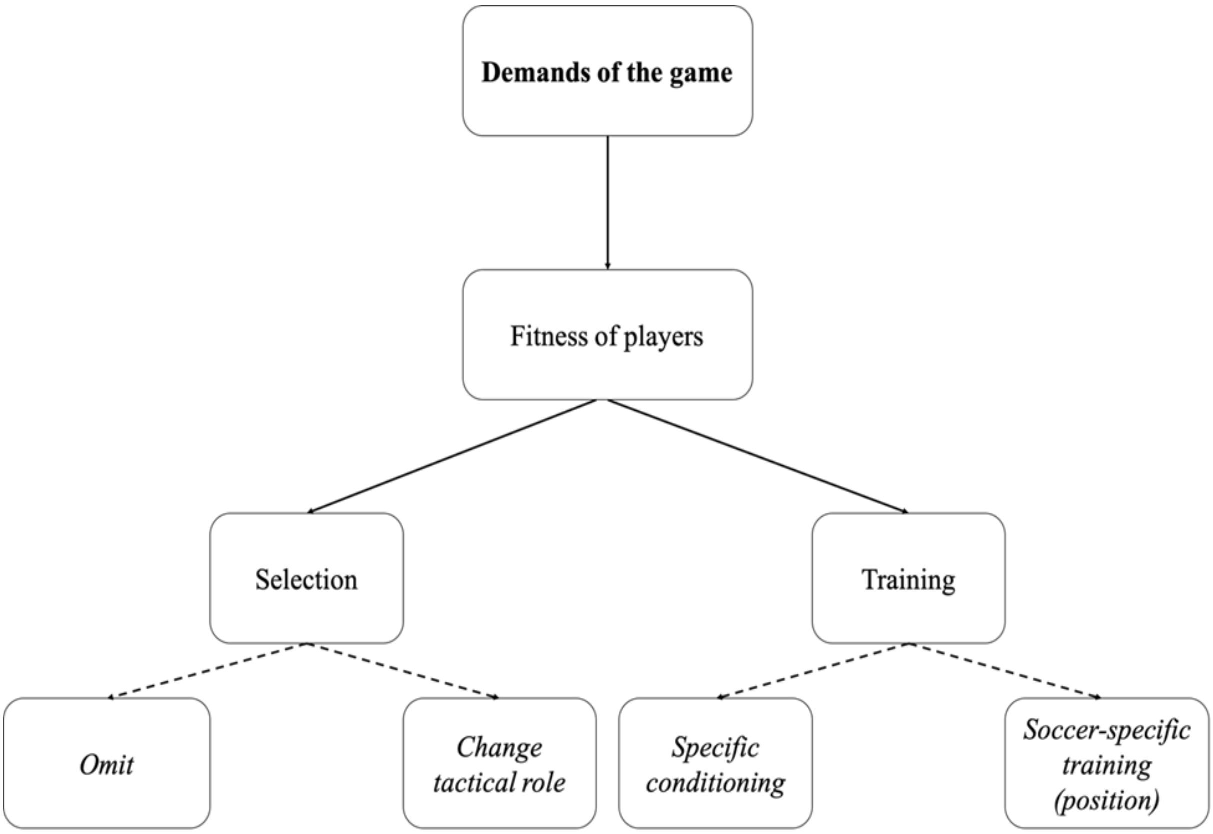


Figure 1 *An ergonomics model for the analysis of football (recreated from Reilly, [25]).*

While there has been progress in female football, and the research conducted, greater investigations are required that consider all elements specific to this population [28]. For example, information of the performance profiles of high-level players are uncommon with the majority of studies focusing on a single physical characteristic or performance testing during one measurement time-point [32-36]. This data provides a lack of information how fitness capacities interact, and are affected by continued exposure to competition and training. Moreover, during investigations of match physical performance, research practices are often directly adopted from male football [14, 37, 38], thus reducing specificity and highlights a potential ignorance to demands specific to the female athlete. Therefore, this information may not be representative to the female population. Examples of this can be observed when comparing genders directly. Bradley et al. [7] observed that males cover greater TD and HI distance during competitive match-play compared to females. Albeit that absolute differences exist, the manner of data collected was somewhat flawed, as the study utilised male orientated speed thresholds during match-play when comparing the movement profiles between genders. This method does not consider inherent physical differences between genders, as females are often not able to achieve the same speed thresholds was their gender counterparts [37]. The use of arbitrary speed thresholds adopted from male football could therefore misinterpret the activity demands of female football match-play and, in turn, underestimate the amount of HI actions, during match-play [37]. However, these practices are not uncommon, thus owing to a need for additional literature within female football to improve population specific practices [14]. It is somewhat disappointing that although it is widely accepted that there are differences between genders in terms of physical [7, 39-41], physiological [39, 41-44], and endocrinological parameters [39, 45], this is often ignored within female football and research.

When investigating the female footballer, gender specific physiology is rarely considered [28]. Specifically, females encounter from the age of menarche (~13 years of age [46]) until menopause (~50 years of age [46]), a monthly interplay of hormones which rise and fall in synergy, known as the menstrual cycle. Throughout the menstrual cycle, hormones fluctuate with the sole purpose of enabling conception. The main hormonal protagonists' oestrogen and progesterone, rise and fall throughout the menstrual cycle, signifying the different phases (Figure 2). When concentrations of oestrogen and progesterone are low, this is referred to as the early follicular phase (EFP), whilst an independent rise of oestrogen signifies the late follicular phase (LFP). After ovulation, both hormones (oestrogen and progesterone) are elevated, this is typically known as the luteal phase (LP). These hormones have been previously attributed to effecting metabolic, thermoregulatory, cardiovascular, and respiratory systems [39, 47-49]. Thus, in turn, potentially influencing football specific performance.

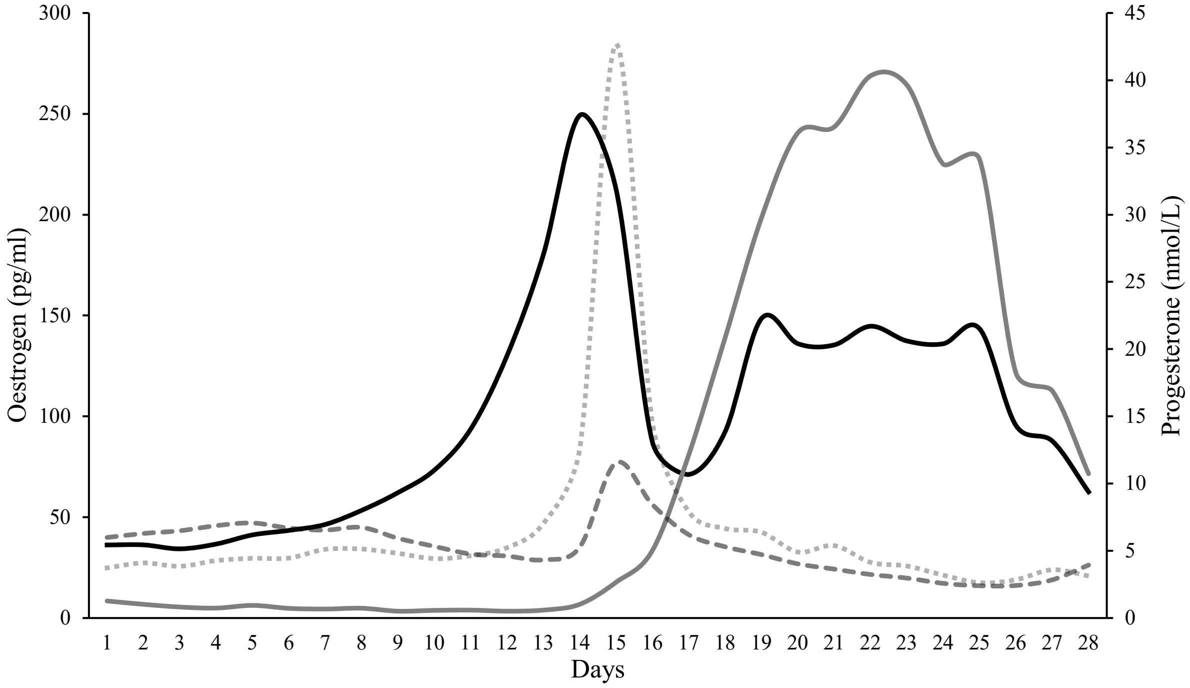


Figure 2 *A schematic representation of the change in female sex hormones (oestrogen, progesterone, luteinizing hormone and follicular stimulating hormone) over an average 28-day menstrual cycle. Recreated from reference values provided by Stricker et al. [50]. Note: Solid black line = Oestrogen; Solid grey line = Progesterone; Dotted grey line = Luteinizing hormone; Dashed grey line = Follicular stimulating hormone.*

Therefore, it is not unreasonable to speculate that the cyclical endogenous hormonal variations throughout the menstrual cycle might influence physical performance in female football players. However, several factors cause a difficulty when monitoring and evaluating the menstrual cycle and its synergistic hormones in this population. Issues such as between player differences in cycle length, and the regularity of testing required to accurately assess menstrual cycle phase (MCP). These reasons amongst others could possibly explain why females are underrepresented within research [51-53] however, as with the developments in the female game in relation to numbers and finances, advancements have also occurred in scientific practice, thus reducing the argument of difficulty in measurements when advancing this area of research.

Currently, there are limited studies describing whether the menstrual cycle influences physical performance in team sport athletes, specifically football [51]. It is, however, important to interpret meaningful changes in physical capacities and performance, to inform practitioners of the current success of their training programs and to understand the 'true' physical demands of match play. Currently, during these processes the physiological responses that are potentially influenced by the variations in endocrine hormones that fluctuate during the menstrual cycle [45] are inherently ignored. Therefore, owing that female players train, are tested, and compete throughout their menstrual cycle, the possible effects on physical capacities and performance should be investigated.

In summary, although there is an exponential increase in popularity, participation, and scientific endeavour within female football, yet there remains a large disparity in research between male and female players. As such, more research is warranted in terms of quantifying the physical attributes of the female footballer to provide sport scientists and practitioners greater specificity for the creation of training plans and monitoring practices. Additionally, there is an absence within the current literature addressing specific female physiology and its potential effects on a players' physical performance. More specifically, whether the menstrual cycle and its associated hormones (oestrogen and progesterone) could potentially influence the physical capabilities of the female footballer. Consequently, research is essential to determine whether this regular occurrence may affect physical performance so that practitioners may monitor MCP during future practice. Consequently, this information could be used when interpreting what is a meaningful change or potential variation based on cycle phase, or to subsequently inform decisions which could be used in player selection or appropriately managing their players.

2.0

REVIEW OF LITERATURE

2.1 PHYSICAL & PHYSIOLOGICAL PROFILE OF THE FEMALE FOOTBALLER

Irrespective of gender, a professional football match comprises of 90 minutes, split into two 45-minute halves, with the main purpose to outscore the opposition and win the match. During this time, players perform an interaction of multiple dynamic physical activities, which in turn results in an array of different physiological responses [2, 28]. Within a match, players are required to tax various physiological systems, but generally due to the relative contribution of low intensity activity and passive recovery, football might be considered an aerobic sport [2]. However, the aerobic component is interspersed with many instances of HI actions and utility movements, including high speed running (HSR), sprints, jumping, accelerations and decelerations in combination with technical actions such as shooting, passing, heading and tackling [1]. In female football, the physical activity profile has been found to change every 4s with approximately 1400 changes in activity per match [4, 13]. In order to prepare players for the physical nature of competitive football, the general physical demands of the game needs to be explored so that training can be modelled in a systematic and pragmatic manner (Figure 1, Chapter 1) [25]. As has been identified in male football, this is an ongoing process, whereby, continued re-evaluation is required to identify developments in the physical demands and newly developed styles of play [10]. Developments can also be identified when evaluating the current level of female football [28], with the relationship between the physical development of the game corresponding with the continued growth in the professionalism and physical abilities of female footballer [29]. The elements of football remain the same for both genders, although there are differences in how they are performed during match play, potentially leading to different physiological responses [7, 28]. Therefore, understanding the physical and physiological profile of the female footballer is important, especially for subsequent tailoring of training, recovery, and injury prevention strategies for the specific population [25]. Moreover, As the popularity of female football and the subsequent financial input increases, this should in turn result in an improvement in training and match-play monitoring via the adoption of access to (access to more sophisticated measured such as GPS). This in turn should start to improve the understanding of female specific responses to football match-play. During match-play there is a complex interplay of different physical actions, which all need to be appropriately conditioned for so that players are prepared to perform to their maximal abilities in a repeated fashion over the course of the season. The complex and sporadic nature of football match-play coupled with physical function specific to the female athlete makes it inherently difficult to monitor the responses to match-play and training; however, by advancing in our knowledge it should be possible to increase our specificity in relation to our conditioning programmes [54]. To begin to understand the specific demands associated with female football we initially need to consider the activity profile of female match-play, thereafter the response to this specific activity profile can be considered.

2.1.1 Notational analyses of female football match-play

Within the scientific literature, TD is the most common metric measured when observing the physical demands of match-play; this could be due to the relative ease of measurement and that it is not dependant on the calculation of different speed zones. The earliest information to note on the distances covered during female football match-play was unpublished data from Ekblom and Aginger [55]. The authors identified that in a cohort of seven Swedish national league players, they performed on average 8.5 ± 2.2km. More contemporary research has observed an increase in TD of ~17%, suggesting that the female footballer covers approximately 10km per match, regardless of playing standard (Table 1; range 9.32 to 10.44km) [4, 7, 12-14, 24, 56-66].

Table 1 *Match physical demands for elite female football players. Data expressed as means ± standard deviation (adapted from Datson et al. [28])*

References	Year	Subject number (n)	Competition level	Nationality/ region	TD (km)	Number of activity changes	Number HI bouts	HI running (km)	VHI running (km)	Sprint distance (m)	Number of Sprint bouts	Method of data capture
Andersson et al. [12]	2010	17	International	Scandinavia	9.90 ± 1.80	1641 ± 41	187 ± 15[e]	1.53 ± 0.10[e]		256 ± 57[g]		Video tape
		17	Domestic		9.70 ± 1.40	1593 ± 30	168 ± 12[e]	1.33 ± 0.90[e]		221 ± 45[g]		
Andersson et al. [56]	2008	21	Highest division	Scandinavia	9.90			1.15[e]				Video tape
Datson et al. [14]	2017	107	International	Europe	10.37 ± 0.95			2.52 ± 0.58[c]	0.78 ± 0.25[f]	168 ± 82[g]		Semi Automated camera
Gabbett and Mulvey [24]	2008	13	vs. male youth	Australia	9.32 ± 0.84			1.86 ± 0.48[a]				Video tape
		13	National		9.70 ± 0.48			2.01 ± 0.30[a]				
		13	International		9.97 ± 1.14			2.46 ± 0.49[a]				
Hewitt et al. [61]	2008	15	International	Australia	9.14 ± 1.03			0.62 ± 0.11[d]			280 ± 80[f]	GPS

Krustrup et al. [4]	2005	14	Highest division	Danish	10.30	1459	125^{e}	1.31^{e}		160^{g}	26^{g}	Video tape
Krustrup et al. [67]	2008	12	International	Scandinavia	10.0 ± 0.5			1.60 ± 0.40^{e}				Video tape
			Domestic		9.70 ± 0.60			1.40 ± 0.40^{e}				
Mohr et al. [13]	2008	19	Highest division	USA	10.33 ± 0.15	1379 ± 34	154 ± 7^{e}	1.68 ± 0.09^{e}		460 ± 20^{g}	30 ± 2^{g}	Video tape
		15	Domestic	Scandinavia	10.44 ± 0.15	1326 ± 24	125 ± 7^{e}	1.30 ± 0.10^{e}		380 ± 50^{g}	26 ± 1^{g}	
Ramos et al. [68]	2017	17	International	Brazilian	10.11 ± 0.87			0.76 ± 0.17^{d}		307 ± 119^{f}		GPS
Ramos et al. [66]	2019	21	International	Brazilian	9.72 ± 0.48			1.24 ± 0.95^{b}	0.70 ± 0.07^{d}	304 ± 54^{f}		GPS
Trewin et al. [62]	2017	45	International	Canadian	9.72 ± 0.90		62 ± 20^{d}	0.93 ± 0.35^{d}			20 ± 9^{f}	GPS

Note: GPS – Global positioning system, HI = High intensity, TD = Total distance, VHI = Very high intensity.

[a] – Descriptive definition, [b] – >12 km·h^{-1}, [c] – >14 km·h^{-1}, [d] – >16 km·h^{-1}, [e] – >18 km·h^{-1}, [f] – >20 km·h^{-1}, [g] – >25 km·h^{-1}

When considering that match-play is completed over a 90 min time-frame, and considering the TD of ~10 km [2], then the average velocity is 6 km·hr^{-1}. This in turn suggests the predominantly aerobic demands associated with football match-play and highlights that the majority of TD is conducted at LI. However, if we only consider the gross demands of the activity profile in relation to TD, we will considerably underestimate the demands of match-play. As further identified in table 1, and previously alluded to, female football match-play comprises of sporadic and a highly intermittent activity profile which equates to a change in activity approximately every 4 seconds. As part of these changes in activity the aforementioned periods of LI are interspersed with acute periods of HI.

It is for this reason that HI actions are often quantified in contemporary literature, especially when considering that the ability to cover a greater distance at HI has been shown to be a useful measure of physical match performance, training status [4], and competition standard [12, 13] in female football. Therefore, understanding the incidence and distances covered at HI are important when conditioning a player, preparing them for peak performance, and to model subsequent training [54]. In early research, HI distance in female football was not often measured in the literature [55] , however, owing to the advances in technology, studies investigating female international footballers have identified HI activity (>18km·h^{-1}) ranging from 1.5 to 1.7km [12, 13, 57]. However, Trewin et al. [62] observed that players covered 930m during 154 individual match observations when using a speed threshold of >4.58m·s^{-1} (>16.5km·h^{-1}). Datson et al. [14] found that the amount of distance during high speed running (HSR) (19.8 to 25.1km·h^{-1}), total HSR distance (>14.4km·h^{-1}) and total very high-speed running (VHSR) (19.8km·h^{-1}) were 608m, 2.52km and 776m, respectively (Table 1).

It is apparent that it is often hard to determine HI distances across female football, due to the differences in terminology (Table 1), in speed definitions, and due to the thresholds used to determine the instance of HI activity. The frequency of HI actions is approximately 5-fold greater than sprinting [38]. Which evokes large physiological stress due to the repeated bouts of anaerobic based activity [21]. One method to potentially quantify the anaerobic demands of match play and assist training prescription is to simply quantify the instances of high-intensity actions per match. There is however some equivocal literature associated with quantifying HI actions with previous literature suggesting 31 ± 19 [69], 62 ± 20 [62], and 169 ± 49 [14] HI running bouts during female match-play. These large differences in metrics could be related to the standard of match-play or could be indicative of the methods and thresholds that are used to record such data. Nevertheless, it is suggested that elite female players can cover up to 1.9 bouts of HI activity per minute of match-play.

Sprinting activity has also been previously proposed to precede crucial moments during matches, such as the movements required to win the ball, avoid opposition players or a goal scoring opportunity [2, 22, 70]. Moreover, a player's ability to reproduce repeated sprinting bouts throughout a match has been further described as a key physical performance indicator [69, 71-73]. In female football, a comprehensive understanding of sprinting performance is still lacking, potentially due to a current lack of consensus on female speed thresholds [33]. Much of the current research conducted utilises male based thresholds to quantify a sprint, meaning that female players are required to reach speeds of greater than 25.1 $km \cdot h^{-1}$ before it is classified as a sprint [74]. However, as can be observed in table 1, the current research suggests that females cover approximately 334m (range: 160 to 615m) per match [12-14, 57, 58, 62, 66, 69, 75]. Whilst, the number of sprints throughout a match are on average 23 ± 6 (range: 19 to 33) [13, 57, 75] accompanied by an average sprinting time of 2.4s per sprint [75]. Sprinting distances have further been described as a discriminating factor between levels of competition, as demonstrated by Krustrup et al. [57], with elite levelled players performing 24% greater distances than sub elite players (460 ± 20m vs. 380 ± 50m; d = 2.2). During match-play, sprints are most commonly performed at less than 10m and are both explosive and leading in nature.

It has been previously proposed that one's ability to quickly recover and maintain maximal performance during multiple sprinting bouts, is an important aspect of football performance [76, 77]. Moreover, understanding highly physically demanding elements of match-play can help in training prescription to manage "worst-case" scenarios and potentially reduce injury risk [38, 78, 79]. Current data identifying sequences of repeated sprint performance during female match-play is limited [38, 69, 80]. Gabbett et al. [69] previously suggested that sequences with two sprints occur ~5 times per match with an average recovery of 10s between the two bouts. However, more recent studies from Nakamura et al. [80] and Datson et al. [38] have observed that the incidence of a two-sprint sequence only occurs 2.5 ± 2.2 and 1.0 ± 1.1 times, respectively. The incidence of repeated sprint sequences in female football does not appear to occur often [38, 80, 81].

The notational demands of female match-play have recently been afforded additional attention and, as such, researchers now possess an increased understanding of these demands to allow for appropriate conditioning practices to be designed and implemented. It should however be considered that the notational match demands and associated variability between literature may be related to a number of contextual factors.

2.1.2 Contextual considerations

Global values of physical performance have been extensively researched [28], however, to assess physical performance with greater accuracy and certainty, it is essential to understand other facets that might affect the physical demands and performance of match-play [3, 17, 62]. This means that factors such as the 'natural' variation in match running parameters and certain contextual variables during match-play need to be considered [65, 82].

Between matches, due to the developments and characteristics of the match itself, variations in running distances are common. However, to identify whether these changes are 'meaningful' and to provide greater information to the players and practitioners, longitudinal repeated measures of match running metrics provide a general recommendation for what could be considered as 'natural' match-to-match variation in physical performance. Typically, this is expressed using the coefficient of variation (CV) as a percentage. Previous information on male footballers suggest that variations in running performance increases with greater running speeds [3, 83]. Currently, there are few studies that have identified the match-to-match variability in match physical performance in female footballers. Nevertheless, one study from Trewin et al. [62] extensively assessed the variations in match running metrics over a five-year period in a cohort of international female footballers. In support of previous findings in male footballers, variation in match-to-match performance increases with intensity. However, this variation is more pronounced in female footballers. The variation in TD is 6.4% [CI 5.8, 7.1], with HSR and the number of sprints varying between 33% [CI 30, 36] and 53% [CI 48, 59], respectively. Therefore, using metrics of HSR and sprinting to assess female physical performance might be [28] difficult due to this large natural variation. However, large variations in performance are "part and parcel" of the game, restricted to not only circadian variations in physical performance [84], but also contextual variables (e.g. playing location, level of opposition) within a match causing performance differences [85].

It has been previously demonstrated that training status is a key protagonist of physical performance in female football, with the differences in physical capacity leading to variations in physical output [4]. Unlike in male football, female players complete greater running and HI running distances in relation to the level of competition [12, 13]. For example, Andersson et al. [12] identified that HI (1.53 ± 0.1km vs. 1.33 ± 0.9km) and sprinting distance (256 ± 57m vs. 221 ± 45m) was significantly greater during international versus domestic levelled football matches. Current research in male football has suggested that factors such as match status (win, lose, draw), location (home vs. away), level of opposition (top, middle, bottom of the league standings), and current score line have impacted physical performance metrics [86-89], yet this has not been extensively investigated in female football. One study from Vescovi and Falenchuk [82] investigated the effects of match location (home vs. away), type of turf (natural grass vs. artificial) and match outcome (win, lose, draw) on match physical performance across 28 outfield footballers. It was identified that there were no differences in physical performance between home and away matches. However, MI (12.1–16.0 $km \cdot h^{-1}$) running (20.0±1.0 m min−1 vs. 16.4±0.9 m min−1) and HI (16.1– 20.0 $km \cdot h^{-1}$) running (8.6±0.4 m min−1 and 7.3±0.4 m min−1) was significantly greater on artificial turf than natural grass. During the various match outcomes, only relative sprint distance was greater during losses in comparison to draws (4.3±0.4 $m \cdot min^{-1}$ and 3.4±0.3 $m \cdot min^{-1}$).

A further study from Trewin et al. [65], investigated the effect of certain situational (match outcome, opposition ranking, and congested schedules) and environmental (altitude and temperature) factors that might influence physical performance. The findings suggested that, when drawing, playing a lower opponent resulted in a moderate increase in HI running (ES = 0.89). Whereas, winning against higher ranked opponents led to a moderately higher TD (ES = 0.75) in comparison to drawing. When investigating the environmental factors, the number of accelerations increased whilst there was a decrease in TD during altitude (>500m), the authors suggested this could be due to the potential decrease in partial pressure of oxygen, meaning that accelerations are easier (less resistance), whilst the reduced TD may be owing to allow for recovery between acceleration efforts. Generally, performance parameters were worse during higher temperatures (mean, 26.5°C), as increasing temperature leads to an increase in core and muscle temperatures and can alter substrate metabolism [90].

An additional contextual factor to consider when attempting to quantify the notational demands of match-play is that of playing position. It is well understood that within a football team there are various playing positions which require different tactical considerations and therefore, potentially differences in physical differences in physical output [1, 3, 7, 17, 91, 92]. The evaluation of each individual playing position is imperative to ensure specificity in training and the selection of players accordingly [28].

Where previous literature has considered differences in the notational demands between playing positions, current findings are somewhat equivocal. Certain research has identified differences between playing position [13, 14, 24, 61], whilst other studies have not identified a difference [7, 58, 62, 66]. The lack of consistency in research findings might be due to variations in methodology, including but not limited to differences in playing standards, and differences data acquisition procedures.

Of the current studies that have suggested differences between playing position, these often use three categories to quantify the different playing positions. Current observations demonstrate that midfielders (MID) cover approximately 10% greater TD per game than defenders (DEF) and forwards (FWD) [24, 61]. This has also been observed in HI distances covered, owing to the tactical demands of a game, DEF have been observed to perform less HI distances than MID and FWD (1.26 ± 0.11 km, 1.65 ± 0.11 and 1.63 ± 0.10 km, respectively), whilst these distances have been observed to be similar between MID and FWD [13]. Moreover, FWD have been observed to perform 17% and 37% greater amount of sprints per game than both MID and DEF, respectively [13]. However, there are differences in the demands due to specific roles within these positions [17]. For example, DEF could be further sub categorised as wide defenders (WD) and central defenders (CD) of which the demands vastly differ. A recent study from Datson et al. [14] evaluated the match physical demands of competitive international match play using five positional categories (CD, WD, central midfielders (CM), WM and FWD). The findings suggested that TD covered was significantly greater for CM (11 km) compared to all other playing positions (CD, 9.5km; WD, 10.3km; FWD, 10.3km) except WM (10.6km).

Datson et al. [14] further observed that CD covered the least distance at higher intensities compared to all other positions (ES 1.6–2.4). Moreover, CD also covered significantly less sprinting distance compared to all other positions, with FWD in absolute values covering the greatest distance (CD, 111 ± 42m; WD, 163 ± 79m; CM, 170 ± 69m; WM, 220 ± 116m; FWD, 221 ± 53m).

Owing to this information, practitioners should consider the effect of various different match conditions and how this might potentially influence physical performance outputs, with future research aiming to further investigate the interaction between contextual factors [65] and the female specific response to match-play and performance [28, 65]. In addition to understanding the notational analysis of female match-play, researchers and practitioners alike need to consider the physical response to these demands and consider these in context to the physical capacity of the players.

2.1.3 Physical responses and capacity of female footballers

As previously discussed, football match-play comprises a predominant aerobic contribution, thus suggesting the need to ensure appropriate aerobic conditioning. Moreover, appropriate aerobic conditioning has been suggested to promote recovery during football-based activity [4]. Therefore, it appears that a high oxygen uptake capacity ($\dot{V}O_{2max}$) is potentially related to a greater consumption and subsequent supply of oxygen, alongside a greater metabolic efficiency which may help delay the onset of fatigue [93]. The measurement of $\dot{V}O_{2max}$ during match-play is impractical and prohibited and, as such, heart rate (HR) measurements may be used to predict the level of energy expenditure via the HR - $\dot{V}O_2$ relationship. Although the HR-$\dot{V}O_2$ relationship may over estimate energy expenditure during football match-play due to changes in HR occurring in isolation to changes in $\dot{V}O2$, a number of previous studies have supported that the HR-$\dot{V}O_2$ relationship is valid method of predicting energy expenditure during intermittent exercise, with various HR indices (average ($HR_{average}$), peak (HR_{peak}), max (HR_{max})) being utilised as suitable and valid alternative to $\dot{V}O_2$ [94]. A study from Krustrup et al. [4] monitored HR in 14 female football players during competitive matches. The results indicated that $HR_{average}$ and HR_{peak} was 167 bpm (152-186 bpm) and 186 bpm (171-205), respectively. These values were equivalent to 87% and 97% of HR_{max}. These findings have also been identified in similar studies investigating match HRs in female footballers [12, 59, 67, 95]. These data would therefore suggest that during competitive match play, players experience moments of near maximal exertion, thus suggesting that although the activity profile is predominantly aerobic in nature, the intermittent and sporadic activity profile, and the interspersing HI activities also elicit a high anaerobic demand. This is further emphasised when considering Mohr et al. [59] suggested that the average oxygen consumption during competitive match play is 80 ± 1% of $\dot{V}O_{2max}$. Whilst, Krustrup et al. [4] has estimated that the average and peak oxygen consumption during match-play is 77% and 96% of $\dot{V}O_{2max}$, again demonstrating that competitive football is highly physically and physiologically demanding.

To directly determine the aerobic capacity of the female football, several studies have conducted $\dot{V}O_{2max}$ measurements [4, 24, 33, 59, 67, 96-102]. The aforementioned studies have identified that $\dot{V}O_{2max}$ values range between 47.0 to 57.6 $mL{\cdot}kg^{-1}$, which is not too dissimilar to that of male football players (55 to 75 mL.kg-1) [2], suggesting that female players have a good level of aerobic conditioning. Interestingly, even though the professionalism and increased sport science support is more apparent in modern female football, $\dot{V}O_{2max}$ values appear to have remained consistent over time [28, 33, 55]. As such, the ability to train $\dot{V}O_{2max}$ per se may be limited in relation to its effects on match-performance; however, improvements in a player's ability to work at a higher percentage of this maximum level may be important.

However, dissimilar to TD during match play, research has suggested that aerobic capacity is linked to competitive standard. Haugen et al. [33] found that senior national-team players (3.58 ± 0.37 L) had a 4.6% higher $\dot{V}O_{2max}$ than first-division players (3.25 ± 0.30 L; $p = 0.042$, d =

0.4), 13.1% higher than second-division players (3.08 ± 0.35 L; $p < 0.001$, $d = 1.2$). Such findings have been generally found across the current literature [4, 59, 102]. When considering the normalisation methods of $\dot{V}O_{2max}$ in relation to an individual's body mass, the observation of playing standard specific differences may be due to anthropometric differences.

The anthropometry of female footballers has been reported to be similar to that of other female team sports, with previous literature identifying that on average 20–27 years old, with a height of 1.61–1.70 m, weighing 57–65 kg, and, with a body fat percentage of 14.6–20.1 % and lean muscle mass of 45.7 ± 3.9 kg [28]. The anthropometry of female players, as with their male counterparts, has been shown to be specific to playing position [103] due to the specific demands and potential considerations associated with talent ID, playing standard [104, 105], and phase of the season when these measures are recorded [96, 106].

Although $\dot{V}O_{2max}$ is considered the criterion measure of aerobic capacity, it remains questionable whether this measure and the methods to collect this information (often maximal incremental running tests) are specific to football activity [5, 107]. As such, "football-specific" field tests have been created and utilised to assess sport-specific aerobic performance. One example, and one of the most commonly investigated in research is the Yo-Yo intermittent test (YYIT); which can be further segregated into the Yo-Yo intermittent recovery test level 1 (YYIRT1) and 2 (YYIRT2), and the Yo-Yo intermittent endurance test levels 1 (YYIET1) and 2 (YYIET2). From the testing protocols it has been concluded that the variations of the YYIET is more aerobic related, whereas the YYIRT is aerobic-anaerobic related.

The YYIET2 appears to be a reliable measure and has been previously suggested to provide an indication of match-specific physical capacity of the female football player [34]. Large correlations were observed between YYIET2 performance and, the TD ($r = 0.55$; $p < 0.05$) and HI ($r = 0.70$; $p < 0.01$) running covered during competitive female football matches [34]. Within the literature, values for elite players during the YYIET range between 1265 ± 133 m to 1774 ± 532m (Table 2). In a study from Bradley et al. [34] YYIET2 performance was found to differ between competitive level, with elite players covering greater distance (1774 ± 532m) compared to elite youth, domestic and sub-elite level football players (1490 ± 447m, 1261 ± 449m and 994 ± 373m, respectively). This information could, therefore, provide guidelines for the expected fitness level of athletes to compete at various competitive standards, albeit from one instance and may not be representative as to changes across the season.

As previously alluded to, the most decisive actions during match-play rely on high anaerobic-energy turnover. One method to quantify the anaerobic response during match-play, is through blood lactate (BLa) measurements [2]. One study from Krustrup et al. [67] suggested that mean BLa concentration was significantly higher after the first half compared to the second half (5.1 ± 0.5 mmol.L vs. 2.7 ± 0.4 mmol.L). Due to the constraints of competitive football, it is extremely difficult to obtain samples during matches, therefore, measurements are only possible during the half time or following the match. Thus, BLa concentrations rather reflect the activity pattern in the minutes prior to the collected sample instead of the demand during the complete half or match., which potentially means that the anaerobic demands of match-play are underestimated. Likewise, as is the case with a lot of female football literature, there is a paucity of research evaluating the anaerobic responses to match-play. Although it could be speculated that this response is similar across genders, additional research specific to female players is required to better inform our understanding of the anaerobic responses to match-play and subsequent management of the conditioning and monitoring practices female footballers.

When considering the decisive actions associated with anaerobic-energy turnover, it is imperative that the anaerobic capacity of female players is appropriately tested and conditioned to aid match performance. The use of the YYIR1 has been suggested to provide a good indication of physical match performance among female players due to its close relationship to the amount of HI distance covered during match play [4]. Meaning that the implementation of the YYIRT1 could provide information supporting the training process, talent identification and player selection [108]. As such, YYIRT1 performance has been quantified during few investigations. Senior domestic levelled players from Denmark, Spain, Germany and Tunisia were found to complete 1379m (range, 600–1960 m), 1224 ± 255m, 1051 ± 399m and 902 ± 405m, respectively [36, 108, 109] (Table 2).

Diversely, the YYIRT2 has been previously validated solely against the demands of male match-play [108], and has been suggested to determine an individual's ability to recover from repeated exercise with a high contribution from the anaerobic system. Female match-play has been observed to be conducted at a lesser proportion of HI action as their gender counterpart [7], thus, the use of the YYIRT2 is rarely performed with female football players. It should be considered that perhaps the YYIRT2 is too physically demanding for female footballers, due to the high starting speed and relatively quick increasing of running speeds.

Table 2 *Football-specific physical performance test results, from high-levelled female football players. Data expressed as means ± standard deviation.*

References	Year	Subject number (n)	Competition level	Nationality/ region	YYIRT (m)	YYIET (m)	10m sprint (s)	20m sprint (s)	CMJ (cm)
Andersson et al. [99]	2008	17	Highest division	Scandinavian				3.18 ± 0.03	30.5 ± 1.2
Bradley et al. [34]	2014	92	National	European		1774 ± 532c			
		46	Highest division			1261 ± 449c			
		42	U20 national			1490 ± 447c			
		19	Lowest division			994 ±373c			
Can et al. [110]	2004	17	Highest division	Turkey					34.48 ± 7.11
Castagna and Castellini [32]	2013	21	National	Italian					31.6 ± 4.8
			U19 national						34.3 ± 3.9
			U17 national						29 ± 2.1
Emmonds et al. [111]	2019	10	Highest division	England			1.87 ± 0.06	3.21 ± 0.07	31 ± 0.40
Hoare and Warr [112]	2000	17	U19 national	Australia			2.01 ± 0.08	3.47 ± 0.14	

Krustrup et al. [4]	2005	14	Highest division	Danish	1379[a]				
Krustrup et al. [57]	2008	12	National	Scandinavian					35.0 ± 1.0
Krustrup et al. [67]	2010	23	Highest division	Danish		1212 ± 90[c]			35.0 ± 1.0
Mara et al. [113]	2015	17	Highest division	Australia	450 ± 118[b]				
McFarland et al. [114]	2016	16	Collegiate	USA			1.92 ± 0.31		41.85 ± 4.98
Mujika et al. [36]	2009	17	Highest division	Spanish	1224 ± 255[a]				32.6 ± 3.7
		17	Second division		826 ± 160[a]				
Sedano et al. [104]	2009	100	Highest division	Spanish					26.1 ± 4.8
Taylor et al. [115]	2013	19	U15 national	England				3.56 ± 0.22	

Tumility. [116]	2000	20	National	Australian	1.91 ± 0.04	3.26 ± 0.06	51.0 ± 5.0
Vescovi and McGuigan. [117]	2008	51	Collegiate	USA			40.9 ± 5.5
Vescovi et al. [118]	2011	113	Collegiate	USA			42.0 ± 5.0
Vescovi [119]	2012	140	Highest division	USA	18.3 ± 0.9	21.6 ± 0.8	

Note: YYIRT – Yo-Yo intermittent recovery test, YYIET – Yo-Yo intermittent endurance test, CMJ – Countermovement jump.

[a] –YYIRT1, [b] – YYIRT2, [c] – YYIET2.

Although the varying YYIT's have been created under the premise that they replicate the physical demands of match-play and that they have been shown to be correlated with some aspects of match activity (e.g. HI running distance); there are still limitations to the test and its design. While it has been created to mimic the intermittent nature of football, the set distance of 20m is relatively arbitrary and the test is still incremental in nature removing a players use of self-paced running to ensure peak performance can be maintained [120]. As such, this limits the physical representation of the test. Moreover, the current investigations are often completed during one-time measurements; such that the fitness level and current conditioning are only representative to the timepoint of measurement within the season. Thus, careful consideration whilst interpreting and evaluating the physical capacity using such tests in relation to the demands competitive match play is required and should be used to supplement in-game measures and help inform practitioners during the training process.

Owing to the potential limitations of some field-based measures to assess physical capacity of players, practitioners and researchers alike utilise methods to acutely assess maximal sprint and lower limb force generation through methods such as, but not limited to, sprint timing activities and jump performance measures. There has been considerable research quantifying the sprinting abilities of the female football during maximal field testing. However, across the research there is a lack of consistent testing protocols (e.g. sprinting distance, stationary vs. flying start) and equipment used. During competitive match-play the average sprint distance has been previously described as ~19m [18], with the majority of these sprinting distances between 10 to 20m [121]. Therefore, during investigations, linear sprinting tests are typically performance over a 10 to 20m distance. Current results suggest that females when measured over a 10m sprint, require on average 1.94s (Min/Max: 2.31 ± 0.25s to 1.67 ± 0.07s) (Table 2) [35, 112, 116, 122-124], this translates to approximately 18.56 $km \cdot h^{-1}$ (Min/Max: 15.58 to 21.56

km·h^{-1}). Whilst measurements conducted over 15 and 20m have provided average sprinting times of 2.38 s and 3.5 s, which represents sprinting speeds of 22.68 km·h^{-1} and 20.52 km·h^{-1}, respectively [36] (Table 2). Alarmingly, as can be observed during maximal sprint testing across the varying distances, players may often not be able to reach the predefined threshold used in some of the previous literature [125], which may potentially underestimate the sprinting demands during match-play. This information suggests that the use of female specific thresholds, which as of yet has not be conclusively determined should be determined and implemented. Moreover, another option would be to implement individualised speed thresholds, to add specificity to the data and to reduce the chance of an underestimation of activity [37].

It should also be considered majority of the research associated with maximal sprint testing utilises a stationary start. Therefore, when measuring maximal sprinting performance using distances such as 10m (although mimics closer to sprinting distances during a match), the acceleration phase often takes several meters to complete, thus limiting the maximal sprinting speeds. Therefore, to ensure that maximal sprinting speeds can be confirmed, practitioners often measure sprinting performance ≥30m. The limited number of studies assessing 30m sprinting time has suggested that 30m-sprinting performance ranges from 4.81 s (22.45 km·h^{-1}) to 5.06 s (21.34 km·h^{-1}) [67, 124, 126]. Whilst, research has suggested that players sprinting time was on average 5.6s and 5.9s over 40m and 40yd, respectively [35, 117, 127, 128].

Although sprinting distance within match play has been a discriminating factor between levels of competition, there is a paucity of research to comparing sprint test performance across differing levels of competition. Of the limited research, Haugen et al. [35] identified that over 40m, national-team players were 5% faster than second-division players ($p < .001$, $d = 1.1$) and first-division players were 3% faster than second-division players (p = .040, d = 0.7). Therefore, more research is required to identify differences between competitive standards, so that information could be used for talent identification and selection purposes.

The use of repeated sprint ability (RSA) testing has been previously conducted to test a player's ability to repeat these HI actions and quantify the rate of fatigue between and throughout the bouts [101]. As such, training interventions could be designed to help prepare players to tolerate the "worst case scenario" during match play. Currently, there is a paucity in research assessing RSA ability during field testing. One study from Gabbett [101] assessed 19 elite female players during 6 x 20-m maximal effort sprints on a 15-second recovery cycle. Time to complete the protocol was found to be 20.9 ± 0.5s for national players, whilst state players took 23.3 ± 0.4s. These results give a basic insight into the differences between competitive standards during RSA testing. As such, future research should look to further investigate the RSA ability of the female footballer to help create a greater overall understanding of the physical capabilities of this population. This should be conducted whilst concurrently acknowledging its limitations during match-play and adjusting the testing accordingly.

In addition to maximal sprint testing, it is also common practice for measures of lower limb strength and power to be performed to assess fatigue induced changes and to quantify training ad recovery needs of players. It is widely accepted that strength and power are important elements of a footballer's physical repertoire has been demonstrated in male football [2, 28, 129, 130]. Many fundamental technical actions require a minimal level of strength and power, such as tackling, duels, jumping and shooting [35, 130]. Moreover, previous research has suggested that there is a significant relationship between team success and average jump height and leg extension power, thus suggesting that strength is an important component for performance [28, 129]. However, at present there is an underwhelming lack of information describing the technical abilities of the female footballer within match-play. Moreover, there is a paucity of research describing the relationship between measures of strength, technical proficiency and the potential link to success. Of the current literature one study from Bradley et al. [7] investigated technical events during elite level female football matches. This study measured the percentage of successful passes, individual time spent in possession of the ball, number of balls lost, mean number of ball touches for each possession, and the percentage of total duels won (heading and ground duels). Although this study provides a descriptive overview of physically inciting events, there is no information regarding physical requirements or demands of such actions. Therefore, future studies should aim to quantify the physical demands of technical actions during match-play, such research could provide greater information to describe the holistic physical demands of competitive female football.

Nevertheless, strength is considered an important aspect of physical performance and protective of potential injury. Therefore, it is an essential element of the female footballers training cycle [28]. Measuring jumping performance has been shown to be a valid, time-efficient and relatively easy measure of lower-limb strength and power [129, 131]. Moreover, jumping performance has been demonstrated to correlate largely with maximal squatting strength in football players [132]. The most commonly used jump in the literature is the CMJ. This is primarily due to CMJ needing less technical ability than other forms of jumps [104]. Moreover, CMJ has been suggested to greater reflect the physical skills executed within football [28, 104]. Subsequently, the current findings suggest that elite female footballers CMJ performance is on average 34.5 ± 6.2cm (range: 26.1 to 51.0cm) (Table 2) [32, 35, 36, 57, 67, 99, 101, 104, 110, 117, 128]. Moreover, though greater physicality has been regularly linked with superior physical performance within female footballer players, studies from England [105] and Spain

[104] have suggested that there are no differences in CMJ performance between competitive standards.

2.1.4 Physical & physiological profile of the female footballer summary

In summary, owing to the large amount of TD (~10km) and the high aerobic energy contribution it might be suggested that female football players require a good level of endurance, which also has been highlighted to benefit recovery between HI bouts. In terms of activities performed at these HI's, the current research demonstrates large variations in performance, potentially due to different methodologies used to collect the data, alongside different speed thresholds used to quantify activity at higher intensities. Although it has been expressed that females require a different set of speed thresholds, the majority of research still uses male based thresholds which might not reflect the true demands of female match-play. Moreover, as expressed previously by Bradley and Vescovi, [37] it would be beneficial to use individualised speed thresholds, which could accurately assess group and individual changes in performance. Finally, there has been extensive research to quantify the physical and physiological demands of match-play, however, much of the research has taken a "one-size-fits-all" approach and has simply reproduced methodologies from male football and utilised them on a different population. As such, female specific physiology and physicality have often been ignored, therefore, understanding the "true" demands and requirements of female footballer to maintain "top-level" match-play still requires greater scientific rigour.

2.2 FEMALE SPECIFIC PHYSIOLOGY (MENSTRUAL CYCLE)

A recent review entitled "Where are all the female participants in sports and exercise medicine research", established that there is a large discrepancy in research conducted on female participants, with only 4 to 13% of articles including female participants [53]. When females are included into performance science research, female hormone fluctuations are often not considered or the testing occurs during the EFP, when hormone levels are low to minimise the effects (Figure 2, Chapter 1) [51, 133]. During a women's reproductive life-span, they are constantly exposed to an ever-fluctuating concentration of hormones. These endocrinological changes manifest themselves for essentially half of a female's lifetime. The key hormones have been associated with regulating and altering an array of physiological responses (Figure 3).

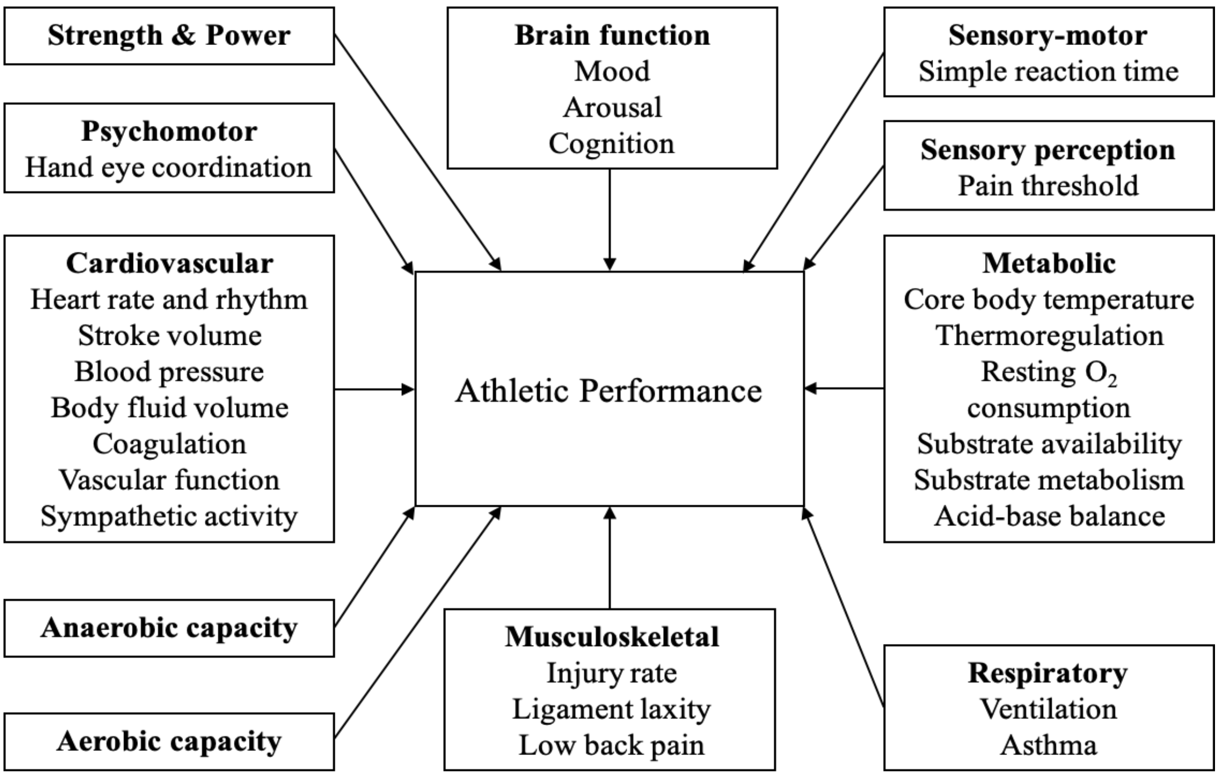


Figure 3 *Components of physical performance that may be affected by fluctuations in endogenous hormones during the menstrual cycle (recreated from Lebrun et al. [49]).*

For example, oestrogen has been previously demonstrated to influence the cardiovascular system, substrate metabolism, and even cognition [42]. Whilst, progesterone, has been suggested to affect thermoregulation, ventilation, and, to a lesser degree, the mode and usage of fuel for energy production [42]. Therefore, female athletes regularly encounter time-points where potentially, peak athletic performance might be compromised. Interestingly, though the participation and professionalism of female sports continually increases, the scientific understanding of the unique nature of female physiology across the reproductive lifespan and performance has not kept pace.

2.2.1 Physiology of the menstrual cycle

From the age of menarche (~11-13 years of age) up until the age of menopause (~50 years of age) females experience a circa mensal biological rhythm known as the menstrual cycle [46]. This cycle, occurs monthly in response to the interactions of hormones produced by the hypothalamus, pituitary, and ovaries (Figure 4). The menstrual cycle can be divided into two key phases: 1) the follicular phase (FP), and 2) LP, which are separated by ovulation. However, further sub phases can be well-defined and are often describable through a predictable pattern of hormonal changes (Table 3) [50, 134, 135], with oestrogen and progesterone being the most dominant of the hormones (Figure 2, Chapter 1). The length of the menstrual cycle is determined by the number of days between the first days of menses (menstrual bleeding) in one cycle to the onset of menses of the succeeding cycle. The median duration has been previously described as 28 days, with most cycle lengths ranging between 21 to 35 days [134]. Cycles below or exceeding this range are classed as polymenorrhagia and oligomenorrhea, respectively [134]. The variability of lengths during the menstrual cycle are typically due to fluctuations in the FP, with the LP remaining relatively consistent, with a duration of 14 days [52, 134, 136].

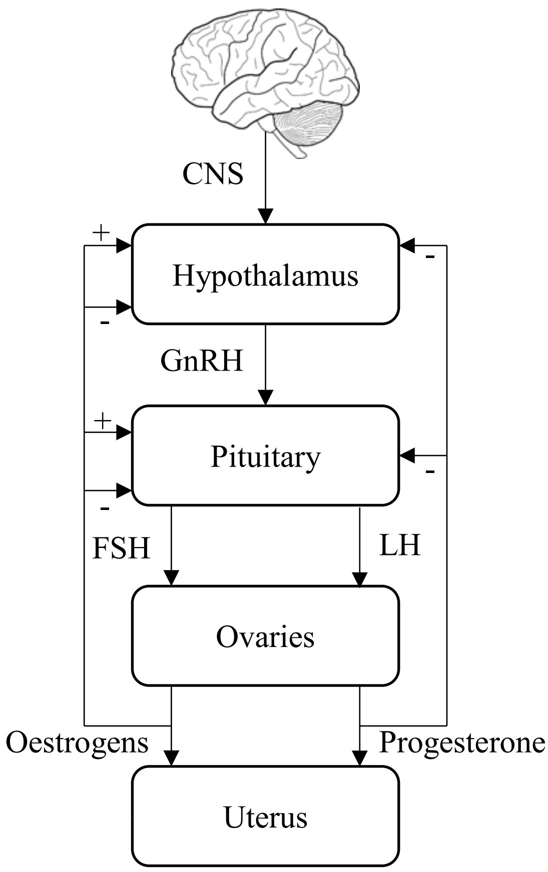


Figure 4 *The hypothalamic-pituitary-ovarian axis (recreated from Reilly, Atkinson & Waterhouse [45]). Note: CNS = Central Nervous System; GnRH = Gonadotrophin Releasing Hormone; FSH = Follicle Stimulating Hormone; LH = Luteinizing Hormone*

The FP begins from the first day of menses, which characteristically lasts for 4-5 days with a typical blood loss of approximately 30 mL [134, 137]. During the EFP, both oestrogen and progesterone are low. Following, this period follicles grow under the influence of follicular stimulating hormone. Oestrogen is secreted from the cells that surround this follicle and gradually increases, inducing the secretion of luteinizing hormone. As oestrogen levels increase further, reaching its peak concentration around days 9-13, a surge of luteinizing hormone is discharged, alongside a steep decrease in oestrogen and approximately one day later ovulation occurs. This signifies the ovulatory phase. A phase which has been suggested to occur 3- 5 days around ovulation [134, 135]. During these phases, the endometrial (the inner walls of the uterus) thickens in preparation for conception.

Table 3 *Menstrual cycle phase terminology with corresponding days of the menstrual cycle, where possible accompanied by an indication of corresponding hormone concentrations of oestrogen and progesterone [135].*

Terminology with corresponding hormone concentrations			Terminology including a range of hormone concentrations
Menstrual cycle phase (days of menstrual cycle*)	Oestrogen concentration	Progesterone concentration	Menstrual cycle phase (days of menstrual cycle*)
Early-follicular (2-7)	Low	Low	Follicular (1-13)
			Pre-ovulatory (1-13)
Late-follicular (9-13)	High	Low	Mid-follicular phase (12-18)
			Ovulatory (3-5 around ovulation)
			Ovulation (14)
Mid-luteal (18-24)	High	High	Luteal (15-28)
			Post-ovulatory (15-28)

*Note: * = Based on a 28-day average cycle*

Once ovulation has occurred (the matured follicle releasing an ovum), a temporary endocrine structure remains (corpus luteum), which is responsible for producing relatively high-levels of progesterone and the increase of progesterone denotes the start of the LP. Increasing levels of progesterone are then produced with a primary role to support the endometrium so that it can house an embryo, which can create the placenta. Peak progesterone concentrations occur around days 21-22, alongside an elevation in oestrogen. However, if conception does not occur, the progesterone secretion from the corpus luteum concludes during the late luteal phase (LLP), with coincident decreasing levels of oestrogen. The endometrium is no longer supported and it sheds off and constitutes menstrual bleeding. Finally, follicular stimulating hormones secretion augments and the cycle begins once again.

Based off the ratios between oestrogen and progesterone, three key phases can be identified: (1) low oestrogen and low progesterone signifying the EFP, (2) high oestrogen and low progesterone levels during the LFP and ovulation, and (3) high oestrogen and high progesterone levels which are present during the LP [135]. These phases are further and clearly defined in table 3.

2.2.2 Effects of menstrual cycle phase on performance

Peak athletic performance is composed of anatomic, physiologic, metabolic, biomechanical, and psychologic elements, which have all been suggested to be affected by various aspects of the menstrual cycle (Figure 3). The relative contribution of each of these elements varies, depending on the type of sport. As mentioned throughout chapter 2, football and the female footballer requires superior physical capacities to perform at the highest levels. However, the menstrual cycle and its fluctuating hormones have been suggested to affect these capacities and therefore, may impact peak footballing performance; at present there is an overwhelming lack of research investigating the effects of female-specific physiology on football performance.

2.2.2.1 Effect of menstrual cycle phase on aerobic performance

Aerobic performance can be crudely defined as performing physical activities that are sufficiently supported by aerobic metabolism and that can be performed for extended periods of time. Several determinants of aerobic performance have been suggested to be affected by oestrogen and progesterone oscillations throughout the menstrual cycle [47, 135]. These hormones interact and affect physiological and metabolic mechanisms [133, 138], examples include alterations in substrate availability and metabolism, core body temperature and respiratory function [39, 47].

$\dot{V}O_{2max}$ is the gold standard physiological indicator of aerobic capacity, thus, research has predominantly investigated the effects of the MCP on $\dot{V}O_{2max}$. Few studies have identified significant effects due to certain MCP. One study from Lebrun et al. [39] investigated 16 trained females during a maximal incremental treadmill test until exhaustion during the FP and LP. They observed a significant decrease during the LP in absolute $\dot{V}O_{2max}$ (-2%), as well as relative $\dot{V}O_{2max}$ (-2%). The authors further stated that there were substantial differences in relative $\dot{V}O_{2max}$ of up to 4 $ml \cdot kg \cdot min^{-1}$ between the phases. Potentially, these differences were linked to the observed changes in core body temperature using basal body charting (BBT), which coincides with increases in progesterone during the LP (approximately 0.3-0.5°C at rest). Such a change may have related to increased cardiovascular strain due to a rise in HR, high skin temperature and skin blood flow [160, 161]. As a result, this may have affected HR responses, which directly influences factors such as the fick equation for oxygen consumption. However, the majority of research using maximal incremental tests to exhaustion to assess $\dot{V}O_{2max}$ across the menstrual cycle, yielded no differences between MCP [139-146]. However, $\dot{V}O_{2max}$ is often less modifiable than other facets of performance, for example, time to exhaustion.

Aerobic performance can often be indirectly determined by means of performance testing (such as time to exhaustion tests) rather than physiological measures [147]. When investigating the effects of MCP on time to exhaustion testing, the results are equivocal. Jurkowski et al. [144] reported that there was an increase in time to exhaustion from 1.57 ± 0.32 min in the FP to 2.97 ± 0.63 min in the LP during cycle ergometer testing. Jurkowski and colleagues [144] attributed this partly due to changes in substrate metabolism, demonstrated by significantly decreased levels of BLa accumulation at exhaustion during the LP (FP, 8.12 ± 0.9 mmol/l; LP, 6.76 ± 0.6 nmol/l), meaning that there was delay in the occurrence of exercise intensity at the lactate threshold. As seen previously (Chapter 2), these BLa values are similar to that during competitive football, therefore, it could be assumed that this potential alteration in substrate utilisation could impact physical performance during football activity. However, Lebrun et al. [39] found that running time to exhaustion at 90% $\dot{V}O_{2max}$ was not significantly different between the FP (735.8 ± 58.8 s) and the LP (769.3 ± 64.1 s). These findings are similar to McCracken et al. [148] who also investigated time to exhaustion during treadmill running at 90% $\dot{V}O_{2max}$ in nine eumenorrheic females comparing similar time frames (mid follicular phase (MFP), 32.5 ± 0.5 min vs. MLP, 32.4 ± 0.5min; $p>0.05$). Although not directly associated with football, the intensities of the activities performed are similar as during football match-play. Therefore, as the gross demands across these tasks are somewhat similar, this could suggest that physical performance in footballers could be compromised in relation to the menstrual cycle.

Another form of aerobic performance measure includes time trial performance, whereby the end point is fixed and the time to achieve this set target is measured. In nature this activity type is less associated with football as the aim is to complete a certain constraint as quickly as possible. However, both exercise types are self-paced, whereby the effects of the menstrual cycle might lead to differences in how the exercise is performed. One study from Campbell et al. [149] investigated eight endurance trained women during the FP and the LP, completing a 4 kJ·kg body weight time trial as quickly as possible. The results suggested that time trial performance was greatest during the FP with the work completed 13% quicker compared to the LP. The authors attributed this to the changes in substrates used for fuel, with the percent contribution from carbohydrates being greater in the FP. That being said, Oosthuyse et al. [150] when investigating three periods on the menstrual cycle, (EFP, LFP, LP) identified a trend for a faster finishing time in a cycling time trial in the LFP (73% of the subjects showed an improvement during this phase of ~5.2 ± 2.9%). Which could be credited to the substrate effects of oestrogen and carbohydrate usage which coincides with the pre-ovulatory surge. However, McLay et al. [151] found no significant difference in 16km cycling finishing time (MF 26.05 ± 1.10 min, ML 26.23 ± 1.33 min; $P = 0.370$). Similar findings have been found during a 2000m rowing ergometer time trial, whereby, no significant difference was found between MCP [152, 153].

The aforementioned literature appears to suggest that any effect of the menstrual cycle appears to be specific to the mode of exercise and the intensity at which it is performed. As such, any effect in female football will be specific to the unique demands of the sport. As previously described, in addition to the aerobic component there is an anaerobic emphasis, therefore, knowledge of how the anaerobic exercise is affected by different stages of the menstrual cycle warrants further investigation.

2.2.2.2 Effect of menstrual cycle phase on anaerobic performance

Anaerobic performance can be simply described as maximal all-out activities which primarily taxes the adenosine triphosphate (ATP) phosphocreatine (PCr) system and anaerobic glycolysis which lasts for several seconds to approximately two minutes [154]. The hormones related to the menstrual cycle have been suggested to affect certain metabolic parameters [133], which in turn may lead to alterations in peak anaerobic performance. For instance, it has been previously documented that PCr recovery rates are greater in eumenorrheic athletes when compared to their amenorrhoeic counterparts following plantar flexion exercises in endurance athletes [155]. Therefore, it could be speculated that the hormones encompassed within a female's menstrual cycle may alter anaerobic energy processes, potentially leading to alterations in performance. As such, research has investigated the effects of the menstrual cycle on varying types of anaerobic performance, including, power output, sprinting performance and jumping performance. Presently, the majority of research has suggested that anaerobic performance is not affected by MCP, however, certain studies albeit sometimes with a lack of standardised and sound methodologies have identified significant differences between phases.

Anaerobic power (also known as power output) and anaerobic capacity are common measures of anaerobic performance. Several studies have attempted to investigate the changes in these parameters across the menstrual cycle. Much of the current research has demonstrated no differences in anaerobic power or capacity in relation to MCP [156-163]. Specifically, one study from Giacomoni et al. [156] observed no differences in maximal cycling power between EFP, MFP and LFP. However, the authors suggested that the presence or absence of premenstrual syndrome (PMS) symptoms might play a role in power output. This finding could be relevant when assessing physical fitness in women suffering from PMS.

Although the vast majority of research has not identified a phase-based difference in anaerobic power and capacity, one study from Masterson [164] conducted a Wingate anaerobic power test during the EFP and MLP. Results suggested that anaerobic power (FP, 532.35 ± 88.77 watts vs. LP, 566.06 ± 86.75 watts) and capacity (FP, 355.7 ± 52.02 watts vs. LP, 393.67 ± 56.44 watts) was significantly greater during the LP. Although the study had some positive elements, such as the counterbalanced design (eliminating sequencing or learning effects) and had relatively large sample size (in comparison to previous research, n = 32), the lack of hormonal documentation and clear definition of phases means these findings are difficult to interpret.

Currently, there are few studies that have investigated the potential effects of the menstrual cycle on repeated sprinting (all-out effort of approximately 30 s or less) performance. Of those that have investigated these differences, the findings in the research are equivocal. Earlier research suggested that single swimming sprint and repeated cycling sprint performance is greater during the FP [165, 166]. Whereas, more recent research has either suggested that no differences are apparent between MCP [157, 167, 168] or that greater performance exists during the LP [169]. Middleton and Wenger [169], found a significant enhancement in average 6-s work over 10 maximal sprints on a cycle ergometer in the LP. Although the authors found only small average differences, the improvement during the LP was exhibited in every subject. Moreover, if these results were translated into running time, this difference in work would translate to approximately one-meter difference in a six second sprint, which in terms of performance is a relatively large difference.

In the limited number of studies that directly measure sprint running performance across the menstrual cycle, the current literature suggests that there is no difference between MCP [109, 167, 168]. For example, a recent study from Tsampoukos and colleagues, [168] investigated sprinting performance and recovery during two 30s all-out sprints during the FP, LFP and the LP. Contrary to Middleton and Wenger [169], the latter did not observed any difference in sprinting performance. One explanation for the potential differences could be the number of sprints performed and the potential physiological response. Tsampoukos et al. [168] only compared performance during two sprints, whereas Middleton and Wenger [169] assessed the performance of 10. Previous research has indicated a greater inflammatory response during the FP in response to prolonged bouts of running [170], which may explain the difference.

An early investigation from Davies et al. [171], suggested that standing jump performance was superior during the EFP compared to the LFP and MLP. Whereas, a more recent study from Thompson et al. [172], assessed CMJ and bilateral hopping performance in 12 active females. Although there were no differences in hopping performance across the menstrual cycle, the results suggested that females are potentially prone to performance decrements as demonstrated by a significantly lower flight time during the CMJ within the EFP in comparison to the LFP (EFP, 421.0 ± 35.6 m·s^{-1} vs. 408.8 ± 31.8 m·s^{-1}; $p = 0.025$, $d = 0.36$).

In contrast to the above research, the majority of research could not identify any difference in jumping performance due to MCP. For example, Giacomini et al. [156] demonstrated that in 17 females, indices of the SJ (contact time, jump height) and multi-jump (maximal jump power, total contact time, total flight time) performance did not differ across three identified phases of the menstrual cycle. Similar findings have also been observed during a five-jump testing and one-legged hop for distance [109, 173, 174].

Collectively, it is important to understand the potential effect of the menstrual cycle on measures that are commonly used within football testing (jumps and sprints). Although such measures provide a means of quantifying performance, a similar outcome can be achieved in different physical states, as such, by a manipulation of technique. Thus, influences of the menstrual cycle on strength performance is oftentimes performed using more controlled measures, which may provide a clearer insight into the "true" effects of the menstrual cycle on strength indices.

2.2.2.3 Effect of menstrual cycle phase on strength

Strength can be simply defined as the ability to apply force under a specified set of movement constraints [175]. Owing to the fact that hormonal signals play a role in anabolic and catabolic processes in the muscle tissue, it is unsurprising that the fluctuations in female hormones may have an effect on muscle properties and strength. With membrane oestrogen receptors located within all skeletal muscle, it could be speculated that oestrogen may affect muscle force production. For example, oestrogen has been known to cause steep increases intracellular calcium concentrations [176, 177], This may potentially enhance actin and myosin binding due to the potential increase in calcium mobilisation and the presence of oestrogen receptors in skeletal muscle [178], thus, evoking a change in the generation of muscle force [179, 180]. However, at present there is a lack of research confirming this potential mechanism. Oestrogen has also been suggested to influence central nervous system functioning [181]. Specifically, it has been shown to bind to receptor sites that reduce the release of gamma-aminobutyric acid, a neurotransmitter whose principal role is reducing neuronal excitability throughout the nervous system [182]. Moreover, oestrogen has been shown to promote glutamate releasing neuron receptors, which further cause an excitatory effect [182]. Contrastingly, progesterone has been suggested to inhibit the central nervous system by enhancing the role of gamma-aminobutyric acid [183], therefore, the ratio between oestrogen and progesterone might be most suggestive to identify individuals who may have differences in strength status. Furthermore, progesterone is known to have a thermogenic response on the body, whereby this increased body temperature may positively affect nerve conductivity [184] and antagonist co-contraction [185], potentially enhancing explosive strength-based tasks. Based off these potential mechanisms, it is not inconceivable that the hormones and the specific MCP may affect strength status during athletic activity.

While the above mechanisms may suggest timepoints where strength is affected by female sexual hormones, present research does not reach a consensus on these or whether strength is affected due to MCP in general. Studies have identified improved muscle strength when oestrogen is elevated [186-188], whilst other research has suggested greater performance when the oestrogen concentration is low [171] or at medium concentration [189]. Furthermore, research has often identified no difference in strength across the menstrual cycle [135].

Early studies using simple handgrip measures of strength, demonstrated significantly greater performances during different stages of the menstrual cycle. Wirth and Lohman [189] demonstrated that grip strength was greater in the MFP when compared to the LP. Whereas, Davies and colleagues [171] identified significantly greater handgrip strength in the EFP. Any strength response will be specific to the measure of contraction (isometric, eccentric, concentric). As such when considering the force velocity profile of muscular contractions, strength assessments should also be conducted across a range of angular velocities during different contraction types.

Consequently, more recent research has attempted to assess the effect of MCP on more sophisticated measures of strength. Bambaeichi et al. [188] investigated peak isometric and isokinetic force of the knee extensors and flexors across five stages of the menstrual cycle. There was no effect of MCP on isokinetic peak torque of the knee extensors or maximal isometric contraction of the knee extensors. However, peak torque of knee extensors was found to differ significantly, with 4.9% greater torque during ovulation compared to EFP. Moreover, significantly greater torque was apparent during the MLP than the EFP (5.1%) and the MFP (6.7%). Maximal voluntary isometric contraction of knee flexors also displayed a significant variation between phases, with significantly higher values observed during ovulation in comparison to MFP (7.0%) and MLP (11.3%). Taken collectively, these findings tend to suggest that strength may be superior when oestrogen concentrations are elevated, which could have implications on sporting performance and training periodisation (Figure 2, Chapter 1). Although the previous literature was not conducted in female footballers, the considerations of the effects of menstrual cycle on thigh musculature (interplay of extensors and flexors) has implications for injury risk in female football players [190].

Similarly, Ekenros et al. [173] identified a 4.3% increase in knee extensor strength during the LP in comparison to the EFP. In addition, a study from Gordon et al. [191] investigated the maximal isokinetic strength of the knee flexors and extensors during four stages of the menstrual cycle. Results suggested that knee extensor strength was significantly greater (2.9%) during the MLP when compared to EFP. Further information supporting the presence of elevated oestrogen concentrations affecting strength measures.

Conversely, Tenan and colleagues [192] compared the maximal strength of the knee extensor muscles during EFP, LFP, ovulatory, MLP and LLP. All maximal voluntary contractions were similar between the EFP, LFP, ovulatory and LLP. However, there were significant reductions in strength during the MLP, when compared to the other three measurement points, specifically, there was a 23% reduction from ovulation to the MLP. Tennan et al. [192] suggested that strength was lower when progesterone is at its theoretical peak, and recovers as progesterone decreases in the LLP. Therefore, it was suggested that changes in strength may have an inverse relationship with progesterone.

However, there is a vast body of literature that has found no difference in isolated strength indices across the menstrual cycle. This has been found in peak torque of the knee extensor and flexor MVC [39, 174, 193-195]. Or in measures of maximal isometric quadriceps strength, fatiguability and electrically stimulated contractile properties, as well as handgrip strength [173, 174, 196-199].

There have also been several studies to investigate indices of ‘functional’ strength, where multiple muscle groups are incorporated to generate force (e.g. bench press). Rodrigues et al. [200] assessed maximal strength during the leg press exercise during EFP, MFP and LLP. There was a significant increase in strength of 8% in the EFP when compared to LLP. Moreover, maximal strength produced post-menstruation was 5% and 11% greater than during menstruation and pre-menstruation periods, respectively. Conversely, Arazi et al. [158] found no significant differences in leg press performance across the menstrual cycle. There were also no significant effects of menstrual cycle found during bench press [158], squatting [201] or maximal lifting [202] performance. Again, although these methods lack specificity, as a similar output can be achieved using various muscle recruitment strategies. Such measures of assessing compound exercises, increases ecological validity and might be closer related to football type activities.

2.2.2.4 Effect of menstrual cycle phase on sport-specific performance

As mentioned previously, athletic performance is a product of an array of factors (Figure 3) [49]. However, at present that vast majority of the literature investigates one specific component of fitness. Therefore, understanding how the menstrual cycle and its accompanying hormones affects different aspects of performance is important for individual and team sport athletes.

Currently, few studies have investigated the direct effects of the menstrual cycle on sport-specific performance (Table 4). Early studies from Brooks-Gunn et al. [203] and Fomin et al. [204] investigated the effects of MCP on swimming and skiing performance, respectively. Swimming times were found to be fastest during the EFP and slowest during the LLP with a mean difference of 1.32 s [203]. Whereas, race times during a 5 km ski race and a 12.5km race on ski rollers were quicker in the LFP and ELP compared to the EFP, MFP and MLP [204]. Although differences in sport specific activity has been found previously, this may not translate to football as the demands of the sports differ. For example, swimming in comparison is relatively short in its duration and activates primarily the upper-body, which are both contrasting to football-based activity.

More recently, investigations have assessed sport specific performance across the menstrual cycle using physical performance testing. Vaiksaar et al. [153] found no effect of menstrual cycle on an array of rowing-specific endurance parameters. Whilst, De Souza and colleagues [142] assessed female runners during maximal and sub-maximal treadmill running at 80% of $\dot{V}O_2max$. There was no observed effect of cycle phase on aerobic parameters or time to exhaustion. Although football is played at a similar intensity, this activity type and characteristics does not represent typical football activity and therefore, the response may differ.

__Table 4__ The effect of menstrual cycle phase on sport specific performance.

Study	Subjects	Sport	Cycle phases investigated	Performance test	Outcome measures	Findings
Brooks-Gunn et al. [203]	6	Swimming	EFP vs. MFP vs. LLP	100 yd freestyle, 100 yd best event	Time to completion (s)	↑ 50m time during EFP ↓ 50m time during LLP
Fomin et al. [204]	164	Skiing	EFP vs. MFP vs. LFP vs. ELP vs. MLP	5 km ski race, 12.5km race on ski rollers	Time to completion (s)	↑ performance during LFP and ELP
de Souza et al. [142]	8	Running	EFP vs. MLP	Maximal and sub-maximal treadmill run @ 80% $\dot{V}O_{2max}$	Time to fatigue, RPE	No effect of menstrual cycle
Vaiksaar et al. [153]	15	Rowing	FP vs. LP	Step wise incremental rowing test	PO, HR	No effect of cycle phase

Štefanovský et al. [159]	8	Judo	MFP vs LLP	SJFT	# of throws[a,b], SJFT index	↑ number of throws in first period during LLP
Tounsi et al. [109]	11	Football	EFP vs. LFP vs. LP	5JT, RSSA, YYIRT1	Jump height, RSSA, YYIRT1	No effect of cycle phase

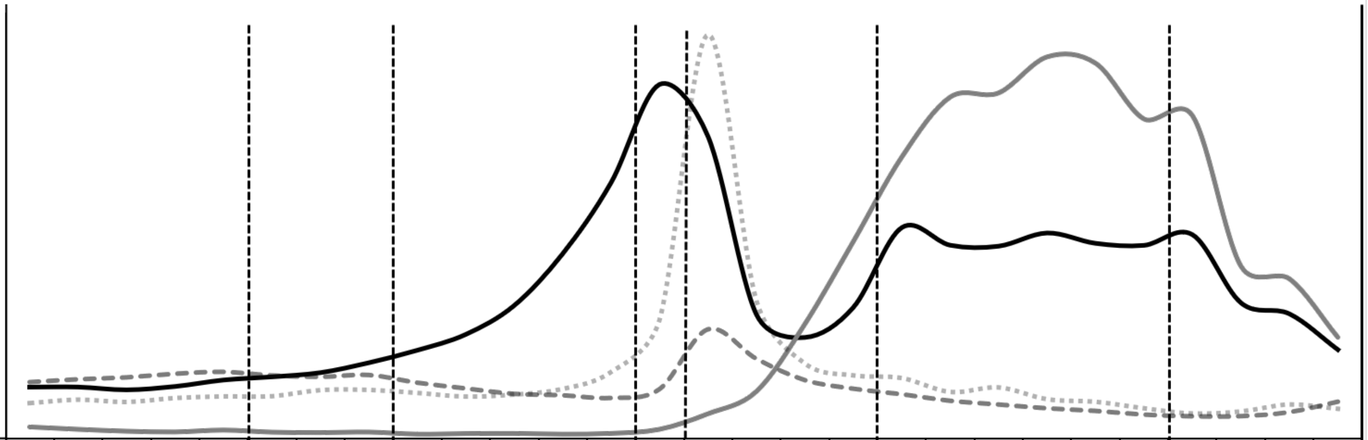
Note: EFP = Early Follicular Phase, MFP = Mid Follicular Phase, LFP = Late Follicular Phase, ELP = Early Luteal Phase, MLP = Mid Luteal Phase, LLP = Late Luteal Phase, SJFT = Special Judo Fitness Test, 5JT = 5 Jump Test, RSSA = Repeated Shuttle Sprint Ability, YYIRT1 = Yo-Yo Intermittent Recovery Test Level 1, ↑ = Greater Performance, ↓ = Reduced Performance

Most recently, Štefanovský et al. [159] conducted a Judo specific fitness test (JSFT) on a sample of eight female Judokas. Results suggested that there were significantly greater number of mean throws during the LP (5.88 ± 0.35) in comparison to the FP (5.38 ± 0.52), during the first of three throwing cycles (each 15s long). Such a finding might have implications on acute changes in sport specific football tasks (e.g. jumping, shooting, change of direction), albeit only being short lived. The authors suggested that improved performance in LP could be a result of potentially higher levels of ATP and PCr stores, which have been previously linked with oestrogen concentration [205]. The findings should be interpreted with caution as cycles were only based off a menstrual cycle calendars; without confirmation of hormones, ovulation, clearly defined phases or an assessment of prior "physiologically normal" cycles

In team-sports, players require multiple physical capabilities for peak performance. Owing to the potentially diverse effects of the menstrual cycle and its hormones, different elements of performance may be influenced over varying time-points across the menstrual cycle. One previous study has investigated the effects of MCP on commonly conducted football-related performance testing. Tounsi et al. [109] assessed lower limb explosive power (five-jump test (5JT), repeated sprint shuttle ability (RSSA), and aerobic performance in 11 high-level Tunisian football players, during the EFP, LFP and MLP. They found no significant differences across the three phases in YYIRT1 distance, absolute height in the 5JT or mean time during six 40-m shuttle sprints interspersed with 20s recovery. The results suggest that MCP does not influence isolated measures of football specific physical performance. However, these responses could be specific to the stage of the season where the measurement was undertaken. Moreover, although considered high-level in their population, this might not reflect the elite level globally and these results might not translate to higher-levelled athletes who may have greater training status and capacities.

Taken collectively, there is limited research investigating the potential effects of the menstrual cycle on sport-specific performance. However, as depicted in figure 5, within the literature and due to the potential accompanying physiological effects, there are timeframes which may be interpreted as potential epochs for differences in performance. As such, future research should aim to further explore the topic area and conduct reproducibility studies to confirm the previous findings. Moreover, due to the vast majority of research conducted during isolated testing related to components of the sports, future research should aim to explore the effects of the menstrual cycle alongside competitive performance in sports.

As shown in the current section, the majority of literature related to athletic performance across the menstrual cycle is often not considered in relation to sport specific tasks or simulations of the sport itself. Moreover, this is often conducted in a one-off measurement during a rested state. Likewise, in sports where it has been conducted in the field, the outcomes are easily quantifiable (e.g. race time). However, with recent advances in technology, performance benefits within football matches can be quantified using micro technologies to assess acute changes in physical performance. As such, future research should further reproduce the findings during isolated testing in football players. Moreover, whether the findings are translatable to match-play, not only acutely but longitudinally.



Menstrual cycle	EFP	MFP	LFP	O	ELP	MLP	LLP
Days	1 2 3 4 5	6 7 8	9 10 11 12 13	14	15 16 17 18	19 20 21 22 23 24	25 26 27 28
Menstrual cycle phases	FP	FP	FP	O	LP	LP	LP
Menstrual cycle sub-phases	EFP	MFP	LFP		ELP	MLP	LLP
Performance variables: Aerobic performance	↔	↔	↑		↔	↓	↔
Performance variables: Anaerobic performance	↔	↔	↔		↔	↑	↔
Performance variables: Strength	↓	↔	↑		↑	↔	↔

Figure 5 *Interpretation of the potential differences in physical performance variables across the menstrual cycle.*

Note: FP = follicular phase; LP = luteal phase; O = ovulation; EFP = early follicular phase; MFP = mid-follicular phase; LFP = late follicular phase; ELP = early follicular phase; MLP = mid-luteal phase; LLP = late luteal phase; ↑ = better performance; ↓ = worse performance; ↔ = indifferent performance, lack of supporting literature or findings too equivocal.

2.2.3 Methodological considerations of menstrual cycle research

Current research investigating the effects of menstrual cycle on physical performance has demonstrated largely equivocal findings. Moreover, much of the research can be difficult to interpret due inconsistencies or limited by methodological flaws. For example, in the only previous study conducted in female football players, Tounsi et al. [109] did not test ovulation and therefore, the inclusion of potentially non-physiologically 'normal' cycles may have been included into analysis. Furthermore, the days of measurement during the LFP were based solely from an average 28-day cycle and therefore, may not have been accurate for every individual [135]. Finally, terminology and measurement timepoint was misleading; the later study suggests that LFP testing was performed between days 7-9 thus missing the apt time for peak oestrogen concentration (Figure 2, Chapter 1) and potentially a misrepresentative measurement time-point. Such inconsistencies can be seen across the literature, certain studies have not confirmed physiologically "normal" menstrual cycle through ovulation testing [156, 158, 159, 164, 171, 188, 192, 194, 197, 200], confirmation of phases via hormonal concentrations [150, 158, 159, 164, 197, 200, 201] or poorly defined MCP [150, 164, 169, 171, 186, 187, 200, 206].

These limitations may lead to the equivocal findings, as well as causing difficulty in interpreting the findings. Moreover, due to the differing methodologies this also increases the difficulties when comparing the results of previous research [39, 48, 52, 135]. Due to the multifaceted nature of the female menstrual cycle and the differences between and within individuals, it is often difficult to use a "one size fits all" approach to conduct menstrual cycle research. However, when possible, there are certain approaches researchers should consider to ensure meaningful and robust conclusions, such as the correct verification of MCPs, ensuring only individuals with known physiological cycles are included into analysis, and that the time points within the menstrual cycle are clearly and logically defined. Previous researchers have attempted to create a consensus explaining "best practice" when including the menstrual cycle into research (Figure 6) [52].

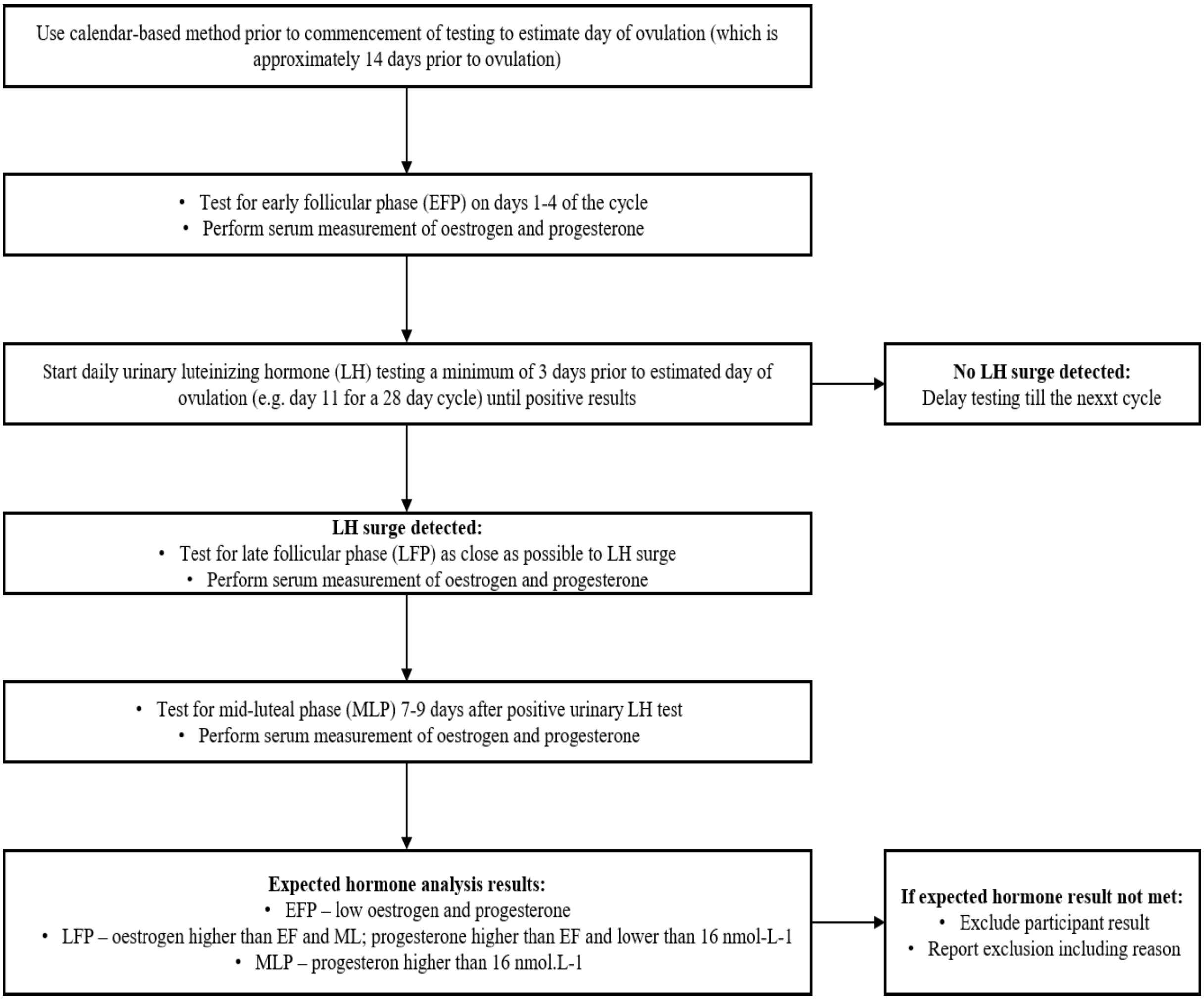


Figure 6 *Flow chart of the methodological steps recommended to verify regular ovulatory menstrual cycle phases (adapted from Janse de Jonge et al. [52]).*

There are different time points across the menstrual cycle, which might be consequential to performance, often these are related to the interaction of sex hormones and their secondary responses [49] . In older studies, the MCP were separated into premenstrual and menstrual [45, 203]. These phases included emotional and physical discomfort which is associated with the

PMS and can occur during menstruation, in particular during the first one to two days where bleeding is heavier. PMS has been previously described as the presence of certain emotional or physical symptoms which occur over consecutive menstrual cycles and occurs five days prior to menstruation [207]. The symptoms of the premenstrual and menses include but are not limited to: breast swelling and tenderness, fatigue, bloating, lack of energy, appetite changes, sleep problems, decreased concentration, and irritability. These factors if severe or in combination could easily be inherent to reduce maximal performance. However, a limitation of such a method is the lack of hormonal information which has been linked to physiological changes that are secondary effects potentially altering performance. Moreover, such a phase determination does not exclude the inclusion of non-physiological 'normal' cycles. Therefore, more recent research has adopted an approach which divides different MCP based on hormonal concentrations (Table 3). This approach compares the time points throughout the menstrual cycle where the magnitude of the differences or ratio in hormones significantly differ, potentially where the largest changes of function might occur [135]. With studies being separated into two (FP and LP), three (FP, ovarian, LP or EFP, LFP and MLP), and even up to five (EFP, LFP, ovarian, MLP, LLP) phases [135].

Across research there has been an array of different methods to ensure that measurements are being conducted within the correct predetermined phase and that cycles are physiologically 'normal', including the use of menstrual cycle calendars, BBT charting, urinary analysis and hormone analysis [52]. Earlier studies used solely menstruation calendars when estimating phases of the menstrual cycle, this approach is conducted by participants identifying the onset of menses (day 1) up until the beginning of the next onset of menses. This provides a guideline for the typical length of the menstrual cycle, owing that there is often more variation in the length of the FP, whereas the duration of the LP is generally fixed [52, 136], repeated measures of calendars counted back retrospectively can provide sufficient information to determine phases [135]. However, this method is limited as you cannot accurately predict ovulation and potentially studies may include individuals with nonovulatory cycles or those who may have luteal phase deficiency (LPD). Moreover, when separating the findings into more than two phases is difficult to do using this method independent of any other modality, therefore, the use of such method in isolation would not be recommended.

A more physiological measure is the use of BBT [52]. This involves participants to provide their body temperature before they get out of bed. The daily body temperature is then monitored over a certain time period as after ovulation body temperature rises by ~0.3°C due to the increase in progesterone [208]. Therefore, this method can provide information on FP and LP length and provide an estimation of ovulation [52]. However, it has been previously documented that females who have ovulated may not necessarily show this increase in temperature [209]. Therefore, the "gold standard" method to measure the correct MCP is the use of hormone measurements [52]. This often requires participants to provide a blood or saliva sample and is measured for the direct concentrations of oestrogen and progesterone. This provides an objective indication of where the females are in relation to MCP (Table 3). Furthermore, this method provides valuable information when determining the relationship between the changes in hormonal concentration and performance.

To ensure that only ovulatory cycles are included into analysis and that correct timing can be calculated for the LP measurement, it is recommended to include ovulation testing. This is often conducted via urinary analysis, whereby, participants urinate onto a device which will provide a positive result when the surge of LH is apparent. This positive result indicates with

95% confidence that within the next 24 hours ovulation will occur [210]. Once ovulation can be confirmed, accurate measurement time points can be created for the LP.

In summary, based from different methods of cycle verification there is sometimes the potential to include nonphysiologically ‘normal’ cycles into analysis, and to measure during an incorrectly predicted timepoint. Therefore, for the cross-comparison between studies, a stricter method of verification should be conducted. Although following a consensus as described by Janse de Jonge et al. [52] and depicted in figure 6 would help to limit the variation in methodologies, there is still discussion as to the accuracy of these recommendations. For example, it is recommended to conduct LFP testing “as close to the luteinizing hormones surge as possible”, however, this timepoint may in fact be too late to detect the peak concentration of oestrogen (Figure 2, Chapter 1). Therefore, future research should clearly identify and explain each step taken when measuring and identifying the menstrual cycle within research.

2.2.4 Basic overview of hormonal contraceptive use in sports

Although the use of hormonal contraceptives will not be investigated within the bounds of this thesis, it is still important to highlight some factors that might influence potential research. Hormonal contraceptives are exogenous steroid hormones that are used to inhibit ovulation and prevent pregnancy [211-213]. The prevalence of hormonal contraceptive across the use has been suggested to be approximately 77% across five major European countries (France, Germany, Italy, Spain, United Kingdom), of the different forms of contraception, the most common delivery system was observed to be oral contraceptive pills (OCP; ~30%) [214]. In comparison to controls, the prevalence of OCP use has been suggested to be greater in elite athletes and has been documented to be approximately 40% [215]. In a study from Sundgot-

Borgen and Torstveit [216], the use of OCP was observed across different sporting types (football, handball and endurance based sports), the prevalence of OCP use was shown to be less common in football players (~36%) than in handball (43.9%) and in endurance athletes (38.2%) (Table 5), however, this value was still observed to be greater than non-active controls (27.8%). There are several suggested benefits from using hormonal contraceptives. For example, reducing the symptoms of dysmenorrhea (painful periods) and shifting the pattern of the menstrual cycle, by self-determining when bleeding will occur. Such actions could be considered advantageous for elite athletes due to their regular schedules of physical training and competition [213, 217]. Within the athletic population, research have confirmed that many athletes use hormonal contraception to manipulate the timing of the menstrual cycle, a recent study from Schaumberg et al. [217] has suggested that over 70% of athletes have used OCP to deliberately change their rhythm, whilst approximately 50% of athletes have used OCP to manipulate their cycles for a specific sporting competition.

Table 5 *Characteristics of the total population of athletes (adapted from Sundgot-Borgen and Torstveit [216]).*

mean (SD)	Football (n= 69)	Handball (n = 60)	Endurance (n = 115)	Controls (n = 607)
Age (years)	19.6 (4.1)	19.9 (3.1)	22.3 (6.3)	27.3 (7.9)
BMI (kg/m^2)	21.5 (1.6)	22.5 (2.0)	20.5 (1.8)	23.3 (4.2)
Training (h/week)	12.3 (3.7)	15.8 (4.2)	13.1 (4.5)	-
Eating disorders*	5.9%†	22.4%	25.7%	21.1%
Current use of contraceptives	35.8%	43.9%	38.2%	27.8%
Current menstrual dysfunction‡	9.3%	18.8%	27.9%	15.2%
Stress fractures	13.6%	23.2%	13.4%	12.4%

*Note: BMI = Body Mass Index, * = Self-reported, † = Football significantly different from the other groups ($p<0.05$), ‡ = oral contraceptives were excluded.*

Whilst research conducted on the effect of OCP on athletic performance is limited, the findings which have been observed are often equivocal. Physiologically, numerous studies have suggested that OCP use reduces $\dot{V}O_{2max}$ [218, 219], potentially due to changes in activation of the sympathetic nervous system [219, 220] or to mechanisms at the level of the muscle (e.g. muscle blood flow, lactate removal, fiber-type recruitment, and mitochondrial respiration) [219]. However, studies conducted in athletes, suggest that $\dot{V}O_{2max}$ is not affected by OCP use [153, 221]. During performance testing, $\dot{V}O_{2max}$ has also been observed to be influenced during both incremental running and submaximal cycling tests, yet, it was not observed to affect performance [218, 219]. Moreover, in eight female rowers during a one-hour rowing ergometer test, there was no influence of monophasic OCP use on any of the exercise performance parameters [153]. These findings have been similarly found in swimming [222], and during isolated performance tests [223, 224]. When identifying changes in strength within the OCP cycle or in comparison to eumenorrheic females, the majority of the research has suggested that OCP use does not significantly affect strength parameters [173, 187, 224-226].

Therefore, knowing that a large population of athletes use contraception, this may limit potential recruitment of athletes for menstrual cycle research, moreover, as OCP use is ever-present, increased research should investigate the effects of its usage on performance.

2.2.5 Female specific physiology (menstrual cycle) summary

Taken collectively, the research would tend to suggest that the menstrual cycle does not overly affect physical abilities. However, the vast majority of the research has been conducted in recreational populations and not during sport specific performance. That being said, several studies have suggested that certain stages of the menstrual cycle might be a mitigating factor for peak performance. These equivocal findings could be due to the current lack of uniformity between studies, utilising the same MCP verifications and terminologies. In team sports there is an interaction of many different components of fitness and the current research inclines to assess the effects of MCP and its synergistic hormones on independent modalities of fitness. Consequently, it has been expressed in the literature there is still an underrepresentation of female team sport athletes in the menstrual cycle research [51, 53], including female football. Thus, the effects of the menstrual cycle on football-specific performance has not been extensively explored and requires further investigation to help lead scientific led practice to ensure the continued development of the sport.

2.3 RESEARCH AIMS AND OBJECTIVES

Taken collectively, the current synthesis of literature led to the series of studies presented in this thesis and were designed to specifically explore the following:

1. Study one may have implications for the longitudinal monitoring of the physical capacities and physiological responses of the female footballer, and to better understand the physical and physiological profile throughout the season. The aim of the study was two-fold; to observe the changes in physical performance and fitness-relevant blood-borne variables of non-professional female footballers using repeated measures within a football season. The specific objectives of this study were:
   - To further assess the physical capacity of the modern female football player
   - Longitudinally assess physical and physiological responses at different timepoints throughout a season
   - Quantify changes in a player's capacity without consideration of female specific physiological implications.

2. Study two has potential implications for improving the physical performance testing of female football players, by incorporating female specific physiology into their monitoring practice. The aim of study two was to determine whether MCP influences a series of football related physical performance parameters in a high-levelled football specific population. The specific objective of the study was:
   - To assess changes in physical capacities during "football-specific" performance tests with consideration for female specific physiology.

3. Study three could have implications for player management strategies, and the monitoring of match-load during the competitive season, in relation to female specific physiology. The aim of study three was to investigate whether MCP affects the match physical performances using GPS monitoring in elite female football players. The specific objective of the aforementioned study was:
    - To quantify the match activity profile during the FP and LP of female footballers longitudinally, considering female specific physiology as a mitigating factor for changes in physical output.

3.0

CHANGES IN PHYSICAL FITNESS AND BLOOD-BORNE PARAMETERS OVER ONE SEASON IN NON-PROFESSIONAL FEMALE FOOTBALLERS

3.1 INTRODUCTION

The number of female footballers continues to expand, suggested by record numbers of players being registered. Of these players, the non-professional footballer encompasses a large volume of the overall community. There seems to exists a relationship between training status (levels of fitness), competitive standard and physical performance [4, 12]. However, comprehensive information describing the physical capacities across an array of competitive standards in female football is still lacking. Though the capacities of the non-professional player have been highlighted in single bouts of testing, it remains relatively unknown whether changes in physical fitness occur over the course of a season. Moreover, continued physical exposure through training and matches, may affect certain blood-borne parameters, such as iron levels, which in turn may affect the physical performance and/or health status of a player. Therefore, coaches and practitioners at the non-elite level (who might not have the opportunity for extensive monitoring) may use this information to help training and create a frame work for testing and monitoring the female footballer. Therefore, the aim was to observe the changes in physical performance and fitness-relevant blood-borne variables of non-professional female footballers using repeated measures within a football season.

3.2 METHODS

3.2.1 Study Design

The study utilised an observational longitudinal design. Whereby, the players underwent a series of physical tests and provided blood samples on the following three time points across the season; pre-season (early August) (M1), beginning of the competitive season (early October) (M2) and end of season (late May/early June) (M3). The pre-season period consisted of 8-weeks training, three times per week. All M2 measurements were conducted within four weeks from the first official game, during this period players trained three times a week and had one official match. Finally, M3 was conducted during the final three weeks of the 22-game season.

3.2.2 Subjects

Thirty-six non-professional female footballers (19 ± 2 y, 164 ± 5 cm, 80 ± 8 kg) competing in the second Frauen Bundesliga, Germany, participated in the study. Only outfield players were considered for the final analysis and participated in all testing periods over the season. Participants were fully informed of all experimental procedures before giving their informed consent to participate. If players were younger than 18 years of age, parental consent was obtained. The current study was conducted in accordance with the declaration of Helsinki and approved by the local ethics committee (Aerztekammer des Saarlandes, approval number 130/14).

3.2.3 Study Procedures

Prior to data collected, players were familiarised with the protocol of each physical test. The physical testing battery was conducted in the present order and included (after appropriate warm-up): CMJ for the analysis of lower limb strength. Characteristics of the CMJ were determined using a force platform (Quattro Jump, Type 9290AD, Kistler Instrument AG, Winterthur, Switzerland) and analysed using professional motion analysis software (Contemplas Bewegungsanalyse, Contemplas Gmbh, Kempten, Germany). Jump height was determined as the centre of mass displacement, calculated from the recorded force and body mass. The mean of the best three jumps was used for the subsequent analyses. 3 x 30 m to measure sprinting capabilities. Splits were set at 5 m, 10 m and 30 m and measured by infra-red single beamed light gates (Brower Timing System, Utah, USA), with a standard error of measurement of 0.03 s [227]. Players had two mins of recovery and then repeated the test for three trials, with the mean of all three 30 m sprints used for analysis. 4 x 5 m change of direction (COD) test was performed, similarly to that described by Sporis et al. [228]. At the start and finish, light gates (Brower Timing System, Utah, USA) were erected and used to assess total time to complete the course. Three trials were conducted with two minutes of passive recovery interspersed, the mean of all three trials was used for analysis. Finally, the YYIET2 was conducted to measure sport-specific endurance capacity. The test was performed outdoors on artificial turf similarly described by Bangsbo et al. [107]. Total distance of completed shuttles was recorded as the test result. HR (≥ 220 – age (years)) and record of perceived exertion (RPE) were used as objective criteria of exhaustion [229] to be included into final analysis. Prior to the physical tests at all three time points over the season, venous blood sampling was obtained from the antecubital vein, following 10-15 min of rest in the supine position. Blood samples were transported immediately to the laboratory for appropriate procedures. Serum tubes were centrifuged at 4000 revolutions per min for 5 min, aliquoted and then stored frozen at -80°C within 2 h of sampling. CK, urea and C-Reactive Proteins were measured using a Unicel DxC600 synchron clinical system (Beckmann Coulter GmbH, Krefeld, Germany). Erythrocytes and Haemoglobin was determined automatically by an ACT 5 Diff AL (Beckmann Coul-ter GmbH, Krefeld, Germany). Ferritin was analysed using an Access 2 Immunoassay system (Beckmann Coulter GmbH, Krefeld, Germany). Data were collected in the early evening (prior to and during normal training sessions). All measures were conducted outside on artificial turf under neutral weather conditions. If weather was deemed unsuitable or at risk of influencing results, or players' welfare, (e.g. any form of rain) the test was either cancelled or if possible, re-scheduled.

3.2.4 Statistical Analysis

The mean ± SD were calculated for each of the three time points across the season. Cohens d ES were calculated with 90% CI and interpreted as; trivial (<0.2), small (>0.2-0.6), moderate (>0.6 – 1.2), large (>1.2 – 2.0), very large (>2.0) [230].

3.3 RESULTS

All physical and blood-borne variables over the three measurement timepoints can be observed in tables 1 and 2, respectively. The course and magnitude of the change can be observed in figures 1 and 2 for physical and blood-borne variables, respectively.

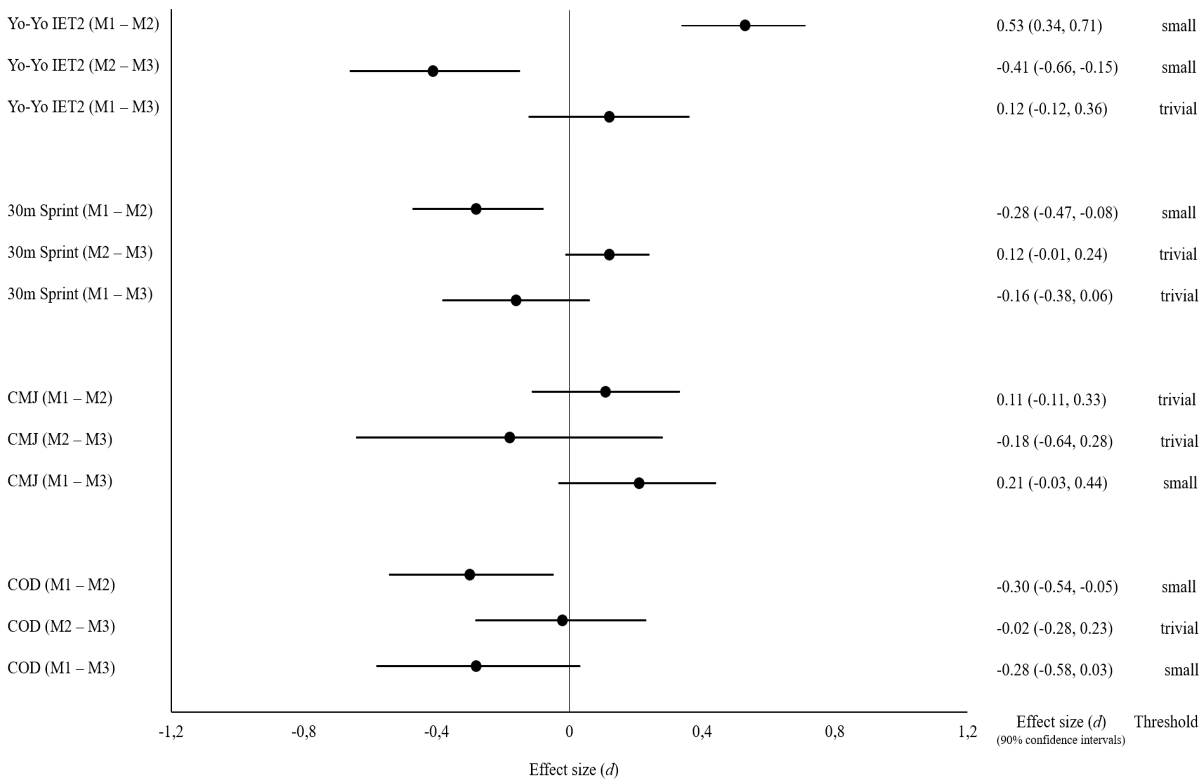


***Figure** 7 Changes in physical performance variables over the course of the season. Data expressed as Cohens d effect sizes and 90% confidence intervals. Note: CMJ = Counter movement jump; COD = Change of direction; YYIET2 = Yo-Yo intermittent endurance test level 2. M1 = Pre-season, M2 = In-season, M3 = End of season.*

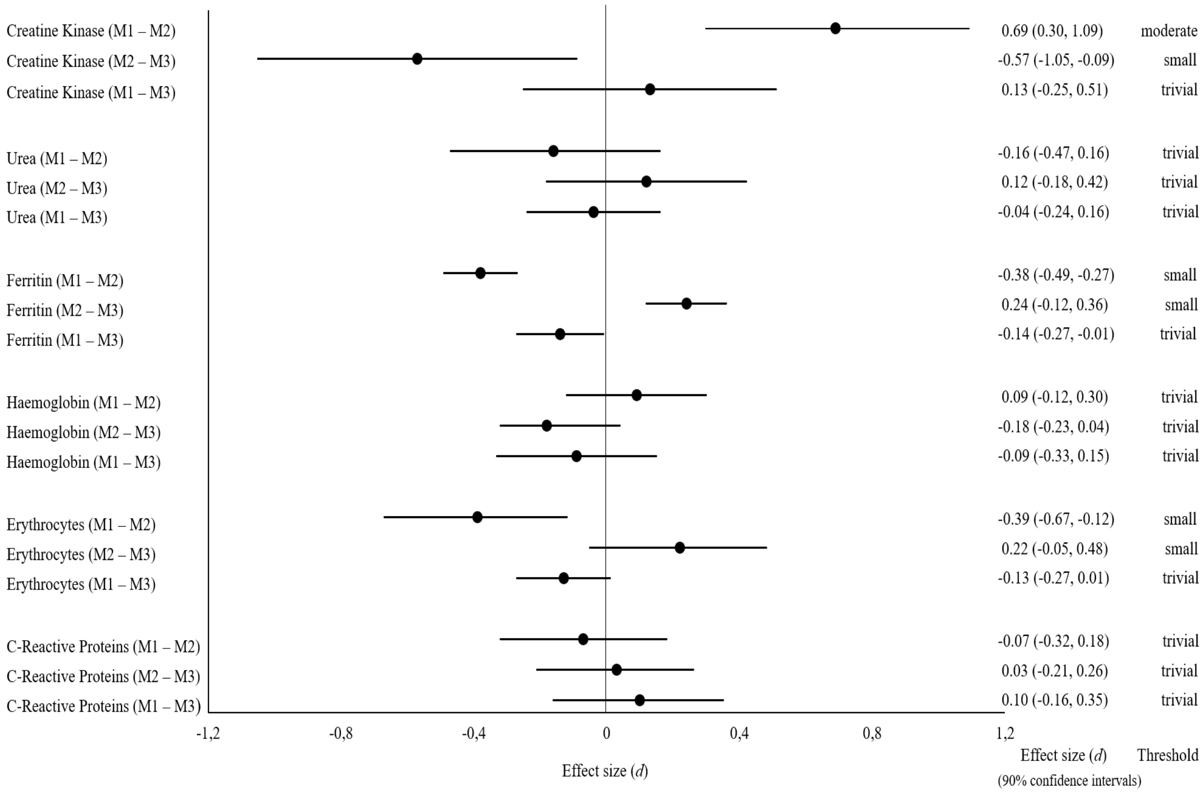


Figure 8 *Changes in blood-borne parameters over the course of the season. Data expressed as Cohens d effect sizes and 90% confidence intervals. Note: M1 = Pre-season, M2 = In-season, M3 = End of season.*

3.4 DISCUSSION

The primary objective of the study was to describe the changes in physical performance within a season of non-professional female footballers. Among the main findings of the study were that small improvements in YYIET2 distance, 30 m sprint time, and COD completion time from pre-season to the start of the competitive season. However, as the season progressed YYIET2 performance declined again, whereas the other physical parameters remained stable across the season. The secondary aim of the study was to detect changes in selected haematological variables. Results indicated that there were moderate to small changes in CK, Ferritin and Erythrocytes from M1 to M2 with a considerable number of players showing indications for iron deficiency. The results of the current study are in line with previous observations [96, 231].

Table 6 *Physical variables and the within season changes across all timepoints. Data is represented as means ± standard deviations.*

Variable	M1	M2	M3
CMJ (cm)	31.3 ± 3.1	31.6 ± 3.0	32.0 ± 3.5
0-30 m sprint (s)	4.8 ± 0.2	4.7 ± 0.1	4.8 ± 0.1
COD (s)	6.0 ± 0.3	5.9 ± 0.3	5.9 ± 0.3
YYIET2 (m)	752.2 ± 233.2	873.3 ± 251.99	779.7 ± 234.1

Note: CMJ = Counter movement jump; COD = Change of direction; YYIET2 = Yo-Yo intermittent endurance test level 2. T1 = Pre-season, T2 = In-season, T3 = End of season.

Table 7 *Blood variables and the within season changes across all timepoints. Data is presented as means ± standard deviations.*

Variable	M1	M2	M3
Creatine kinase (U/L)	194.3 ± 87.7	256.5 ± 118.2	205.7 ± 118.8
Urea (mg/dl)	29.4 ± 6.2	28.4 ± 9.0	29.1 ± 6.0
Erythrocytes (Mio/μ)	4.5 ± 0.2	4.4 ± 0.3	4.5 ± 0.2
Ferritin (ng/ml)	32.5 ± 14.9	26.7 ± 13.6	30.4 ± 14.8
Haemoglobin (g/dl)	13.6 ± 1.3	13.7 ± 1.0	13.5 ± 0.9
C-Reactive Protein (mg/l)	1.2 ± 2.9	0.9 ± 1.6	1.0 ± 1.6

Note: M1 = Pre-season, M2 = In-season, M3 = End of season.

Physical performance was observed to increase during pre-seasonal period, which is often designed to increase physical capacities for the upcoming season whilst, small decreases in physical performance was apparent as the season progressed. Moreover, there are large inter-individual differences at each time point and intra-individually over the course of the season (illustrated in figure 9). There are several factors which might cause these changes in physical performance. Oftentimes non-professional female football teams have an inability to effectively monitor training, with financial constraints, suboptimal education level of coach/practitioners and limited availability of staff leading to such insufficiencies.

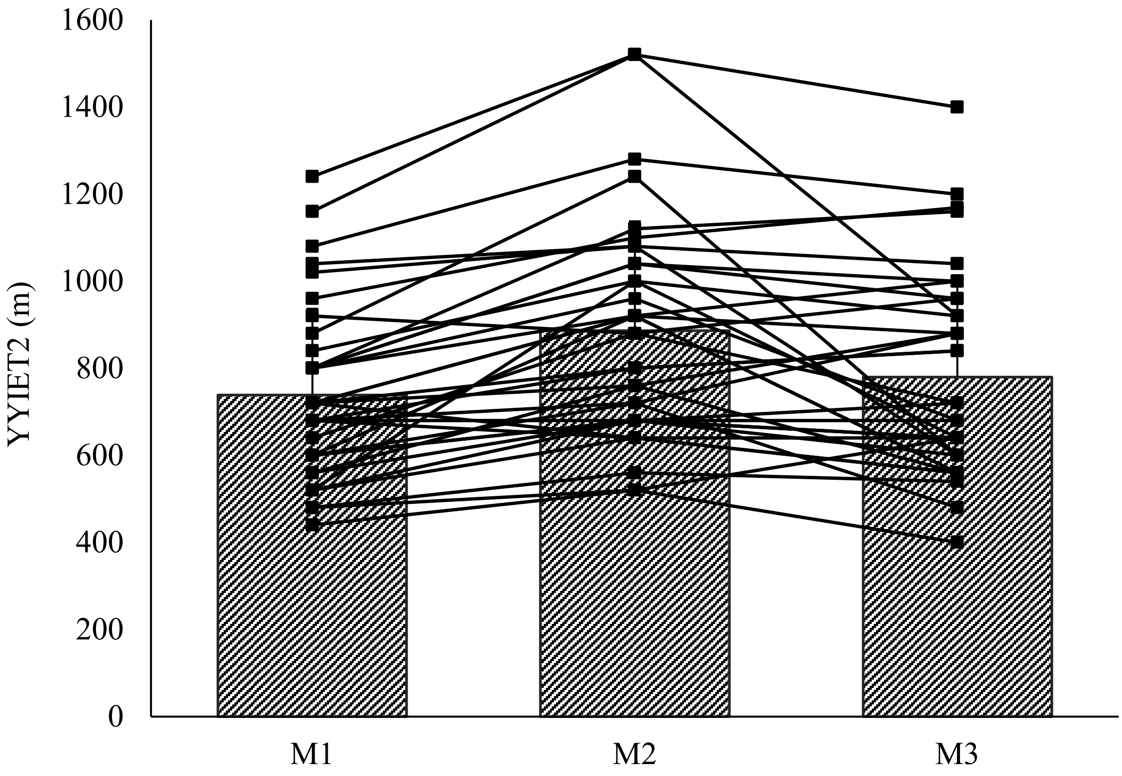


Figure 9 *Group and individual changes in Yo-Yo intermittent endurance level 2 (YYIET2) across the season. Note: M1 = Pre-season, M2 = In-season, M3 = End of season*

As such, inadequate training stimulus might be imposed upon the players or a monotonous training routine, which loses its ability to maintain a peak level of physical performance [232]. Relatively low values for CK (indicator of microdamage to muscle fibres) and urea (indicator for metabolic load) seem to be in favour of this interpretation. This neglect on physical loading could in part, be due to the shift in focus towards the tactical element and preparation for games. Moreover, due to playing weekly matches, alongside other obligations (work, studies) this may possess an issue to ensure that satisfactory levels of physical load can be transferred onto the player to maintain physical performance. Recent monitoring frameworks suggest that a lack of loading may not only affect physiological parameters, but potentially leads to a lack in biomechanical load which may in turn increase injury risk [233]. As such, it could be recommended that basic testing, with limited "necessary" equipment (such as YYIT) is implemented more regularly to help respond to the needs-analysis for effective training in non-professional populations. The current selection of blood-borne markers has been suggested to provide an overview of the physical state of a footballer [234]. When evaluating, it is imperative to understand the magnitude of the change and understand what is beyond a 'normal' change. Therefore, these findings can provide a larger pool of information for baseline and within-season changes for the non-professional female footballer. It should be noted, that large variations within and between individuals are observed, even when experiencing a similar training stimulus [235]. As such, when possible, consistent testing of each individual is warranted to ensure the physical state can be maintained throughout the season. Interestingly, on the group level the results indicated that there were small ($d = 0.38$) changes in ferritin from M1 to M2. Reduced levels of ferritin have been linked to negatively affecting physical performance [236] and health of the female population. This proposes the need for more consistent monitoring to identify those that are at potential risk and thus one can effectively intervene by giving dietary advice or supplement iron. These findings are similar to that of 28 elite-female footballers, whereby 57% were iron deficient and 29% had iron deficiency anaemia in the run up to the FIFA Women's World Cup [237] signifying that individualised monitoring is important. Therefore, future research should further investigate whether the female footballer is exposed to an increased risk of reduced iron storage and explore whether there is an effect on subsequent performance.

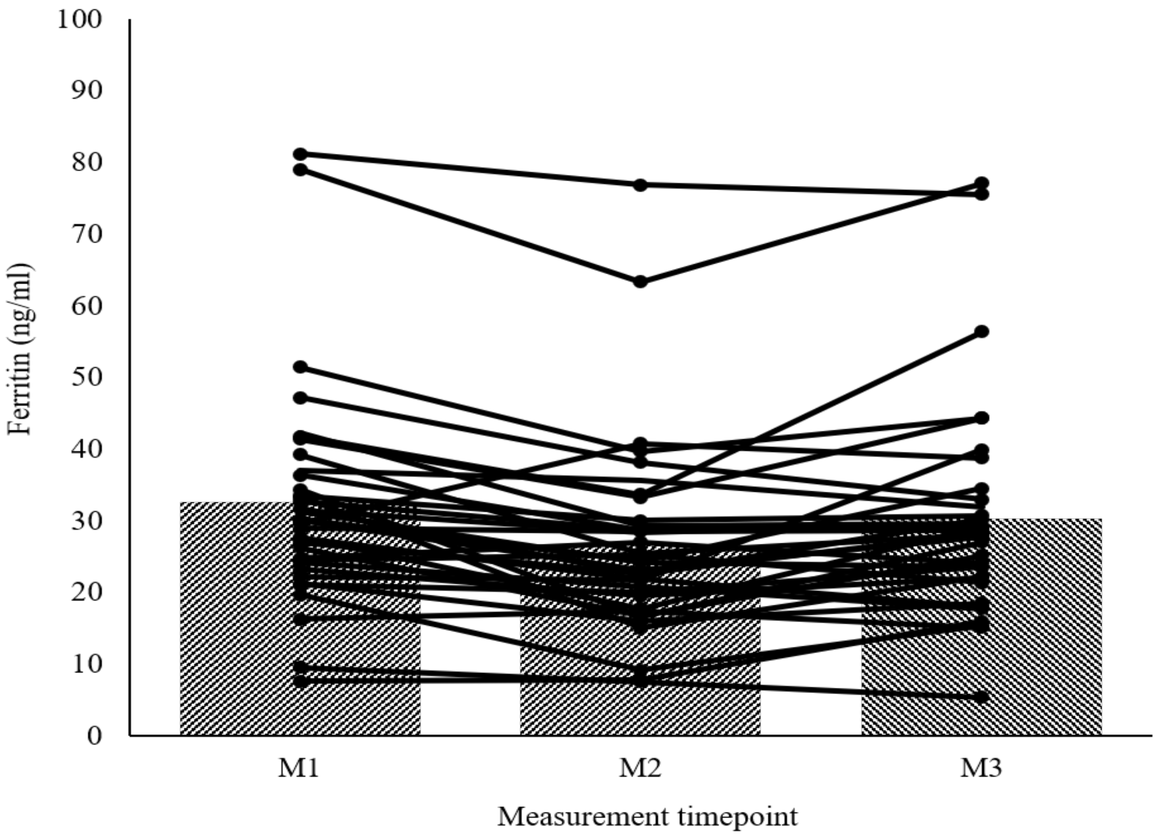


Figure 10 *Group and individual changes in Ferritin concentrations across the season.*
Note: M1 = Pre-season, M2 = In-season, M3 = End of season.

3.5 PRACTICAL APPLICATIONS

- The results suggest that practitioners may target increasing the physical capacities during the pre-season period and then possibly slightly neglect them as other priorities such as, match preparation may be determined as "more important". Therefore, a detraining effect may occur which could potentially affect peak performance during match play.
- It might be advisable for practitioners to assess the physical capacities of the players throughout the season (at multiple time points) and manipulate training plans accordingly, to maintain peak physical condition.
- The results indicate that it would be worthwhile in future research to assess the ferritin levels (as well as a full blood count to detect possible iron deficiency anaemia) of the female

footballer on a more rigorous routine to identify those at potential risk and to help to create a consensus on appropriate courses of action.

3.6 LIMITATIONS

- The current study only involved one team which has a certain player pool and one method of training. However, this study represents the first to repeatedly monitor physical and haematological parameters in non-professional female footballers and could provide an insight into the possible challenges with this population.
- Low number of testing time points, without mid-season measurement timepoint.
- Female specific physiological changes, i. e. the menstrual cycle, were not considered during the current study which has been previously observed to affect maximal performance [238].

4.0

THE EFFECTS OF MENSTRUAL CYCLE PHASE ON PHYSICAL PERFORMANCE IN FEMALE FOOTBALL PLAYERS

4.1 INTRODUCTION

The professionalism and interest in female football has rapidly increased over the last decade, which has led to an exponential rise in research within many realms of the game. Despite the increasing amount of scientific work surrounding female football, gender-specific aspects of physiology, particularly the menstrual cycle and its effects on the physical performance has been unaccounted for and remains fundamentally unknown [28, 51]. The menstrual cycle encompasses two main phases, the FP and the LP. The FP can split further into two sub phases; the EFP, which is characterised with low concentrations of both the key hormones oestrogen and progesterone; and the LFP where oestrogen is high independently from progesterone. The LP is typified by high concentrations of both oestrogen and progesterone. These two main phases are separated by a steep surge in luteinizing hormone triggering ovulation. These cyclical changes are said to be often predictable whilst spanning over the reproductive years [48] .

Besides from reproductive function, female sex hormones are further known to affect numerous cardiovascular, respiratory, thermoregulatory and metabolic parameters. This may plausibly be expected to have implications on exercise physiology, for example via fluid retention, changes in body temperature, and energy metabolism [47]. Currently, much of the research has investigated these effects on endurance performance, and the results thus far have been highly equivocal [139, 140, 142, 144, 149, 239-242]. The differing results of the studies to date may be due to methodological differences. Inconsistent time points within the menstrual cycle when performance had been assessed, the method of assessment (varying performance tests), the verification of cycle phases and/or assessment of physiological cycles, could be factors for the varied findings.

Currently, to the authors knowledge only three studies have evaluated the effect of MCP on intermittent activity and related performance [167, 169, 243]. Two of the three studies indicated no differences between phase. However, Middleton and Wenger [169] found a significant enhancement in power output during ten, six second maximal sprints on a cycle ergometer in the LP. Although the authors found only small average differences in power output, the improvement during the LP was exhibited in every subject. Moreover, if these results were translated into running time, this difference in work would translate to approximately one-meter difference in a six second sprint, which in terms of football, could make the difference in reaching the ball at important stages in a match. However, throughout these studies, the population sample was primarily healthy active females and not those of a highly competitive level and studies were not related to football.

The current study aims to determine whether MCP influences a series of football related physical performance parameters in a high-levelled football specific population. Based on the above considerations, the designs involve a strict protocol for the assessment of physiological cycles and distinct timing of performed tests in relation to MCP.

4.2 METHODS

4.2.1 Subjects

Thirty-five high-level female football players agreed to participate in the study. Formal sample size calculations were not performed, all players of the local second league female football team who fulfilled the inclusion criteria were enrolled for the study. The inclusion criteria for participation in the study were: 1) the absence from any form of contraception (OCP, implanted, injected, IUD, patches), 2) regular cycle of physiological length (24 – 35 days [39]), 3) free from any illness or disease which could affect performance and/or health, 4) not suffering from an injury which would affect their performance, 5) participated in competitive football for a minimum of five years. A further inclusion criterion was that the testing must be completed over the 24 – 35-day cycle (reducing the influence of confounding variables associated with time, including but not limited to: humidity, ambient temperature and stage in the season). Therefore, nine participants (age: 18.6 ± 3.8 y, height: 161.2 ± 6.6 cm, weight: EFP 59.1 ± 7.7 kg, mid LP 58.8 ± 7.5 kg) were suitable for analysis; the flow of the participants is represented in figure 11. These players all competed in the second women's league (2nd Frauen-Bundesliga), Germany. The players were fully informed of all experimental procedures before giving their written informed consent to participate. If players were younger than 18 years of age, parental written consent was obtained. The current study was conducted in accordance with the declaration of Helsinki and approved by the local ethics committee (Aerztekammer des Saarlandes, approval number 130/14).

4.2.2 Study design

The observational design implemented in this study investigated the differences in physical performance parameters during two distinct phases of the menstrual cycle. The participants completed a battery of physical tests, measuring several components of football specific physical performance capacity. These included; lower limb power, sprinting capability and endurance capacity [2]. The participants did not conduct any vigorous exercise on the days of testing and the training the day before testing days remained consistent; training comprised of low/moderate aerobic training for 30-45 minutes followed by 30 minutes of strength training.

The tests were conducted during two time points, representative of the EFP and MLP, respectively (EFP days 5-7 and MLP days 21-22 [135]), where hormonal concentrations differ greatest between the two time points (Figure 2, Chapter 1) [45, 135]. Data was collected in the early evening (during their normal training session), and training time was kept consistent for the duration of the study. Testing sessions were conducted during the second half of the season for over an 8-week period (March - May). All measures were conducted outside on artificial turf under similar weather conditions. If weather was deemed unsuitable and at risk of influencing results, and or to players' welfare, (e. g. any form of rain) the test was either cancelled or if possible re-scheduled.

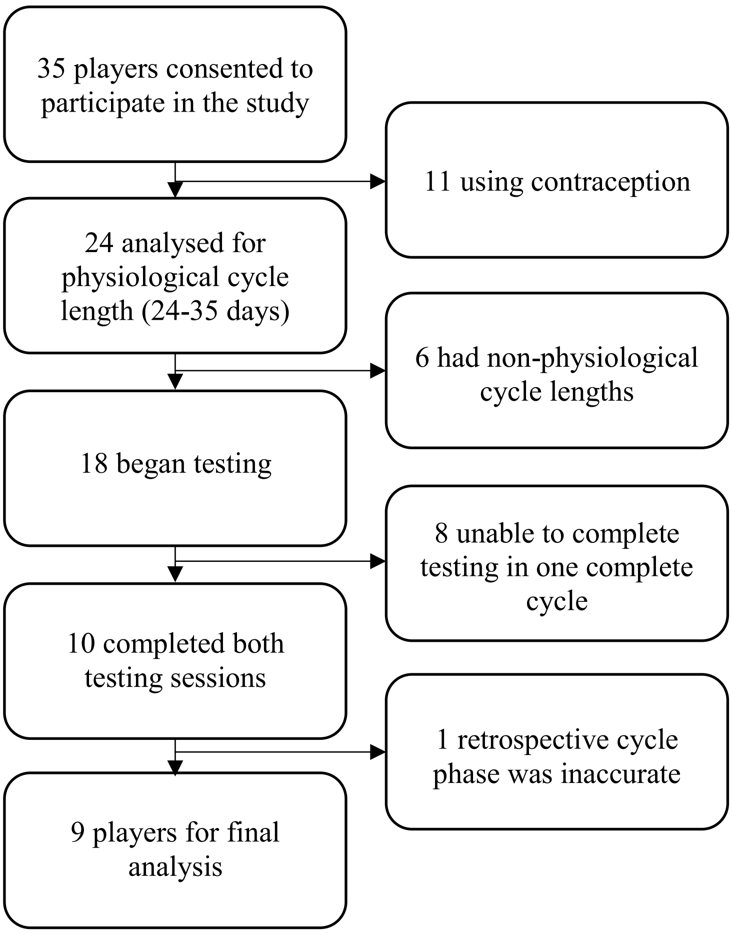


Figure 11 *Details of the participants' timeline throughout the study.*

4.2.3 Determination of menstrual phase

Twice a month, participants completed a menstruation diary, which included: date of menses, length of menses, and severity of blood flow and discomfort. This information was recorded for a minimum of 6 months, for a valid characterisation of an individual's menstrual cycle [135]. From retrospective analysis, (counting back from previous menses, from month to month, indicating the length of the cycle) the next cycle phase was then prospectively determined [135] and time-point was calculated. For final confirmation of LP sampling time, the participants completed menstruation diaries during the course of testing. Consequently, one player dropped out due to LP sampling time being incorrect (Figure 11). Furthermore, serum oestrogen and progesterone were used to verify physiological reproductive function and verify timing of tests with respect to the cycle phases[135].

4.2.4 Outcome measures

The following panel of established tests for components of football specific physical performance capacities were applied and conducted in this order (Figure 12): 1) Jump height during a CMJ (five trials, mean of the best three) was implemented as an indicator of lower-limb power [244], 2) 30 m (recording set at 5, 10 and 30 m) sprint time was employed as a parameters of sprint capability (fastest of three trials) [245], 3) running distance in the YYIET1 was used as a measure of football-specific endurance capacity [6].

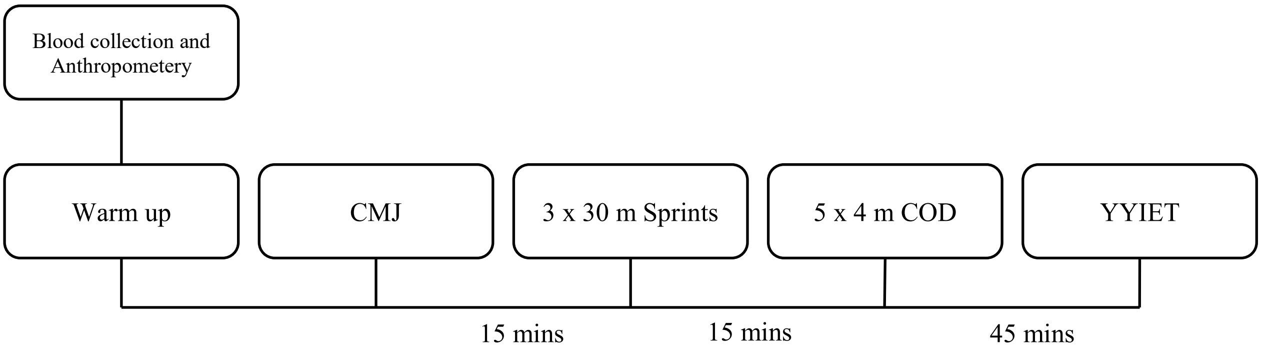


Figure 12 *Timeline of the testing protocol.*

4.2.4.1 Counter movement jump

Characteristics of the CMJ were determined using a force platform (Quattro Jump, Type 9290AD, Kistler Instrument AG, Winterthur, Switzerland) and analysed using professional motion analysis software (Contemplas Bewegungsanalyse, Contemplas Gmbh, Kempten, Germany). Jump height was determined as the centre of mass displacement, calculated from the recorded force and body mass. The CMJ began from an upright position, making a downward movement to a knee angle of approximately 90° and simultaneously beginning to push-off, whilst hands are placed upon their hips, with a rest period of 30 s between efforts.

4.2.4.2 3x30m sprints

Players were asked to stand with their toe behind a white line (touchline of the football pitch), and ran maximally straight ahead for 30m. 30 m sprint recording times at 5 m, 10 m and 30 m was employed and measured by light gates and a hand-held monitor (Brower Timing System, Utah, USA). Once a sprint was completed the players had 2 mins of recovery and then repeated the test following the same procedure for all three trials.

4.2.4.3 Yo-Yo Intermittent Endurance Test Level one

The test was performed outdoors on artificial turf similarly described by Bangsbo et al. [107]. Concisely, the test consisted of repeated 2 x 20 m runs with an 180° turn in between at a progressively increasing speed controlled by audio beeps. Between each running bout, the players had a 5 s rest period, during the active break in the form of a 2.5 m walk. Termination of the test occurred when a player had twice failed to reach the finish line, in time. Total distance of completed shuttles was recorded as the test result. All players had been familiarised with the test procedures previously. HR was measured before the warm-up period (rest) and was continuously measured using commercial heart rate monitors (Polar, Kempele, Finland) until exercise cessation (HR_{peak}). BLa was collected in 25µl samples of capillary blood, withdrawn from the earlobe and were subsequently measured using an enzymatic method (Super GL, Rolf Grenier, Biochemica, Flacht, Germany). BLa was collected prior to exercise (rest value), then further collected immediately after, 1 min, 3 min and 5 min post-test. HR (≥ 220 – age (years)), BLa concentration (≥ 8 mmol/l) and RPE were used as an objective criteria of exhaustion[229].

4.2.4.4 Blood collection

Venous blood samples were collected on the day of testing prior to any form of activity. These samples were obtained from the anti-cubital vein by a standard protocol, following 10-15 min of seated rest. The participants did not perform any vigorous exercise on the day of blood sampling. Blood samples were transported immediately to the laboratory for appropriate procedures. Serum tubes were centrifuged at 4000 revolutions per min for 5 min, aliquoted and then stored frozen at -80°C within 2 h of sampling. Hormones were measured in an accredited medical laboratory. Oestrogen was measured using an Estradiol II electrochemiluminescence immunoassay ECLIA (IBL International GMBH, Hamburg, Germany) with intra- and inter-assay coefficients of 4.13 - 6.81% and 6.72 - 9.39%, respectively. Progesterone was measured using a 17-OH-Progesterone enzyme immunoassay ELISA (IBL International GMBH, Hamburg, Germany) with intra- and inter-assay coefficients of 2.8 - 4.9% and 5.8 - 9.2%, respectively.

4.2.5 Statistical Analysis

Data analysis was conducted using the statistical software package SPSS v.21 (SPSS Inc., Chicago, IL, USA). All data were normally distributed (Kolmogorov-Smirnov) and expressed as means ± SD. Students paired *t*-tests were conducted to determine the difference in mean response between phases for all variables. The magnitude of the mean differences between phases were expressed as ES [246]. The extent of the ES are classified as follows: trivial (<0.2), small (>0.2-0.6), moderate (>0.6 – 1.2), large (>1.2 – 2.0), very large (>2.0), based on updated guidelines from Batterham and Hopkins [247]. Magnitude-based differences (MBD) were conducted to determine the possible benefit, in terms of beneficial or harmful effects, of the MCP. This approach represents a contemporary method of data analysis that uses confidence intervals to calculate the probability that a difference is practically meaningful. For assessing the difference in team performance smallest worthwhile change was set at Cohen effect size of 0.2 [247]. When clear interpretation was possible, a qualitative descriptor was assigned to the following quantitative chances of performance effect: 0.5% to 5%, very unlikely; 5% to 25%, unlikely; 25% to 75%, possibly; 75% to 95%, likely; 95% to 99.5%, very likely; and >99.5%, most likely [247].

4.3 RESULTS

The participants' body mass and sum of four skin folds were not significantly different between MCP (Table 8). Oestrogen and progesterone levels of the players were significantly higher in the MLP compared to the EFP.

Table 8 *Anthropometric and hormone characteristics of females involved in the study. Comparison between follicular and luteal phase.*

Variable		Follicular Phase	Luteal Phase
	Means ± SD	Means ± SD	Means ± SD
Age (y)	19 ± 4		
Height (cm)	161.3 ± 6.6		
Body mass (kg)		59.1 ± 7.7	58.8 ± 7.5
Body fat (%)		17.7 ± 2.5	17.8 ± 2.2
Oestrogen (pg/ml)		26.8 ± 22.1	109.8 ± 55.6*
Progesterone (nmol/l)		2.2 ± 0.6	6.5 ± 2.0*

Note: Body fat calculated by the sum of four skinfolds (biceps, triceps, subscapular, suprailiac).
*Note: * = significant difference between phases, P < 0.05.*

In YYIET1 performance, MBD intimated the qualitative inference that there was a possibly harmful effect for maximal performance in the LP, whereas, all other performance variables were found to be unclear. Moreover, the ES for the YYIET1 was small to moderate, whereas all other variables were trivial (Table 9). For all performance variables, there were no significant differences revealed, although a trend towards significance was observed in the YYIET1 between phases ($p = 0.07$) (Figure 13). There was a likely beneficial inference for HR pre-values tending towards the EFP, whereas the HR post values were deemed unclear. RPE was also deemed unclear. Lactate values were inferred to be unclear pre-and 1 min post the

Yo-Yo test. However, at 3- and 5-min post, the MBDs suggested that it was very likely harmful during the early FP, where lactate values were higher. The ES for all variables ranged from trivial to moderate (Table 10). There were significant differences between phases for HR prior to exercise and lactate values at minutes 1 and 3 post YYIET1 ($p = 0.04$, $p = 0.01$ and $p = 0.03$, respectively). For HR post, lactate pre-and lactate 1 min post YYIET1 and RPE there were no significant differences observed ($p > 0.05$).

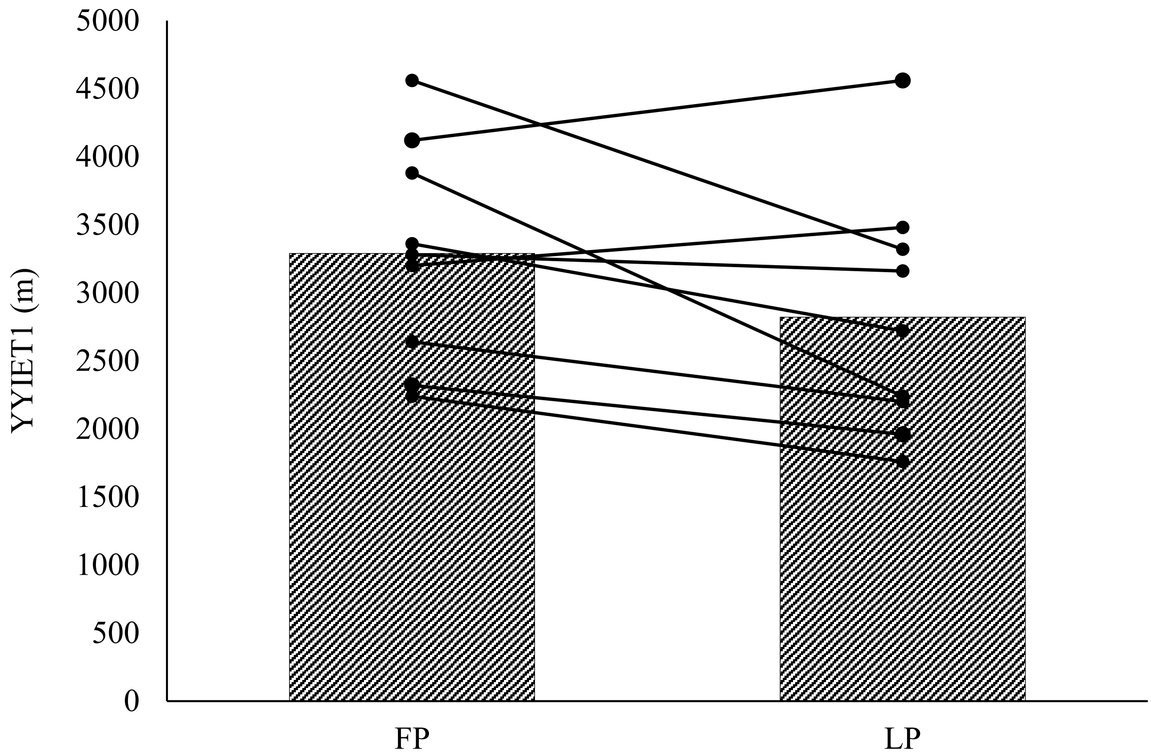


Figure 13 *Mean meters of completion during the Yo-Yo intermittent endurance test level one (YYIET1) during the two phases of the menstrual cycle. The data for each individual has been superimposed onto each group mean. Note: FP = Early follicular phase; LP = Mid luteal phase.*

Table 9 *Performance outcomes for all parameters measured during the follicular phase and luteal phase. Results expressed as means ± standard deviations, statistical significance, effect sizes and magnitude-based differences.*

Variables	EFP	MLP	*P*	ES	% Chance +/trivial/-	Qualitative Inference
0-5m (s)	1.1 ± 0.1	1.1 ± 0.1	1.00	0.00	50/0/50	Unclear
0-10m (s)	1.9 ± 0.1	1.9 ± 0.1	0.25	0.18	10/7/83	Unclear
0-30m (s)	4.7 ± 0.1	4.7 ± 0.1	0.96	0.00	48/0/52	Unclear
CMJ (cm)	29.0 ± 3.9	29.6 ± 3.0	0.33	0.16	8/24/68	Unclear
YYIET1 (m)	3289 ± 801	2822 ± 896	0.07	0.56	0/61/39	Possibly harmful

Note: EFP = Early follicular Phase, MLP = Mid luteal Phase, ES = Effects Size

***Table** 10 Internal load measures during the follicular phase and luteal phase. Results expressed as means ± standard deviations, statistical significance, effect sizes and magnitude-based differences.*

Variables	EFP	MLP	*P*	ES	% Chance +/trivial/-	Qualitative Inference
HR Pre (bpm)	97 ± 16	105 ± 12	0.04	0.45	91/9/0	Likely beneficial
HR post (bpm)	194 ± 4	193 ± 5	0.90	0.05	27/37/36	Unclear
RPE (AU)	18.7 ± 0.7	18.6 ± 0.9	0.76	0.14	23/31/46	Unclear
Lactate pre (mmol L^{-1})	2.3 ± 0.8	2.1 ± 0.8	0.65	0.21	17/25/58	Unclear
Lactate 1min post (mmol L^{-1})	9.3 ± 1.4	8.6 ± 0.7	0.20	0.41	6/28/66	Unclear
Lactate 3min post (mmol L^{-1})	8.8 ± 2.2	7.6 ± 1.6	0.01	0.75	0/1/99	Very likely harmful
Lactate 5min post (mmol L^{-1})	8.7 ± 2.2	6.9 ± 1.9	0.03	0.93	1/4/95	Very likely harmful

Note: EFP = Early follicular Phase, MLP = Mid luteal Phase, ES = Effects Size, HR = Heart rate, RPE = Record of perceived exertion.

4.4 DISCUSSION

The purpose of the study was to investigate whether MCP influences different facets of physical performance in a group of high-level football players. The main finding of the study suggested that YYIET1 performance was affected during the LP of the menstrual cycle. However, in all other aspect of measured performance were not different between phases.

To the authors knowledge this is the first study to investigate the effects of MCP on different sport specific physical parameters in female football players. In the nine players, MCP appeared to have an effect on maximal endurance capabilities, as demonstrated through the YYIET. It has been suggested that physical performance in elite female football is highly related to their trained status and maximal capacities [4], therefore, it may be postulated that the maintenance of these high levels throughout the entire cycle is important for success. In 78% of our population, a reduction of meters completed was observed in the MLP (Figure 13). The results of the current study are in line with the extensive work of Lebrun et al. [39]. Lebrun and colleagues used a cohort of 16 trained athletes (age 27.6 ± 3.8 y, height 167.9 ± 5.3 cm, mass 59.6 ± 6.7 kg, $\dot{V}O_{2max}$ 53.7 ± 0.9 $ml \cdot kg^{-1} \cdot min^{-1}$), who participated in a variety of sports including running, cycling, triathlon, squash, cross-country skiing, ultimate frisbee and rowing. This study contained a strict phase confirmation comparable to our trial. Through the use of a progressive and continuous treadmill running test, the results indicated that maximal endurance capacity was reduced in the LP.

Differences in endurance performance during various phases of the menstrual cycle have been postulated to be due to differences in heat regulation, substrate availability, and metabolism [140]. It is well documented that a female's BBT can differ between phases in the region of

0.3-0.5°C [135]. This elevation in temperature has been attributed to the surge of progesterone exhibited during the LP. The associated increase in body temperature has been suggested to limit prolonged exercise capabilities and increase cardiovascular strain [135]. This has been seen in previous studies, presenting higher $\dot{V}O_2$ values, accompanied with higher heart rates and RPE values during exercise at given percentages of $\dot{V}O_{2max}$ [248-250]. Although BBT was not measured in the present study, cardiovascular strain may have been present in our study and thus could influence performance. Conversely, it has been shown in previous studies, that exercise time to exhaustion is improved during the LP. This has been speculated that the role oestrogen plays on enhanced lipid metabolism, with spared glycogen and usually a supressed lactate response to exercise in the LP [133] . As seen in the present study, significant differences were found in BLa concentration between phases at 3- and 5-min post exercise. Therefore, there is the potential that the energy pathways may have been taxed differently between phase, for example it has been implicated previously that during the LP there is a lower contribution of anaerobic glycolysis and hence reduced BLa values [133] . A conclusive statement about this, however, is beyond the current scope of this study and perhaps requires deeper investigation with more sophisticated methodology (e. g. indirect calorimetry). Moreover, the present data, however, did not indicate any differences in lactate production at rest or at maximal BLa concentration, which was similarly found in female rowers completing a one-hour row at 70% $\dot{V}O_{2max}$ [251].

It is important to consider the specific type of sports to validly assess if and how MCP potentially affects physical performance. However, in football (which is intermittent in nature), there is a special challenge to evaluate sport-specific physical performance, this is due to the current difficulty to establish valid and reliable tests sensitive to the sport, unlike in endurance sports. The YYIET1 has been suggested to replicate physical patterns in female football [252], however, factors such as motivation have been suggested to cause differing results when being assessed at multiple time points, however, the exhaustion criteria were achieved in all players. In the present study, a reduction during the LP was observed in the clear majority of the players, with similar HR_{max}, BLa and RPE values. Therefore, it can be postulated that although maximal effort was elicited in both phases, there was an observed reduction in maximal performance during the LP.

Sprinting performance in the present study did not differ between cycle phases at either split or full 30 m distance (Table 9). The absence of the menstrual cycle affecting sprinting performance in the present study concurs with previously published literature [168]. It had, however, been suggested previously that sprinting performance may differ between phases. An improvement in sprinting performance being attributed to the increase in BBT during the LP [253]. However, a study from Somboonwong and colleagues [253] suggested that, although BBT was significantly greater in the LP, sprinting performance over 40-yd (36.6m) was not affected. Unlike the present study, Somboonwong et al. [253] only measured 40-yd sprinting time and did not investigate differing split times or acceleration. From the present study's results, it could be postulated that MCP does not influence sprinting ability at many various aspects of acceleration and velocities. This was seen as 5 m, 10 m and 30 m sprint time was not affected by MCP. In football, a players' ability to perform multiple HI actions and sprinting ability has been considered to be more important for overall performance than endurance capacity [23], therefore, the understanding that MCP does not affect sprinting behaviour is important for players and practitioners. This being said, in the present study and in the work from Somboonwong et al. [253] sprinting performance was measured from singular sprints and not RSA. RSA is another facet of football which has been suggested to be important for football performance. The only study to assess a repeated sprint and the effects of MCP, was conducted by Tsampoukos et al. [168]. In the aforementioned study, eight females conducted two 30 s sprints with a 2 min passive recovery in between; the results indicated no effect of MCP on repeated sprint performance. However, this study, only employed sport science participants, not those of a sport specific population and, furthermore, only completed two sprints. Therefore, for future research, due to the lack of understanding and studies conducted in football, the investigation of MCP on RSA is warranted.

The menstrual cycle has been suggested to alter motor control and muscular strength. However, similarly to the present study Janse de Jonge et al. [254] found no differences between phases in 19 normally menstruating females for any strength parameter, including maximal isometric quadriceps strength with superimposed electrical stimulation, isokinetic knee flexion, and handgrip strength. It has been previously proposed that oestrogen, may have a strengthening action on skeletal muscle [186], although the mechanisms of this effect has not become clear. During the present study, the time points of measurements, were at minimal oestrogen (EFP) and when both progesterone and oestrogen were elevated (MLP). Therefore, it can be speculated that the levels of oestrogen were not elevated enough to cause such an effect during EFP. Moreover, the antagonistic effect of progesterone, may have restricted the effects of oestrogen on muscular strength during LP [255]. Altogether, the current results suggest that CMJ was not affected by cycle phase, however, due to the assumptions of the effect of oestrogen on strength, future investigations should target other time points within the menstrual cycle, where oestrogewn is independently elevated, i.e. in the rise prior to ovulation.

A limitation to the current study is that it did not deploy a randomised counter balanced design, whereby all participants first completed the testing in the EFP followed by the MLP, this was due to the current method of MCP determination. Although the participants were well accustomed to this form of testing, confounding factors that may influence the current findings need to be considered. As recommended in previous literature, solely conducting menstruation calendars does not provide all relevant information regarding MCP [256]. Therefore, hormone values of oestrogen and progesterone were measured. However, concentrations were found to be lower than those previously described in the general population. In regards to research a progesterone limit has been recommended conservatively at 16 nmol/L [135]. It must be noted, that unfortunately at present reference values for athletic populations have yet to be defined and are not available. Furthermore, although the levels were not at the expected magnitude, changes in hormone values were observed; thus, the associated change in phase could be verified (Table 8). Moreover, the current data have been taken from the EFP and the MLP; the associated hormones also differ just before ovulation in the LFP, where oestrogen is elevated without a pronounced presence of progesterone. Therefore, the independent effects of oestrogen have not been assessed where these results could also differ. Finally, although the subject number is in the range of previous studies [167, 169, 243], the results may not accurately represent a wider female football population.

4.5 CONCLUSION

The current study indicates that there is potentially a reduction in maximal endurance performance during the LP of the menstrual cycle. However, this reduction in performance was not observed for jumping and sprint performance. Therefore, due to the findings of the current study, practitioners should keep MCP constant when completing routine physical assessments with their players, to ensure that changes in performance are consistent with the outcome and not due to the effects of the menstrual cycle. Alternatively, the cycle phase should at least be recorded and taken into account when interpreting the results.

5.0

MENSTRUAL CYCLE PHASE AND ELITE FEMALE FOOTBALL MATCH-PLAY: INFLUENCE ON VARIOUS PHYSICAL ACTIVITY OUTPUTS

5.1 INTRODUCTION

An exponential increase in women's soccer participation has resulted in a plethora of research focussing on the characteristics of female match-play [28]. However, studies in female soccer have generally replicated research practices conducted on male players, thus lacking sex specificity. It is well established that females exhibit hormonal and physiological differences and thus it is surprising that these are not reflected in the research thus far [51, 53, 257]. Arguably one of the most important factors when conducting research with female athletes is the potential influence that the menstrual cycle may have on athletic performance. A recent study from Bruinvels and colleagues (2016) [51] reported that 41.7% of exercising women believe that the menstrual cycle negatively affects training and performance. However, at present there is a paucity of investigations that explain these effects or to what magnitude the menstrual cycle affects physical performance. As such, there has been an increased interest and outcry for research to evaluate the possible effects of the menstrual cycle across various sports [258].

The menstrual cycle is a vital biological rhythm that occurs monthly from ~13 years of age [259]. This can be separated into specific MCP that are based on the magnitude of respective hormones. The FP is defined as the onset of menses until ovulation. This phase can be divided into early FP (both key hormones oestrogen and progesterone are minimal) and mid FP (an increased concentration of oestrogen with a low concentration of progesterone). Typically, the LP is associated with an elevation of both oestrogen and progesterone; therefore, progesterone is often measured to confirm the transition between the FP to the LP [135]. These hormones interact and affect physiological and metabolic mechanisms [49]. For example, substrate availability and metabolism have appeared to be affected across the menstrual cycle [47], with increased rates of glycogen depletion observed during the FP [149, 260]. In view of previous literature suggesting that muscle glycogen is likely the prominent substrate for energy production in soccer [261], and the link to reductions in HI distance [17, 20]; it could be suggested that the changes in substrate metabolism could relate to changes in physical performance.

Due to the potential alterations in female physiology over the menstrual cycle, it could be assumed that this may lead to changes in physical abilities of soccer players. At present, research related to the effects of MCP on physical performance are equivocal [47]. For example, studies have suggested there is a decrement in aerobic capacity and exercise performance during the LP [39, 149]. Nevertheless, one study found an overall improvement of 13% in time trial performance during the FP in a population of eight healthy (24 ± 2 yr), endurance-trained women [169]. In terms of sprinting performance, a study investigating six physically active young women demonstrated greater average power output over a series of ten intermittent sprints during the LP [169]. However, Tsampoukos., et al. (2010) [168] found no significant differences between MCPs during two 30 s sprints in 14 highly active sports science students. It should be noted that there were apparent limitations to certain research methodologies - for example, each study had limited samples sizes across varying populations; although, the populations were all of similar maturity and were classed as healthy, the athletic standard ranged from physical active and sport science students, to trained individuals. Moreover, certain studies have not measured ovulation [169, 262] and based the timing of measurement from a 28-day cycle and/or did not consider fluctuations in individuals cycle lengths [169].

Two recent studies in female soccer players explored the impact of MCP using isolated physical performance tests [238, 255]. Dos Santos Andrade and colleagues [255] found that female soccer players had a lower hamstring-to-quadriceps peak torque strength ratio during the FP. This reduced strength ratio may have implications on certain technical performance parameters, such as shooting [263] and has been previously linked to a greater risk of sustaining a lower limb injury [264]. Moreover, endurance performance was influenced by MCP as evidenced by a reduced YYIET performance in the LP [238]. Although apparent differences exist in these isolated tests, there are no studies which have assessed the influence of MCP on physical performance during match-play. Based on the results of discrete physical performance tests, MCP may influence match physical performance.

With recent changes in FIFA regulations, the use of GPS technology is now increasingly common and offers a method of quantifying physical loading [265]. There is a plethora of studies on the physical match demands of elite male players using GPS technology but limited research exists for females. The scant literature on female players typically uses absolute locomotion thresholds adopted for male players, thus raising pertinent questions about the application of such data. Likewise, a recent criticism of absolute locomotion categories used in male soccer is that they often underestimate HI locomotion. Whereby, individual differences in maximum locomotion capacity relative to the arbitrary thresholds (which commonly defines HI actions) are not considered, which could potentially be further exaggerated in female players who have been previously shown to possess lower physical capacities [37]. Thus, the utilisation of individualised thresholds (IND) could be beneficial for more accurate measurements of HI actions in soccer [37, 266]. Thus, the purpose of the study was to investigate whether MCP affects the match physical performances using IND GPS monitoring in elite female soccer players.

5.2 METHODS

5.2.1 Study design

The observational design aimed to investigate the differences in match physical performance due to MCP (FP v LP). Matches were assessed over a four-month period during the 2015/16 competitive season (February – May 2016). Match physical performance was assessed via GPS devices for players participating in a minimum of 75 min during at least two matches in each MCP

5.2.2 Participants

Eighty-three elite female soccer players from the first (n=68) and second (n=15) German league initially agreed to participate in the study. However, after applying strict inclusion criteria, 15 players remained for final analysis (Figure 14; 23 ± 4 yr, 1.69 ± 0.8 m, 64.3 ± 8.2 kg). The inclusion criteria consisted of: 1) having a regular cycle of physiological length (24 -35 days [39], 2) free from use of contraceptives, 3) having ovulatory cycles, 4) hormonal change (progesterone) being of sufficient concentration to confirm LP in at least one menstrual cycle [135], 5) having played in the same playing position and played a minimum of 75 min in at least two matches during each phase. The players were fully informed of all procedures and provided written informed consent to participate. The current study was conducted in accordance with the declaration of Helsinki and approved by a local ethics committee.

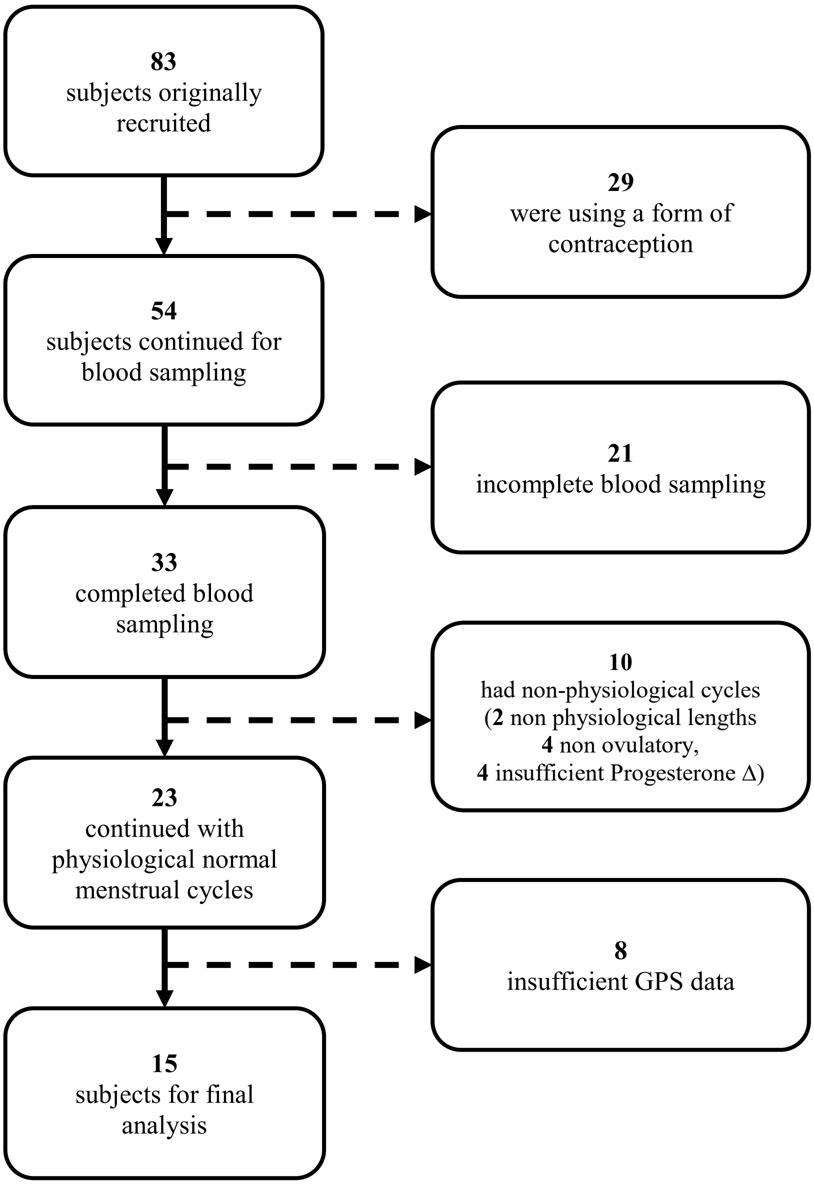


Figure 14 *Flow diagram detailing the participant's timeline throughout the study*

5.2.3 Study Procedures

5.2.3.1 Verification of Menstrual Cycle Phases

All players were initially assessed to establish physiologically 'normal' menstrual cycle (cycle length of 24 -35 days; presence of ovulation; significant change in progesterone). Players provided information via email regarding their onset of menses. Once confirmed, players were scheduled for FP blood sample (days 3-5). Once this sample was collected, players were instructed from day 11 (post menses), to urinate on the provided ovulation test strips (Cyclotest, UEBE Medical GmbH, Wertheim, Germany). Ovulation was confirmed by the player and then corroborated by the primary investigator via photo image (test line being of the same shade or darker than the control line). Ovulation testing was conducted until either a positive ovulation test was found or until day 17. Ovulation tests detect the luteinizing hormone surge, allowing an accurate prediction of ovulation with a confidence of up to 95% [135]. Regular menstruating women have been observed to have intermittent anovulatory cycles [267], therefore, if a player did not receive a positive ovulation test, the FP blood sampling and ovulation testing were conducted a second time for verification. When confirmed, 7 days post ovulation the LP blood sample was collected. A minimum limit of 1.83 ng/ml for progesterone was accepted as a change from FP to LP (Analysis spectrum and reference values, Saarland University Clinic). All blood samples were collected via an anti-cubital vein by a standard protocol, following 10-15 min of supine rest. The players did not perform any vigorous exercise on the day of blood sampling. Blood samples were transported to the laboratory for appropriate procedures. Serum tubes were centrifuged at 4000 revolutions per min for 5 min, aliquoted and then stored at -80°C within 2 h of sampling. Oestrogen was measured using an Estradiol II electrochemiluminescence immunoassay ECLIA (IBL International GMBH, Hamburg, Germany) with intra- and inter-assay coefficients of 4.13–6.81% and 6.72–9.39%, respectively. Progesterone was measured using a 17-OH-Progesterone enzyme immunoassay (IBL International GMBH, Hamburg, Germany) with intra- and inter-assay coefficients of ~3-5% and 6-9%, respectively. These initial cycles were verified through ovulation testing and the determination of oestrogen and progesterone concentrations via venous blood sampling in addition to the continuous use of menstruation diaries [135]. The continuous blood collection after every match and/or every cycle phase throughout the whole period of data collection would not have been accepted by players and coaches. Additionally, teams were spanned across the whole of Germany thus, obtaining regular venous blood samples would have been logistically difficult.

5.2.3.2 Match Physical Performance

Match physical performance was quantified using 5Hz GPS devices (Tracktics TT01, Hofheim, Germany) which has been previously found to be reliable, with a CV of 2.0% (CL 1.3; 5.9) for TD covered and test-retest differences in velocity within limits of agreement (0.08 - 0.55 m.s-1; own unpublished observation). Bradley & Vescovi [37], suggest that an IND approach may provide a more representative indicator of a female players' physical match exertion. Thus, players activities were split into four intensity zones using an IND approach, based on Hunter et al., [268]. These were determined individually using: <lactate threshold speed (LTS), LTS-maximal aerobic speed (MAS), MAS-29% anaerobic speed reserve (ASR) and >30% ASR. LTS, MAS, and ASR were determined during an incremental field-based lactate test and a 30-m sprinting test, whereby the average velocity during the final 10 m of the test was used as the maximal sprinting speed (MSS). The ASR was calculated as the difference between MAS and MSS [268].

The range of IND thresholds were: low intensity (Zone 1; $<13.20 \pm 0.71$ $km \cdot h^{-1}$), HI (Zone 2; $13.20 \pm 0.71 - 16.69 \pm 1.09$ $km \cdot h^{-1}$), VHI (Zone 3; $16.69 \pm 1.09 - 19.94 \pm 0.88$ $km \cdot h^{-1}$) and sprinting (Zone 4; $>19.94 \pm 0.88$ $km \cdot h^{-1}$). The number of HI runs and sprints were also recorded based on a minimum dwell time of 0.6 s. VHI bouts were calculated from the instance where the velocity reached the threshold for Zone 3, until the instance where the velocity was below this threshold. The measures of physical match-play were expressed in meters per minute ($m \cdot min^{-1}$) [269]. Throughout the study period, players were contacted twice per week to establish their onset of menses. When confirmed, cycle length for the month could be established. Subsequently, cycle phases (FP = day 1 to 14, LP = day 15 to 28; example based off an average 28-day cycle) were calculated retrospectively [135]. This was considered using the physiologically 'normal' cycle which was used as the criterion cycle. Meaning that based off this cycle the "normal" cycle length of each player was determined. Then after data collection we retrospectively analysed which of the two phases (based off retrospective counting from previous onset of menses) the match was played FP (days 1-14; based on an average 28-day cycle) or their LP (days 15-28; based on an average 28-day cycle)). If the length of the cycle was different to that of the 'criterion' cycle, any changes were manipulated from the FP, as it has been previously documented that the length of the FP is more variable than the LP [134].

5.2.4 Statistical Analysis

All data are reported as means ± standard deviation (SD). Prior to analysis, data were analysed for normality using the Kolmogorov-Smirnov test. All statistical methods were conducted using the statistical software package SPSS v.25 (SPSS Inc., Chicago, IL, USA). The change in hormone concentration between FP and LP was assessed using a paired samples *t-test*. Pearson product-moment correlation was used to explore the relation between changes in hormone concentration and performance from the FP to LP. The magnitude of the correlation effect statistic were: <0.1 trivial, ≥0.1 small, ≥0.3 moderate, ≥0.5 large [247]. To determine differences in physical match performance between the FP and LP, a mixed linear model was conducted. MCP was included as the fixed effect, whilst the players' identity, phase-by-subject ID interaction and the residual error were random effects. An α-level of $P \leq 0.05$ indicated significance. Furthermore, the change in physical performance with 95% confidence intervals

(CI) was calculated and the magnitudes of the changes were expressed as Cohen's d effect sizes using the following scale: <0.2 trivial, ≥0.2 small, ≥0.6 moderate, ≥1.2 large [247]. For individual analysis, percentage (%) change between phases were calculated and compared to the physical categories coefficient of variation (CV).

5.3 RESULTS

5.3.1 Hormone Changes

Players hormonal concentrations were found to be greater in the LP compared to the FP (oestrogen: FP, 52.5 ± 37.3 $pg \cdot mL^{-1}$ vs. LP, 189.6 ± 64.0 $pg \cdot mL^{-1}$, $p<0.01$, $d=2.6$;. progesterone: FP, 0.4 ± 0.3 $ng \cdot mL^{-1}$ vs. LP, 6.4 ± 3.9 $ng \cdot mL^{-1}$, $p<0.01$, $d=2.2$). There were large ranges in hormonal concentrations in both phases (FP: oestrogen: 19.0-158.0 $pg \cdot mL^{-1}$, progesterone: 0.2-1.0 $ng \cdot mL^{-1}$ vs. LP: oestrogen: 82.0-280.0 $pg \cdot mL^{-1}$, progesterone: 1.9-15.7 $ng \cdot mL^{-1}$). Moreover, there were large minimum and maximum changes (Δ) in hormone concentration on the individual level from FP to LP (min: oestrogenΔ: 39.0 $pg \cdot mL^{-1}$, progesteroneΔ: 1.4 $ng \cdot mL^{-1}$; max: oestrogenΔ: 261.0 $pg \cdot mL^{-1}$, progesteroneΔ: 15.4 $ng \cdot mL^{-1}$).

5.3.2 Differences in Match Physical Performance Metrics

Overall, a total of 76 individual match observations (ranging between four matches to seven matches per player, 36 in FP and 40 in LP) were recorded. The discrepancies between the number of matches played between players were either due to not starting in same playing position, or not completing the minimum 75 min for inclusion. All match physical performance metrics are presented in Table 11. The distance covered per min within Zone 3 was greater in the LP than the FP ($p = 0.02$, $d = 0.41$), with a between-subject SD of 61.2% and within subject-SD of 38.8%, however, the distances covered in Zones 1, 2 and 4 did not differ (p = 0.29-0.36, *d* = -0.08-0.12). With between subject-SD ranging from 52.3-62.2%, within subject-SD ranging from 29.6-47.7% and true between subject-SD ranging from 0.4-8.4%. Furthermore, TD covered was not different between FP and LP (*p* = 0.53; *d* = 0.08), with a between subject-SD of 33.6% and within subject-SD of 66.4% observed. HI and sprinting bouts were not different between FP and LP (*p* = 0.09-0.241; *d* = 0.20-0.22). The between subject-SD was 60.1% and 51.9%, the within subject-SD was 32.6% and 47.3% and the true between subject-SD was 7.3% and 0.8%, respectively.

5.3.3 Relationships between Hormone Concentrations and Physical Performance

No significant correlations were observed (p > 0.05). Only oestrogenΔ and Zone 1, and progesteroneΔ and Zone 1 and Zone 2 showed a moderate correlation (r = -0.32, r = 0.34 and r = -0.32, respectively). Whereas values were small (r = 0.12 – r = 0.29) to trivial (r = -0.03 to r = 0.08) for all other variables.

5.3.4 Individual Effects of MCP on Match Physical Performance

On an individual basis, there was large match-to-match variability in physical performance metrics in general (CV for Zone 1-4 = 7.0-56.0%), and in relation to MCP (Figure 16). One player (#8, Figure 15) systematically performed more distance during the LP in all metrics (TD: +14.0%, Zone 1: +6.6%, Zone 2: +39.8%, Zone 3: +199.7%, Zone 4: +82.9%). All individual changes in performance between FP and LP compared to the match-to-match variability are presented in Figure 16.

Table 11 *In match performance outcomes for each physical category, during the follicular phase and luteal phase. Results expressed as means ± SD*

Variables	FP	LP	Δ in mean (95% CI)	*P*	ES
Total distance ($m \cdot min^{-1}$)	103.00 ± 7.69	103.89 ± 6.84	-0.89 (-3.90 to 2.10)	0.53	0.08
Zone 1 ($m \cdot min^{-1}$)	82.03 ± 7.47	81.65 ± 5.73	0.38 (-0.48 to 1.20)	0.36	-0.08
Zone 2 ($m \cdot min^{-1}$)	11.41 ± 3.40	11.61 ± 3.27	-0.20 (-0.59 to 0.19)	0.29	-0.03
Zone 3 ($m \cdot min^{-1}$)	5.90 ± 2.16	6.64 ± 2.72	-0.74 (-1.30 to -0.14)	0.02	0.41
Zone 4 ($m \cdot min^{-1}$)	3.73 ± 2.38	3.99 ± 2.03	-0.26 (-0.79 to 0.27)	0.31	0.12
High intensity bouts (#)	150 ± 29	156 ± 29	-6 (-13.1 to 1.10)	0.09	0.22
Sprinting bouts (#)	23 ± 13	25 ± 12	-2 (-5.50 to 1.50)	0.24	0.20

Note: FP = Follicular phase, LP = Luteal phase, Δ = Change, CI = Confidence interval, ES = Effect size.

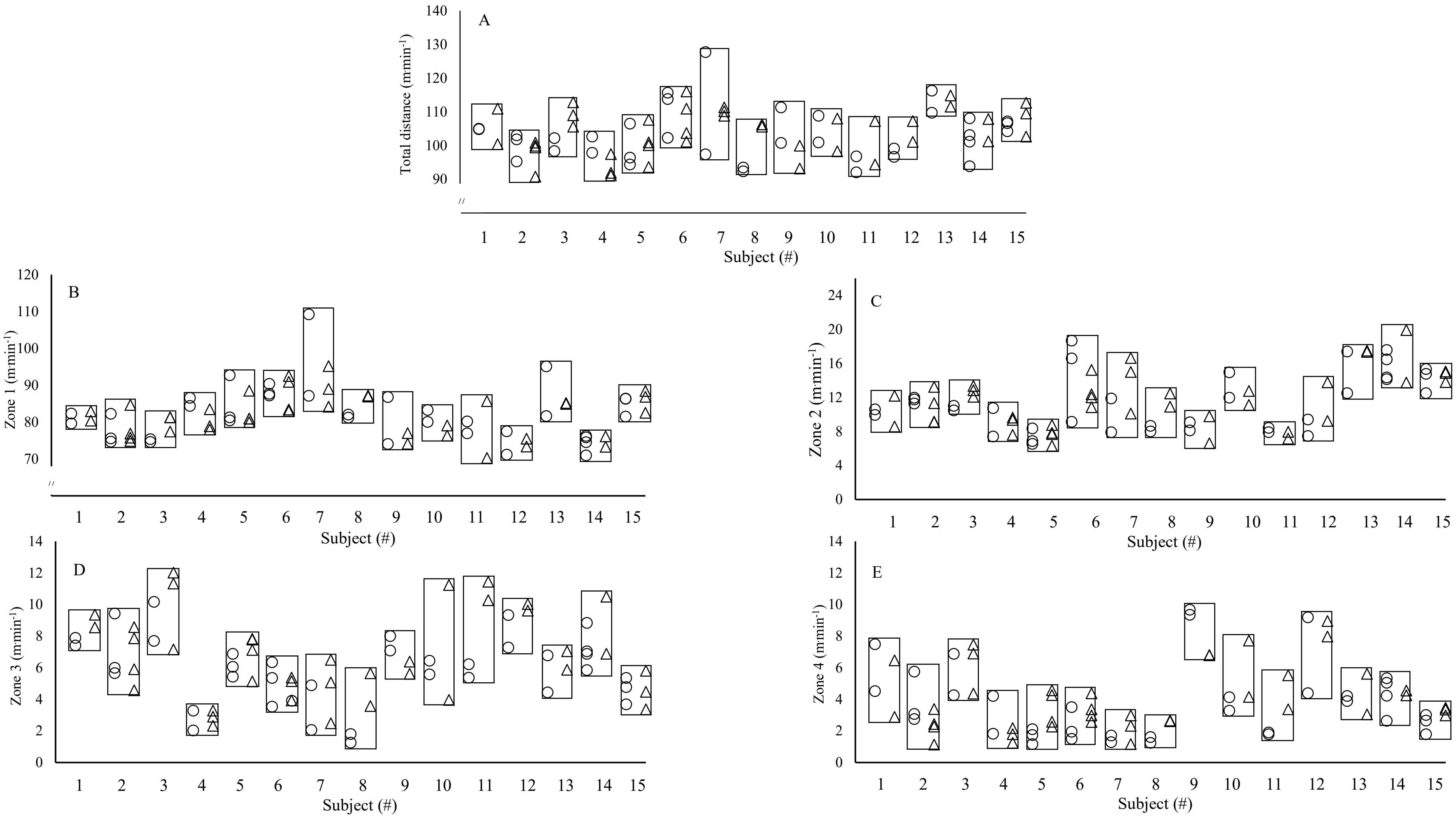


Figure 15 *Individual variability plots for each participant in each speed zone in both the follicular and the luteal phase using individualised (IND) thresholds. Note: Circles = follicular phase, Triangles = luteal phase*

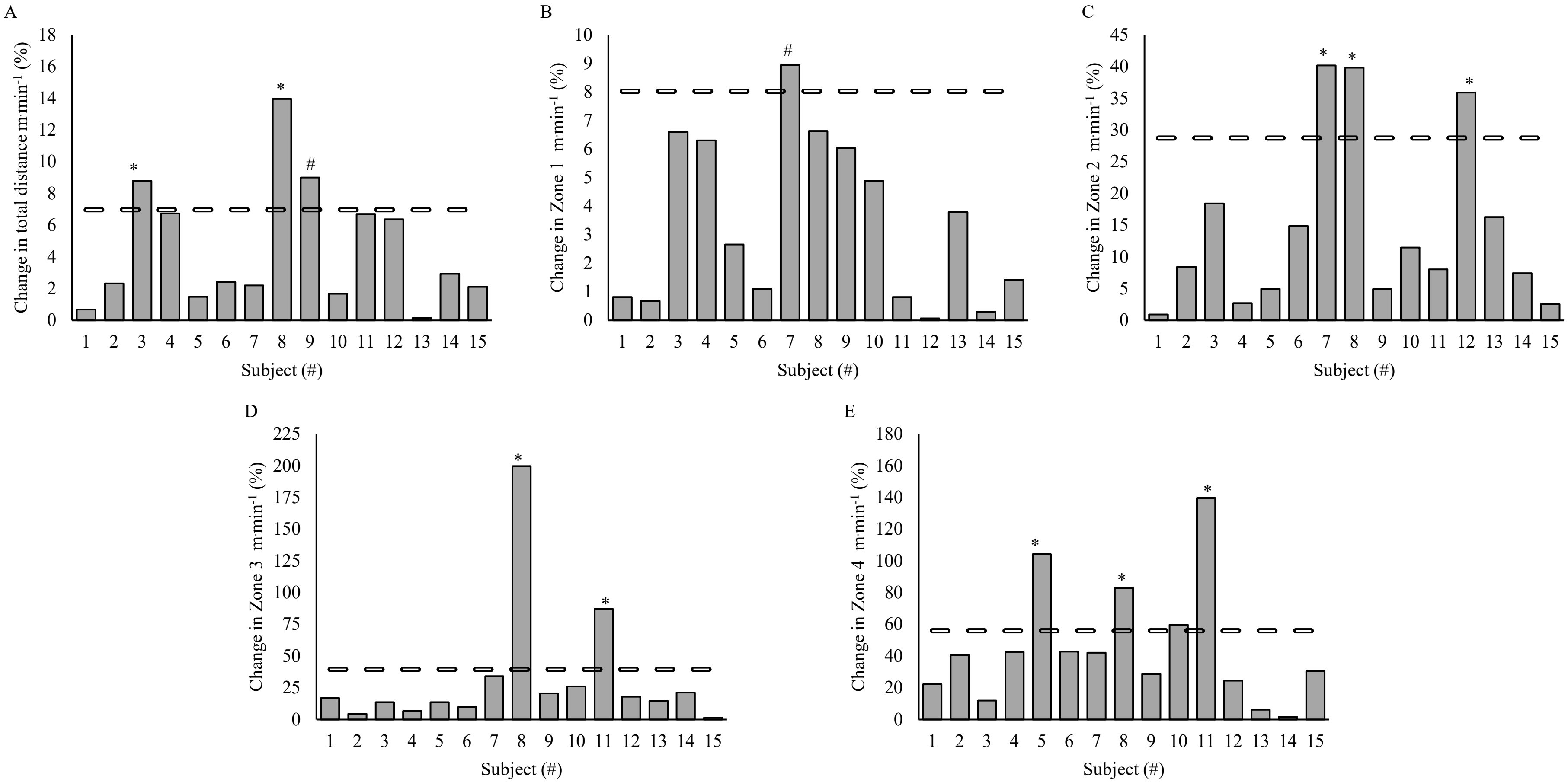


***Figure 16** Individual changes in physical performance using individualised (IND) thresholds, expressed as percentages, between the follicular phase and the luteal phase in comparison to the speed zones coefficient of variation (CV).*

Note: Hashtag = improved performance during follicular phase; Star = improved performance during luteal phase; Dotted line = coefficient of variation.

5.4 DISCUSSION

The findings of this study suggest that VHI running distance covered per minute was significantly greater during the LP in this population of elite female soccer players. However, all other variables were not found to be different between MCPs. Nevertheless, due to the intricacies of the menstrual cycle, multifaceted nature of soccer and the logistical problems which currently lie within female soccer, these findings are difficult to interpret. Furthermore, this study exemplifies the complexities and difficulties of considering the menstrual cycle as a factor, when assessing competitive physical match performance in elite female soccer players.

Physical demands imposed upon a player, have been shown to be modulated by several contextual factors [83, 88, 270]. For example, match location, opposition standard and current score line. The current results indicate a large level of match variation (Zone 3, CV=39.5%). This variation is similar to that of previous research, with Trewin et al., [62] demonstrating match-to-match variations of 33% in high speed running distances across 55 international soccer matches. In terms of practical implications, the potential effects of MCP on physical performance appears to be difficult to interpret with such values of match-to-match variability within competitive match play. Therefore, important decisions based off of specific MCPs, for example player selection, appear unfounded.

Although in our fifteen elite female players, MCP was suggested to have an influence on distance covered in Zone 3, with 0.74 $m \cdot min^{-1}$ more distance covered in the LP. It remains difficult to draw any concrete conclusions from the current data. For example, there were only two players which completed greater high-intensity distances during the LP (Figure 16). However, as mentioned previously, there was large match-to-match variation within this locomotive category, which may have been more indicative of the differences between menstrual phases. Typically, existing literature suggests that MCP does not affect anaerobic performance [47]. However, when differences have been observed, the greatest HI performances generally occur in the LP [164, 169]. Middleton and Wenger [169] observed enhanced power output during repeated bouts of cycling in a non-elite population. Whilst Masterson [164], concluded that anaerobic capacity, peak power and level of fatigue during a Wingate Test were all superior in the LP in 32 active females. Although the competitive standard and exercise modalities were not comparable, energy production and metabolic function during these tasks can be similar. It has been previously suggested that oestrogen and its relationship with progesterone, potentially alters the metabolic pathways and manipulate the sources of energy during exercise and could be a potential source for a change in performance [133]. As demonstrated in male soccer, creatine-phosphate utilization and glycolysis are frequently high, as evidenced by reductions of muscle creatine-phosphate and glycogen, as well as high concentrations of blood and muscle lactate after intense periods of match play [271, 272]. As such, owing to the fact that female footballers complete similar movement patterns, it could be assumed that such substrates are essential for the female game. While this potential mechanism might explain a latent source of difference between phases, it is beyond the scope of the current study to conclusively address such a finding. To ensure accurate measurements of cycle phase and relate to the specific effects of each hormone, sampling is required prior to each match. Such an invasive measure would not be logistically possible (with current lack of sport science support staff) or financially viable to incorporate into the daily practice.

A large contribution to the TD covered within a soccer match requires activity conducted at lower intensities and for extended periods of time [2, 273]. It has been suggested that there is a relationship between overall physical performance and fitness status in female players [4]. Recent evidence indicates endurance capacity of female players may be compromised during the LP [238]. However, soccer match-play is understood to be self-pacing [274, 275], whereby, players will self-moderate their fluctuating activity profiles to ensure optimal performance can be maintained. As such, owing that female soccer players train and compete throughout each stage of the menstrual cycle over the course of the season, they may have adapted personal strategies to cope with such demands.

As previously stated, the hormones related to each MCP have been suggested to affect different aspects of athletic performance [49] . There was no or a small relationship between the degree of change in hormone (both oestrogen and progesterone) concentration and physical performance (oestrogen, r=-0.29; progesterone, r=0.05). Furthermore, there were no large or meaningful relationships between oestrogen or progesterone and any other of the physical parameters. Consequently, these findings would suggest that the hormones evaluated in the current study were not related to any changes in performance. Other confounding factors that are often attributed to the menstrual cycle, such as bleeding-associated pain or feelings of discomfort which do not appear to be due to abnormal concentrations, but the symptoms triggered by fluctuations of such hormones [276] should be monitored and considered as they may also lead to changes in physical performance [156].

When observing individual responses, it was shown that one player (#8) demonstrated a consistent systematic difference in physical performance between the FP and LP (Figure 15). Furthermore, these changes in performance were observed to be greater than the CV (%) for each of the zones (Figure 16). Currently, it would be difficult to make recommendations based on these findings, due to the prevailing lack of studies and inconsistencies in methodologies which have investigated individual effects of MCP on sport-specific performance. Currently there are no guidelines regarding those athletes who may be more susceptible to physical performance changes. Furthermore, though suggestions such as player rotation or change in personal tactics could be an option, it is of increased difficulty in elite female soccer to make such recommendations. Restrictions, due to an initial inability to distinguish those at risk or a lack of depth in squad may limit the efficacy of such ideas.

Further analyses of individual responses indicate that ten demonstrated a consistent pattern of difference in physical performance. This difference occurs during at least one of the measured outcomes in relation to the FP or LP (Figure 15). Of these individuals, seven players exhibited larger changes in performance compared to the match-to-match variability as demonstrated through the CV. Interestingly, of these seven players, two players elicited these improved performances during the FP. As described in previous research, the magnitude of the effect of MCP on the changes in physical performance varies substantially between individuals [262], which although cannot conclude that this was specifically due to MCP, it does additionally demonstrates the difficulty to assess the menstrual cycle alongside competitive match performance.

Due to the highly-applied nature of the current study, there were apparent (and unavoidable) limitations to the study design; however, they shed a light on the complexity of trying to address the menstrual cycle in relation to match-performance. Though the study held some strict criteria for inclusion, the determination of hormone concentrations and verification of ovulation was conducted only once during the present study. It is known that women with physiological 'normal' cycles and accompanying menstruation, still sometimes encounter anovulatory cycles [135] and hormone concentrations have been noted to not only differ greatly between participants but also within a subject over several menstrual cycles [49] . These factors may have caused greater variability which was not accounted for in the present study. However, due to the elite level of our sample, extensive blood sampling (e. g. after each match) and monthly ovulation testing was not feasible.

Additionally, MCP was only separated into two phases (FP and LP), due to the nature of the secretion of hormones these phases may ignore certain physiological differences due to increased or supressed hormone concentrations associated with different times of the cycle, for example low levels of both oestrogen and progesterone during menses [135]. Owing to the timing of the matches (which was not subject to study-related changes) and the chosen method of cycle calculation, the individual (sub-)phases could not be precisely determined and therefore, were not segregated. This method does however, provide an overview between the pre- and post-ovulatory phases; which (when being tracked by players without ability to assess hormones or specific phases) can produce basic information for the players and coaches. However, based on our method of analysis (counting retrospectively across multiple cycles, based on each individual player's average cycle length), 19, 8 and 9 matches were played during the early, mid and late FP, respectively. Whilst, 13, 21 and 6 matches were played during the early, mid and late LP, respectively.

In the current study, although there were strict inclusion criteria, the concentration of progesterone to confirm the LP was less conservative than previous studies [52]. However, at present reference values for athletic populations have yet to be defined and are not available. Moreover, with strict criteria you may remove those who otherwise were physiologically “normal”. Therefore, with the confirmation of ovulation and a significant change in hormone values, the associated change in phase could be verified.

Although initially large subject numbers were recruited, a further limitation is the low sample size analysed in this study. However, within professional environments these sample sizes are common, specifically in this type of research there are various issues that may increase drop-out rates. As can be observed in figure 14, the drop-out rate from the originally recruited participants in the current study was 82%. Primarily, this was influenced by those using a form of contraception. In soccer, previous reports have suggested that approximately 36% of players use oral contraception [216]. Similarly, in the current study ~35% of the original population were excluded due to using a form of contraception. This limits potential sample sizes during such investigations. A further factor that contributed to this high drop-out rate was the confirmation of cycle phases. Blood sampling was required to be conducted at very specific timepoints (to accurately account for the changes in oestrogen and progesterone). Twenty-one participants were lost due to incomplete blood sampling (Figure 14). In the current study it was difficult to complete both blood samples at the specific timeframes. The most common factor was due to other time commitments (e.g. jobs, university classes) meaning samples were unable to be completed within one cycle, at the predefined time periods. This is often why in previous research female participants are tested solely in the EFP of their menstrual cycle, to minimise the possible impacts that hormones may have on study outcomes [133]; thus reducing the difficulty of measurements.

Surprisingly, there were a large proportion of players who had non-physiologically “normal” menstrual cycles (ten from 33). In a previous sample of 44 female soccer players only four individuals were shown to have menstrual dysfunctions [216]. The issues surrounding normal menstrual function provide additional sources of consideration for research and within practice. Nonetheless, it should be acknowledged that elite female players were used in the study and observed throughout an extended time during the season.

In addition, the observational design of the study limits the possibilities to understand causal factors for the alterations in physical performance, which would be interesting in future research. Taken collectively, such information illustrates the difficulties in conducting such research and the potential implementation into daily practise.

5.5 CONCLUSION

This study adds to the growing body of research investigating the effects of MCP on sport specific physical performance. The current results suggest that MCP does not contribute largely to the changes in physical performance, in comparison to the potential effects of individual and match variation. Therefore, interventions or other methods of adaptation do not seem necessary on a group level. Inter-individual variability in response may potentially call for sport scientists and other practitioners to consider MCP when assessing performance among those individuals who have been identified as "responders". However, owing to the multifaceted nature of soccer and the difficulties of monitoring the menstrual cycle in relation to match performance, caution must be implemented when interpreting these findings. Nevertheless, this study exemplifies the complexities when attempting to conduct such research. Future research should attempt to reproduce the current findings; using larger sample sizes, spanning across multiple seasons, or using more controllable measures, such as match simulations. This increased volume of information would help support these initial findings and could be used to further inform practise and support the progression of female soccer players.

6.0

GENERAL DISCUSSION

6.1 DISCUSSION INTRODUCTION

The series of investigations within this thesis were designed to investigate the potential fluctuations in physical performance of the female footballer, with special reference to female specific physiology, in particular the menstrual cycle. The current studies were conducted in both a controlled (Chapters, 3 & 4) and ecologically valid (Chapter, 5) environments, which aimed to provide a comprehensive overview of the different settings, which are specific and translatable to practice when performing physical testing and assessment of match-specific performance in this population. Specifically, the aims of this thesis were to: (1) Identify variations in physical and surrogate markers of performance across the competitive season during football-specific isolated testing; (2) To determine whether these physical capabilities are affected due to two specific phases of the menstrual cycle, where theoretically the largest differences in hormones are apparent (EFP vs. MLP); (3) To observe whether physical match performance was influenced between the FP and LP of the menstrual cycle. Thus, the following chapter will initially aim to reiterate the conclusions of the experimental studies and then collate the discussion points pertinent to the current thesis.

6.1.1 Study 1 – Changes in physical fitness and blood-borne parameters over one season in non-professional female footballers

Physical parameters including YYIET2 distance, 30m sprint time and COD were found to improve from pre-season to the first weeks of the competitive season (ES, 0.3 to 0.5). Whereas, these improvements were seen to decline towards the end of the season in YYIET2 performance. These data suggest that endurance training might have been negated throughout the season to maintain performance, moreover, suggests that practitioners of female football teams might not be able to monitor training effectively. Secondary findings, demonstrated that relatively low values for CK (indicator of microdamage to muscle fibres) and urea (indicator for metabolic load) were apparent, further confirming that inadequate training stimulus might have been imposed onto the players. Finally, changes in ferritin concentration was demonstrated, which might have direct consequences to physical performance. Importantly, the findings suggested that there are certain individuals who were below the "normal" reference ranges, thus, regular monitoring of iron status in female footballers is required.

6.1.2 Study 2 – The effects of menstrual cycle phase on physical performance in female football players

The results suggested that there was no significant effect of MCP on any of the isolated tests of physical performance (YYIET1, 30m sprint, CMJ). However, there was a trend to significance, with a decrease in YYIET1 distance, suggesting that maximal aerobic performance during the MLP (~14%, p = 0.07, ES = 0.56) was possibly compromised. As such, it is potentially important to consider and keep consistent the MCP when measuring endurance capacity in female football players during football-specific endurance testing.

6.1.3 Study 3 - Menstrual cycle phase and elite female football match-play: influence on various physical performance outputs

Overall, the findings suggest that the menstrual cycle does not contribute largely to the changes in physical performance. Although not directly measured, other factors such as game-to-game variation, appear to be more influential. The data suggested that only zone 3 (13.4-18.9 – 17.4-21.6 km·h-1) was significantly affected by MCP (FP vs. LP: -0.74m/min, p = 0.02, ES = 0.41). However, these values should be interpreted with caution as only 13% of the population demonstrated this change greater than the CV (game-to-game variation). Consequently, the inter-individual variability in response may potentially call for sport scientists and other practitioners to consider MCP when assessing performance among those individuals who have been identified as ‘responders. However, taken collectively, the incorporation of the menstrual cycle into assessing match performance appears limited due to the complex nature of both the sport and female physiology.

6.2 GENERAL DISCUSSION

Physical fitness has been determined as a prerequisite to high-levelled footballing ability [2, 8, 16, 28, 129]. Due to the diligent work of previous researchers, a large volume of research has been conducted describing the general physical capacities of the female footballer [28]. However, less information has been provided relating to the potential changes in physical capacities over course of the season. There are instances where players face potential barriers to maintain peak physical capabilities. Such as during periods of recovering from injury, changes in training loads, amount of competitive playing time amongst others [277]. Therefore, conducting regular assessments of the physical capacities may provide practitioners and coaches with invaluable information on the current status of their pre-planned training program or whether adaptation is needed [277, 278]. Although, this is oftentimes more difficult in female football, due to the financial constraints within the sport, meaning there is a current lack in number and in competency of support staff.

Conventionally, the target of the pre-season is to prepare and condition the footballer for the demands of the upcoming season. These adaptations have attributed to the high and low intensity aerobic focused training performed during traditional pre-seasons [96, 279]. The results of the first study are in line with previous literature which observes that there are improvements in physical capabilities from preseason to the beginning of the season. The results of chapter 3 showed an increase of approximately 16% for endurance performance from the preseason until the beginning of the season. A similar change in YYIET2 performance has been observed previously, with Bradley et al. [34] observing a 13% improvement within the season (1767 ± 539 m) compared with the preseason (1564 ± 504 m). However, this finding was not replicated in a population of elite Australian female footballers using the YYIRT2 [113].which could be explained due to the different nature of the tests, whereby, the YYIRT2 contains a higher anaerobic component with greater accompanying speeds and may be too intense for the population. Interestingly, Mara et al. [113] suggested that potentially the pre-season period did not provide the desired stimulus, highlighting the importance of the pre-season as the key developmental phase of conditioning and within the season there should be greater focus on maintaining performance.

A similar trend was observed across all physical parameters throughout this study. This could suggest that there was a large devotion to the development of these parameters during the pre-season; however, the physical loading was neglected throughout the season to maintain this performance. Previous research has suggested that inadequate training stimulus and monotonous training regimes may lead to an inability to maintain peak levels of performance [113, 280]. In the current study, there was no information provided to indicate the training program and periodisation used by the coaches. However, load is oftentimes measured using internal measurements [281-283]. These can be particularly attractive because of their obvious objectivity, high accuracy and precision, with the minimal interference with the training process [235]. Measures such as RPE, HR and surrogate blood markers have all been previously used to describe the physical responses to football activity [281-285]. Chapter 3 used blood-borne markers to support the physical responses during these measurement time points. It has been previously demonstrated that CK is related to acceleration-based load in team sport athletes

[286, 287], signifying that CK can describe the physical load during exercise based on the accumulation of tissue trauma as a direct indicator of muscle damage [235, 288]. The findings of the study suggest that inadequate training stimulus might have been imposed upon the players throughout the season, due to the relatively low CK and urea (a marker of protein breakdown) concentrations were observed. A recent framework has highlighted that insufficient training may eliminate the performance benefits of previous training programs, thus, players should be adequately exposed physically through appropriate loading and periodisation of their activities to achieve the desired adaptations and maintain peak physical performance [233]. The data associated with chapter 3, therefore has potential implications for the design and micro management of training and schedules. Whereby, practitioners and coaches should maintain a good-level of training stimulus after the pre-season, to avoid unsatisfactory stimulus and the reduction in peak performance benefits.

Physical capacities were shown to fluctuate across the season, this is often attributed to insufficient or incorrect periodisation of training and load management. However, in females there are large changes in hormones which occur regularly throughout the menstrual cycle; each of which have been suggested to alter varying physiological, metabolic and thermoregulatory factors, among others [39, 47, 48]. Yet, when conducting physical performance testing, this facet is often ignored. Thus, the aim of study chapter 4 was to quantify whether two specific phases of the menstrual cycle (with opposing hormonal profiles) elicited different physical performance profiles.

To evaluate the potential differences in physical performance profiles across the menstrual cycle, a series of “football-specific” components (YYIET, CMJ, 30m-sprint, COD) were measured during two distinguishable phases. The first in the EFP, where both oestrogen and progesterone concentrations are minimal and the second within the MLP, where both aforementioned hormones are elevated (Figure 2, Chapter 1). It was observed that CMJ and 30m sprint performance was not different between MCP's. However, maximum endurance performance (YYIET1 distance) was diminished during the mid LP. Such findings could question the sensitivity of such measures, e.g. CMJ, whereby similar performance might be maintained by changes in technique rather than changes in strength due to MCP. On the other hand, this may also indicate that activity type was potentially affected due to MCP; with physical actions over a longer duration affected greater than acute actions, which have a greater emphasis on power. As such, this could have implications during prolonged performance, such as during match-play, or during physical assessment and monitoring of female footballers.

Although there was no significant difference in YYIET1 performance between phases (P = 0.07), there was a moderate effect size (Cohen's d = 0.56) which could indicate a potential difference between cycle phases, especially due to the low sample size (n = 8). The current study is one of two investigations that have attempted to observe the effect of the menstrual cycle of football performance. Tousni et al. [109] suggested that there was no difference in YYIRT1 performance between the EFP (905.45 ± 386.69) and MLP (894.55 ±305.30). The contrasting findings between the two studies could potentially be due to the different YYIT protocols deployed. While both the YYIET1 and YYIRT1 have been classified to assess the endurance capacity of the footballer [289], the duration and intensity differs. The intermittent nature of the YYIT taxes both the aerobic and anaerobic energy system, but the relative contribution of each system differs. With a larger anaerobic component within the YYIRT compared to the YYIET; as such, this could be indicative as to why the findings differ. Nevertheless, the findings of the current study suggest that it is advantageous to keep MCP consistent when measuring maximum aerobic performance, minimising potential sources of variation. Moreover, due to the potential effect of the menstrual cycle during prolonged activity, this may have consequences during match-play, as football is often considered an aerobic sport.

Accordingly, due to the findings of chapter 4 and the potential effect of the menstrual cycle on maximal aerobic performance, it would appear imperative to observe whether certain phases of the menstrual cycle could affect physical match performance. Activity types during a 90 minute match, such as TD and HI distance have often been described as important indicators of physical match performance [290], which both rely on the training status (level of physical capacity) of the female football player [4]. Therefore, the aim of chapter 5 was to determine whether there are differences in various physical performance outputs between the FP and the LP of the menstrual cycle during competitive match-play.

Currently, this is the first study that has attempted to identify differences in physical performance between MCP during competitive football match-play. Interestingly, the results of chapter 5 suggested that there was significantly greater distance covered per minute whilst performing VHI running (13.4-18.9 – 17.4-21.6 $km{\cdot}h^{-1}$) during the LP than the FP (6.64 ± 2.72 m·min vs. 5.90 ± 2.16 m·min, $p = 0.02$, $d = 0.41$). As can be observed in chapter 2, typically, anaerobic and repeated HI performance has not been observed to be affected based on MCP. That being said, research that has identified a difference noted greater performance during the LP [164, 169]. For example, Middleton and Wenger [26] observed an enhancement in power output during ten, six second maximal sprints on a cycle ergometer. Although the authors found only small average differences in power output, the improvement during the LP was exhibited in every subject. Conversely, in the current study only four players consistently performed greater distances at this intensity during the LP, with only two of those demonstrating a larger difference in performance than the inherent match-to-match variation measured during the current study (CV = 39.5%). However, it must be noted that the results from Middleton and Wenger [26] was conducted during isolated testing, not accounting for additional movement activities and contextual additions that come with match-play. Furthermore, the absolute number of sprints conducted in the aforementioned study (ten) was over 50% less than conducted during match-play. Although the relative findings might indicate that VHI actions are affected by MCP, due to the contrasting methodologies it is difficult conclusively compare the findings.

As mentioned previously, two players demonstrated a greater distance covered during VHI, however, only one player demonstrated a consistent systematic difference in all physical performance outputs between MCPs (Figure 15, Chapter 5). This finding would suggest that there is an inherent between- and within- subject response to the potential effects of MCP on physical performance. Consequently, identifying those individuals who may be at greater risk of changes in physical output, could be advantageous for practitioners.

When identifying the differences in physical outputs between the FP and the LP, there appears to be little effect. Physical demands imposed upon a player, have been shown to be modulated by several contextual factors, such as, match location, opposition standard and current score line [83, 88, 270, 291]. The current results demonstrated large match-to-match variations (CV= 7.0-56.0%), in the measured running metrics, which were much greater than that observed previously in an elite international female team (1.7 – 9.4%) [62]. These large differences could be due to the specific philosophies of each team, or could be due to the individualised nature of the speed thresholds. Unlike the vast majority of the research in female football, the current study personalised the speed thresholds during physical tests based on the model from previous research [268] . This provides the current study with greater accuracy in terms of identifying fluctuations and changes in running performance, be it due to the standard constraints and variations of football or other specific confounding factors such as the menstrual cycle. In terms of practical implications, the potential effects of MCP on physical performance appear to be less influential than the match-to-match variability within match play. Therefore, important decisions based off MCP in terms of player selection, or adaptations to training in the run up to competitive matches appear in the most part unnecessary.

In addition, this research has also identified the complexity of assessing the menstrual cycle alongside physical performance in female football (Chapter 5). What is already described in previous literature are several factors that can add to the difficulty of assessing physical performance during match-play, such as level of opponents or variation of playing position [86-89]. However, when attempting to include the menstrual cycle, there are several problematic factors which become apparent; such as the use of contraceptives, within- and between- subject variation of the menstrual cycle (magnitude of hormones, length of cycle, severity of symptoms), inclusion of anovulatory or otherwise irregular cycles (e.g. LPD, endometriosis). Consequently, study 3 (Chapter 5) highlights the difficulties when attempting to include the menstrual cycle into scientific research (Figure 14, Chapter 5) and subsequent possible implementation into daily practice.

6.3 DIRECTIONS FOR FUTURE RESEARCH

Whilst synthesising the current literature and conducting the present research, there were some clear areas of potential research that appear to be warranted. Although there is a reasonable amount of literature describing the physical profile of the female football, it gives the impression that there is sound knowledge on the topic. However, there is a current lack of position specific information. It was identified in chapter 3 that players are exposed to variations in physical capacities throughout the season, however, it has not yet been established whether this occurs generally across all playing positions. It has been previously shown that the amount of load varies across playing position in training [54] and during competitive match-play [270], as such those positions who are exposed to these greater levels may not have such a change in physical capabilities and as a result, a greater training stress (top ups) could be applied to certain positions similarly to that of unused substitutes and squad players [292]. Consequently, future research should try to identify whether these changes in physical capacities differ across playing position and whether this is directly related to the amount of training and match load.

Interestingly, the data from chapter 3 identified that certain players demonstrated a potentially reduced iron status and that this should be further monitored. It would be of interest to determine whether continued application of training and match load is related to changes in a player's iron status and how this could be potentially avoided or supplemented. As such, this change in iron status could also be linked to changes in peak physical performance and subsequently warrants further research.

The current studies in chapter 4 and 5 are the first to observe the potential effects of the menstrual cycle on football-related physical performance, therefore, many potential avenues of future research are needed to fully understand how female specific physiology might impact football performance. In chapter 4, it was documented that there was a potential trend for a reduction in endurance performance during the LP. However, the current phases used during this study were EFP (both oestrogen and progesterone low concentration) and the MLP (both oestrogen and progesterone elevated concentration). Therefore, it is not sure whether synergistic interaction of oestrogen and progesterone may have led to the potential change, or whether, it was the effects of oestrogen. During the menstrual cycle, there is a timepoint where oestrogen is independently elevated (LFP) and therefore, future research could investigate whether football-specific endurance capacity is reduced during each of the three main phases of the menstrual cycle (Table 3, Chapter 2). Moreover, a greater investigation into the responses in those with a skewed oestrogen/progesterone ratio, could also shed a light on certain responders; this could potential suggest whether there is a large imbalance between the protagonist and agonist leading to a change in performance. Additionally, in general, there is a current lack of research investigating the effects of the menstrual cycle on sport-specific physical capacities, thus, future research should aim to reproduce the current findings to provide a clear consensus on the effect, and if possible within a larger cohort with stricter MCP verification (addition of ovulation testing).

Similarly, chapter 5 is the first study attempting to observe whether MCP impacts physical performance during match-specific scenarios. The current information suggests that there are potentially those who may have a greater individual response to others. However, this requires further reproducibility studies, potentially with greater subject number and a closer (case study like) investigation into how those who are suggested as "responders" react throughout the season. Currently, due to the design (as explained in chapter 5) it was difficult to segregate the phases into their specific timepoints within the menstrual cycle, therefore, it would be of interest to identify whether the different specific phase may affect physical performance. This could be conducted using football-simulations to provide a more controlled environment, where testing could be easier to organise [293]. However, one benefit of the current study is that it was conducted in a highly ecologically valid setting, as such, it could be conducted over a shorter time frame (two months) where players and coaches might be more accepting of more regular but limited testing.

Chapter 5 identified that variations in physical performance are more likely due to general match-to-match variability and contextual variables, however, residual fatigue could also play a role as performance has been previously observed to remain reduced 96 hours preceding a competitive match [99]. Due to the physiological changes owing to the hormones of the menstrual cycle, it would be of interest for future research to identify whether different phases (with relative hormones) alter the pathways for recovery, meaning that subsequent recovery time course and modalities can be potentially tailored to the menstrual cycle.

Finally, the physical component is only one element of football performance. There is a current lack of research comprehensively describing the technical performance of female football players. Moreover, due to the supposed changes in coordination across the menstrual cycle [39], it would be of interest in future research to determine the effects of the menstrual cycle on technical proficiency. Understanding first the overall technical competencies and the potential changes in such performance could help practitioners and coaches to maximise technical abilities and maintain peak match performance.

6.4 CONCLUSION

Collectively, the current thesis provides further insight into the physical profile of the female footballer and is the first to consider female specific physiology as a factor that could potentially limit performance. The present findings highlighted that variations in physical performance exist across the season, and that specific MCP may influence isolated endurance performance testing. Thus, when monitoring and assessing a female footballers' capacities to evaluate adaptations to training, MCP should remain consistent, if possible. However, such changes in physical performance was not observed during competitive match-play. The results suggest that

MCP, even though it might play a slight role, is not a major contributing factor to variations in physical performance, it has been observed to be extremely difficult to assess performance alongside the menstrual cycle during competition. As such, other factors including match-to-match variation and other contextual factors might be more influential. Nevertheless, it still could be advantageous to identify those who potentially demonstrate a "response" to the fluctuations in performance during certain MCP, so that appropriate actions can be made to support peak performance.

7.0

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8.0

CURRICULUM VITAE

The curriculum vitae was removed from the electronic version of the doctoral thesis for reasons of data protection.

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APPENDICIES

Changes in physical fitness and blood-borne parameters over one season in non-professional female footballers.

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Women | Soccer | Blood | Fitness

Headline

The number of female footballers continues to expand, suggested by record numbers of players being registered. Of these players, the non-professional footballer encompasses a large volume of the overall community. There seems to exists a relationship between training status (levels of fitness), competitive standard and physical performance (1, 2). However, comprehensive information describing the physical capacities across an array of competitive standards in female football is still lacking. Though the capacities of the non-professional player have been highlighted in single bouts of testing, it remains relatively unknown whether changes in physical fitness occur over the course of a season. Moreover, continued physical exposure through training and matches, may affect certain blood-borne parameters, such as iron levels, which in turn may affect the physical performance and/or health status of a player. Therefore, coaches and practitioners at the non-elite level (who might not have the opportunity for extensive monitoring) may use this information to help training and create a frame work for testing and monitoring the female footballer.

Aim of the paper

The aim was to observe the changes in physical performance and fitness-relevant blood-borne variables of non-professional female footballers using repeated measures within a football season.

Methods

Design. The study utilised an observational longitudinal design. Whereby, the players underwent a series of physical tests and provided blood samples on the following three time points across the season; pre-season (early August) (M1), beginning of the competitive season (early October) (M2) and end of season (late May/early June) (M3). The pre-season period consisted of 8-weeks training, three times per week. All M2 measurements were conducted within four weeks from the first official game, during this period players trained three times a week and had one official match. Finally, M3 was conducted during the final three weeks of the 22-game season.

Subjects. Thirty-six non-professional female footballers (19 ± 2 y, 164 ± 5 cm, 80 ± 8 kg) competing in the second Frauen-Bundesliga, Germany, participated in the study. Only outfield players were considered for the final analysis and participated in all testing periods over the season. Participants were fully informed of all experimental procedures before giving their informed consent to participate. If players were younger than 18 years of age, parental consent was obtained. The current study was conducted in accordance with the declaration of Helsinki and approved by the local ethics committee (Aerztekammer des Saarlandes, approval number 130/14).

Methodology. Prior to data collected, players were familiarised with the protocol of each physical test. The physical testing battery was conducted in the present order and included (after appropriate warm-up):

Counter movement jump (CMJ) for the analysis of lower limb strength. Characteristics of the CMJ were determined using a force platform (Quattro Jump, Type 9290AD, Kistler Instrument AG, Winterthur, Switzerland) and analysed using professional motion analysis software (Contemplas Bewegungsanalyse, Contemplas Gmbh, Kempten, Germany). Jump height was determined as the centre of mass displacement, calculated from the recorded force and body mass. The mean of the best three jumps was used for the subsequent analyses.

3 x 30 m to measure sprinting capabilities. Splits were set at 5 m, 10 m and 30 m and measured by infra-red single beamed light gates (Brower Timing System, Utah, USA), with a standard error of measurement of 0.03 s (3). Players had two mins of recovery and then repeated the test for three trials, with the mean of all three 30 m sprints used for analysis.

4 x 5 m change of direction (COD) test was performed, similarly to that described by Sporis et al (4). At the start and finish, light gates (Brower Timing System, Utah, USA) were erected and used to assess total time to complete the course. Three trials were conducted with two minutes of passive recovery interspersed, the mean of all three trials was used for analysis.

Finally, the Yo-Yo Intermittent Endurance Test level 2 (YYIET2) was conducted to measure sport-specific endurance capacity. The test was performed outdoors on artificial turf similarly described by Bangsbo et al (5). Total distance of completed shuttles was recorded as the test result. HR ($\geq 220 -$ age (years)) and RPE were used as objective criteria of exhaustion (6) to be included into final analysis.

Prior to the physical tests at all three time points over the season, venous blood sampling was obtained from the antecubital vein, following 10-15 min of rest in the supine position. Blood samples were transported immediately to the laboratory for appropriate procedures. Serum tubes were centrifuged at 4000 revolutions per min for 5 min, aliquoted and then stored frozen at -80°C within 2 h of sampling. Creatine kinase (CK), urea and C-Reactive Proteins were measured using a Unicel DxC600 synchron clinical system (Beckmann Coulter GmbH, Krefeld, Germany). Erythrocytes and Haemoglobin was determined automatically by an ACT 5 Diff AL (Beckmann Coul-ter GmbH, Krefeld, Germany). Ferritin was analysed using an Access 2 Immunoassay system (Beckmann Coulter GmbH, Krefeld, Germany).

Data were collected in the early evening (prior to and dur-ing normal training sessions). All measures were conducted outside on artificial turf under neutral weather conditions. If weather was deemed unsuitable or at risk of influencing re-sults, or players' welfare, (e.g. any form of rain) the test was either cancelled or if possible, re-scheduled.

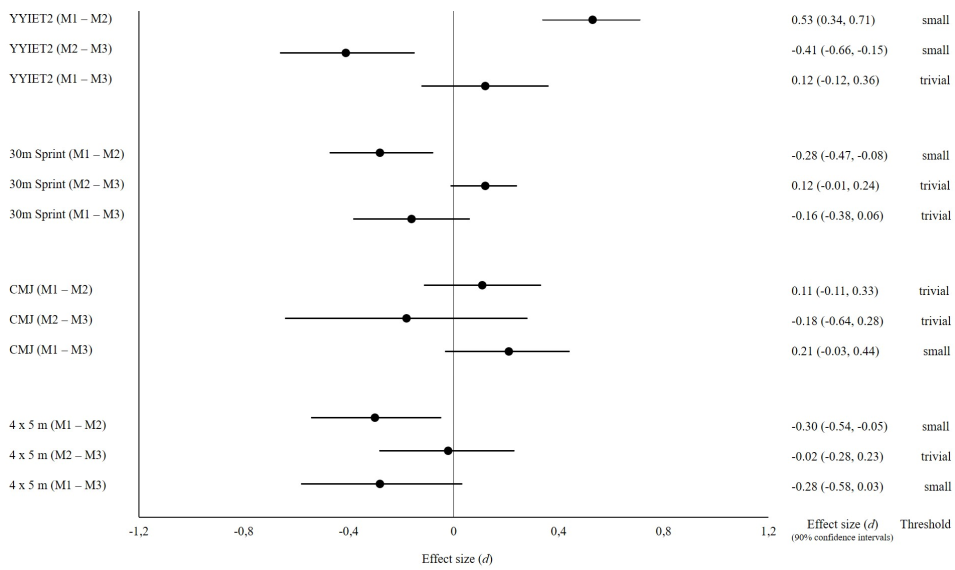

Fig. 1.

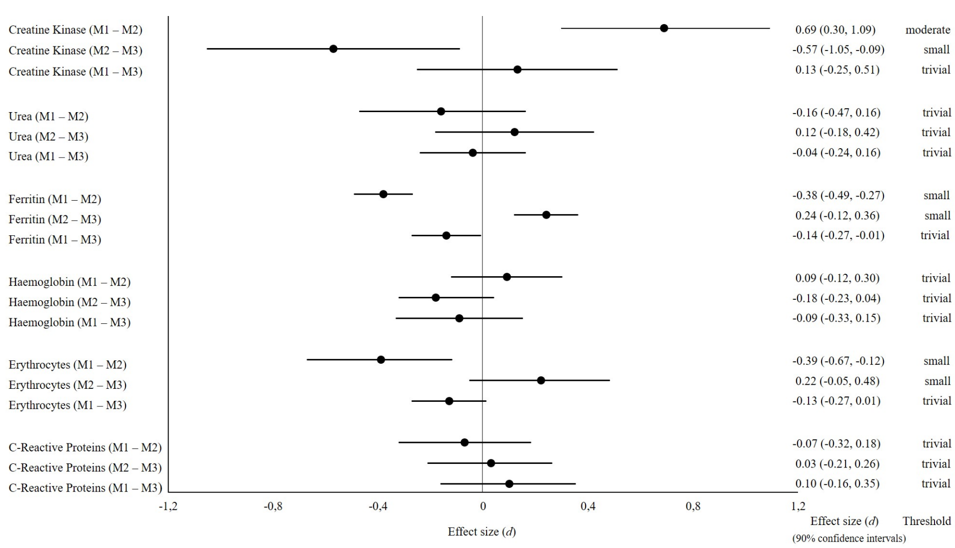

Fig. 2.

Statistical analysis. The mean ± SD were calculated for each of the three time points across the season. Cohens d effect sizes (ES) were calculated with 90% confidence intervals (CI) and interpreted as; trivial (<0.2), small (>0.2-0.6), moderate (>0.6 – 1.2), large (>1.2 – 2.0), very large (>2.0) (7).

Results

All physical and blood-borne variables over the three measurement timepoints can be observed in tables 1 and 2, respectively. The course and magnitude of the change can be observed in figures 1 and 2 for physical and blood-borne variables, respectively.

Discussion

The primary objective of the study was to describe the changes in physical performance within a season of non-professional female footballers. Among the main findings of the study were that small improvements in YYIET2 distance, 30 m sprint time, and COD completion time from pre-season to the start of the competitive season. However, as the season progressed YYIET2 performance declined again, whereas the other physical parameters remained stable across the season. The secondary aim of the study was to detect changes in selected haematological variables. Results indicated that there were moderate to small changes in CK, Ferritin and Erythrocytes from M1 to M2 with a considerable number of players showing indications for iron deficiency.

The results of the current study are in line with previous observations (8, 9). Physical performance was observed to increase during pre-seasonal period, which is often designed to increase physical capacities for the upcoming season whilst, small decreases in physical performance were apparent as the season progressed. Moreover, there are large inter-individual differences at each time point and intra-individually over the course of the season (illustrated in figure 3). There are several

Table 1. Physical variables and the within season changes across all timepoints. Data is represented as means ± standard deviations.

Variable	M1	M2	M3
CMJ (cm)	31.3 ± 3.1	31.6 ± 3.0	32.0 ± 3.5
0-30 m sprint (s)	4.8 ± 0.2	4.7 ± 0.1	4.8 ± 0.1
COD (s)	6.0 ± 0.3	5.9 ± 0.3	5.9 ± 0.3
YYIET2 (m)	752.2 ± 233.2	873.3 ± 251.99	779.7 ± 234.1

Note: CMJ = Counter movement jump; COD = Change of direction; YYIET2 = Yo-Yo intermittent endurance test level 2. M1 = Pre-season, M2 = In-season, M3 = End of season.

Table 2. Blood variables and the within season changes across all timepoints. Data is presented as means ± standard deviations.

Variable	M1	M2	M3
Creatine kinase (U/L)	194.3 ± 87.7	256.5 ± 118.2	205.7 ± 118.8
Urea (mg/dl)	29.4 ± 6.2	28.4 ± 9.0	29.1 ± 6.0
Erythrocytes (Mio/μ)	4.5 ± 0.2	4.4 ± 0.3	4.5 ± 0.2
Ferritin (ng/ml)	32.5 ± 14.9	26.7 ± 13.6	30.4 ± 14.8
Haemoglobin (g/dl)	13.6 ± 1.3	13.7 ± 1.0	13.5 ± 0.9
C-Reactive Protein (mg/l)	1.2 ± 2.9	0.9 ± 1.6	1.0 ± 1.6

Note: M1 = Pre-season, M2 = In-season, M3 = End of season.

factors which might cause these changes in physical performance. Oftentimes non-professional female soccer teams have an inability to effectively monitor training, with financial constraints, suboptimal education level of coach/practitioners and limited availability of staff leading to such insufficiencies. As such, inadequate training stimulus might be imposed upon the players or a monotonous training routine, which loses its ability to maintain a peak level of physical performance (10). Relatively low values for CK (indicator of microdamage to muscle fibres) and urea (indicator for metabolic load) seem to be in favour of this interpretation. This neglect on physical loading could in part, be due to the shift in focus towards the tactical element and preparation for games. Moreover, due to playing weekly matches, alongside other obligations (work, studies) this may possess an issue to ensure that satisfactory levels of physical load can be transferred onto the player to maintain physical performance. Recent monitoring frameworks suggest that a lack of loading may not only affect physiological parameters, but potentially leads to a lack in biomechanical load which may in turn increase injury risk (11). As such, it could be recommended that basic testing, with limited "necessary" equipment (such as Yo-Yo testing) is implemented more regularly to help respond to the needs-analysis for effective training in non-professional populations.

The current selection of blood-borne markers has been suggested to provide an overview of the physical state of a footballer (12). When evaluating, it is imperative to understand the magnitude of the change and understand what is beyond a 'normal' change. Therefore, these findings can provide a larger pool of information for baseline and within-season changes for the non-professional female footballer. It should be noted, that large variations within and between individuals are observed, even when experiencing a similar training stimulus (13). As such, when possible, consistent testing of each individual is warranted to ensure the physical state can be maintained throughout the season.

Interestingly, on the group level the results indicated that there were small (d = 0.38) changes in ferritin from M1 to M2. Reduced levels of ferritin have been linked to negatively

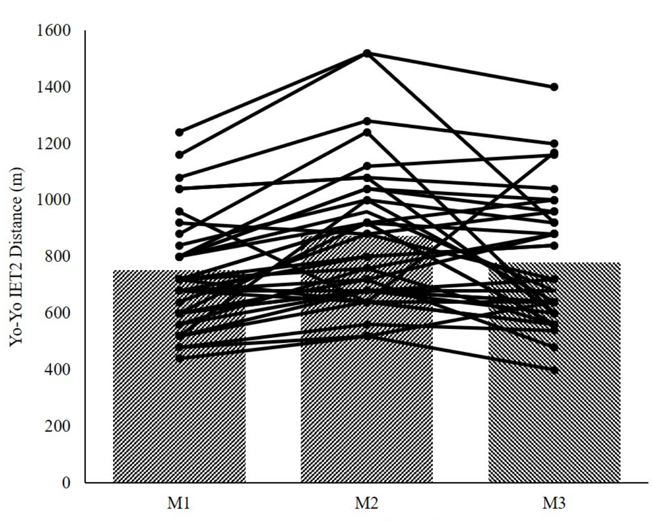

Fig. 3.

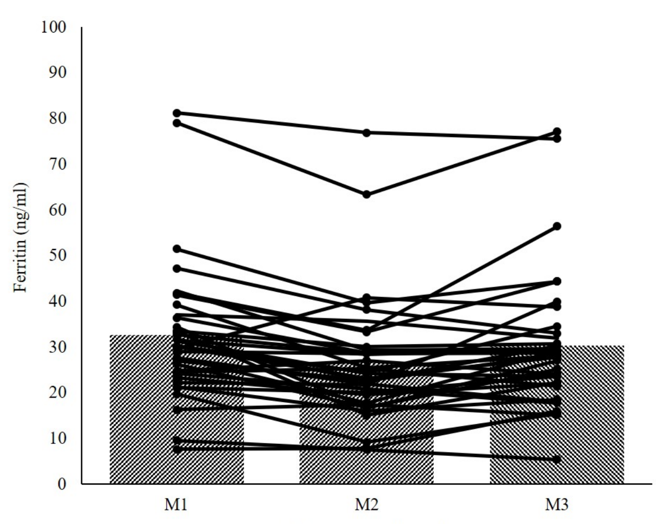

Fig. 4.

affecting physical performance (14) and health of the female athlete (15). However, this tendency was not observed in the raw values of change in this population. On the group level, the findings suggest that mean values were not beyond the standardised "normal" ranges of ferritin (22 – 120 ng/ml), however, there was a relevant number of players (n = 17%) with suboptimal values albeit without over anaemia (Figure 4). This proposes the need for more consistent monitoring to identify those that are at potential risk and thus one can effectively intervene by giving dietary advice or supplement iron. These findings are similar to that of 28 elite-female footballers, whereby 57% were iron deficient and 29% had iron deficiency anaemia in the run up to the FIFA Women's World Cup (16) signifying that individualised monitoring is important. Therefore, future research should further investigate whether the female footballer is exposed to an increased risk of reduced iron storage and explore whether there is an effect on subsequent performance.

Practical Applications

- The results suggest that practitioners may target increasing the physical capacities during the pre-season period and then possibly slightly neglect them as other priorities such as, match preparation may be determined as "more important". Therefore, a detraining effect may occur which could potentially affect peak performance during match play.
- It might be advisable for practitioners to assess the physical capacities of the players throughout the season (at multiple time points) and manipulate training plans accordingly, to maintain peak physical condition.
- The results indicate that it would be worthwhile in future research to assess the ferritin levels (as well as a full blood count to detect possible iron deficiency anaemia) of the female footballer on a more rigorous routine to identify those at potential risk and to help to create a consensus on appropriate courses of action.

Limitations

- The current study only involved one team which has a certain player pool and one method of training. However, this study represents the first to repeatedly monitor physical and haematological parameters in non-professional female footballers and could provide an insight into the possible challenges with this population.
- Low number of testing time points, without mid-season measurement timepoint.
- Female specific physiological changes, i. e. the menstrual cycle, were not considered during the current study which has been previously observed to affect maximal performance (17).

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RESEARCH ARTICLE

The effects of menstrual cycle phase on physical performance in female soccer players

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Abstract

Background

Female soccer has grown extensively in recent years, however differences in gender-specific physiology have rarely been considered. The female reproductive hormones which rise and fall throughout the menstrual cycle, are known to affect numerous cardiovascular, respiratory, thermoregulatory and metabolic parameters, which in turn, may have implications on exercise physiology and soccer performance. Therefore, the main aim of the present study was to investigate potential effects of menstrual cycle phase on performance in soccer specific tests.

Methods

Nine sub elite female soccer players, all of whom have menstrual cycles of physiological length; performed a series of physical performance tests (Yo-Yo Intermittent endurance test (Yo-Yo IET), counter movement jump (CMJ) and 3x30 m sprints). These were conducted at distinct time points during two main phases of the menstrual cycle (early follicular phase (FP) and mid luteal phase (LP)) where hormones contrasted at their greatest magnitude.

Results

Yo-Yo IET performance was considerably lower during the mid LP (2833±896 m) as compared to the early FP (3288±800 m). A trend towards significance was observed ($p = 0.07$) and the magnitude based inferences suggested probabilities of 0/61/39 for superiority/equality/inferiority of performance during the mid LP, leading to the inference of a possibly harmful effect. For CMJ (early FP, 20.0±3.9 cm; mid LP 29.6±3.0 cm, $p = 0.33$) and sprint (early FP, 4.7±0.1 s; mid LP, 4.7±0.1 s, $p = 0.96$) performances the results were unclear (8/24/68, 48/0/52, respectively).

Conclusion

The results of this study are in support of a reduction in maximal endurance performance during the mid LP of the menstrual cycle. However, the same effect was not observed for jumping and sprint performance. Therefore, consideration of cycle phase when monitoring a player's endurance capacity may be worthwhile.

Introduction

The professionalism and interest in female soccer has rapidly increased over the last decade, which has led to an expediential rise in research within many realms of the game. Despite the increasing amount of scientific work surrounding female soccer, gender-specific aspects of physiology, particularly the menstrual cycle and its effects on the physical performance, has been unaccounted for and remains fundamentally unknown[1, 2]. The menstrual cycle encompasses two main phases, the follicular phase (FP) and the luteal phase (LP). The follicular phase can split further into two sub phases; the early FP, which is characterised with low concentrations of both the key hormones oestrogen and progesterone; and the mid FP where oestrogen is high independently from progesterone. The LP is typified by high concentrations of both oestrogen and progesterone. These two main phases are separated by a steep surge in luteinizing hormone triggering ovulation. These cyclical changes are said to be often predictable whilst spanning over the reproductive years[3].

Besides from reproductive function female sex hormones are further known to affect numerous cardiovascular, respiratory, thermoregulatory and metabolic parameters. Which may plausibly be expected to have implications on exercise physiology, for example via fluid retention, changes in body temperature, and energy metabolism[4]. Currently, much of the research has investigated these effects on endurance performance, and the results thus far have been highly equivocal[5–14]. The differing results of the studies to date, may be due to methodological differences. Inconsistent time points within the menstrual cycle when performance had been assessed, the method of assessment (varying performance tests), the verification of cycle phases and/or assessment of physiological cycles, could be factors for the varied findings.

Currently, to the authors knowledge only three studies have evaluated the effect of menstrual phase on intermittent activity and related performance[15–17]. Two of the three studies indicated no differences between cycle phase. However, Middleton and Wenger[15] found a significant enhancement in power output during ten, six second maximal sprints on a cycle ergometer in the LP. Although the authors found only small average differences in power output, the improvement during the LP was exhibited in every subject. Moreover, if these results were translated into running time, this difference in work would translate to approximately one-meter difference in a six second sprint, which in terms of soccer, could make the difference in reaching the ball at important stages in a match. However, throughout these studies, the population sample was primarily healthy active females and not those of a high competitive level and studies were not related to soccer.

The current study aims to determine whether menstrual cycle phase influences a series of soccer related physical performance parameters in a high levelled soccer specific population. Based on the above considerations, the designs involve a strict protocol for the assessment of physiological cycles and distinct timing of performed tests in relation to cycle phases.

Materials and methods

Subjects

Thirty-five high-level female soccer players agreed to participate in the study. Formal sample size calculations were not performed, all players of the local second league female football team who fulfilled the inclusion criteria were enrolled for the study. The inclusion criteria for participation in the study were: 1) the absence from any form of contraception (oral, implanted, injected, intrauterine devices, patches), 2) regular cycle of physiological length (24–35 days [8]), 3) free from any illness or disease which could affect performance and/or health, 4) not suffering from an injury which would affect their performance, 5) participated in competitive soccer for a minimum of five years. A further inclusion criteria was that the testing must be completed over the 24–35 day cycle (reducing the influence of confounding variables including but not limited to: humidity, ambient temperature). Therefore, nine participants (age: 18.6 ± 3.8 y, height: 161.2 ± 6.6 cm, weight: early FP 59.1 ± 7.7 kg, mid LP 58.8 ± 7.5 kg) were suitable for analysis; the flow of the participants is represented in Fig 1. These players all competed in the second women's league (2nd Frauen-Bundesliga), Germany. The players were fully informed of all experimental procedures before giving their written informed consent to participate. If players were younger than 18 years of age, parental written consent was obtained. The current study was conducted in accordance with the declaration of Helsinki and approved by the local ethics committee (Aerztekammer des Saarlandes, approval number 130/14).

Study design

The observational design implemented in this study investigated the differences in physical performance parameters during two distinct phases of the menstrual cycle. The participants completed a battery of physical tests, measuring several components of soccer specific physical performance capacity. These included; lower limb power, sprinting capability and endurance capacity[18]. The participants did not conduct any vigorous exercise on the days of testing and the training the day before testing days remained consistent; training comprised of low/moderate aerobic training for 30–45 minutes followed by 30 minutes of strength training. The tests were conducted during two time points, representative of the early follicular and mid luteal phases, respectively (early FP days 5–7 and mid LP days 21–22[19]), where hormonal concentrations differ greatest between the two time points[19, 20]. Data was collected in the early evening (during their normal training session), and training time was kept consistent for the duration of the study. Testing sessions were conducted during the second half of the season for over an 8-week period (March—May). All measures were conducted outside on artificial turf under similar weather conditions. If weather was deemed unsuitable and at risk of influencing results, and or to players' welfare, (e. g. any form of rain) the test was either cancelled or if possible re-scheduled.

Determination of menstrual phase

Twice a month participants completed a menstruation diary, which included: date of menses, length of menses, and severity of blood flow and discomfort. This information was recorded for a minimum of 6 months, for a valid characterisation of an individual's menstrual cycle[19]. From retrospective analysis, (counting back from previous menses, from month to month, indicating the length of the cycle) the next cycle phase was then prospectively determined[19] and time-point was calculated. For final confirmation of LP sampling time, the participants completed menstruation diaries during the course of testing. Consequently, one player dropped out due to LP sampling time being incorrect (Fig 2). Furthermore, serum oestrogen and progesterone were used to verify physiological reproductive function and verify timing of tests with respect to the cycle phases[19].

Outcome measures

The following panel of established tests for components of soccer specific physical performance capacities were applied and conducted in this order (Fig 2): 1) Jump height during a

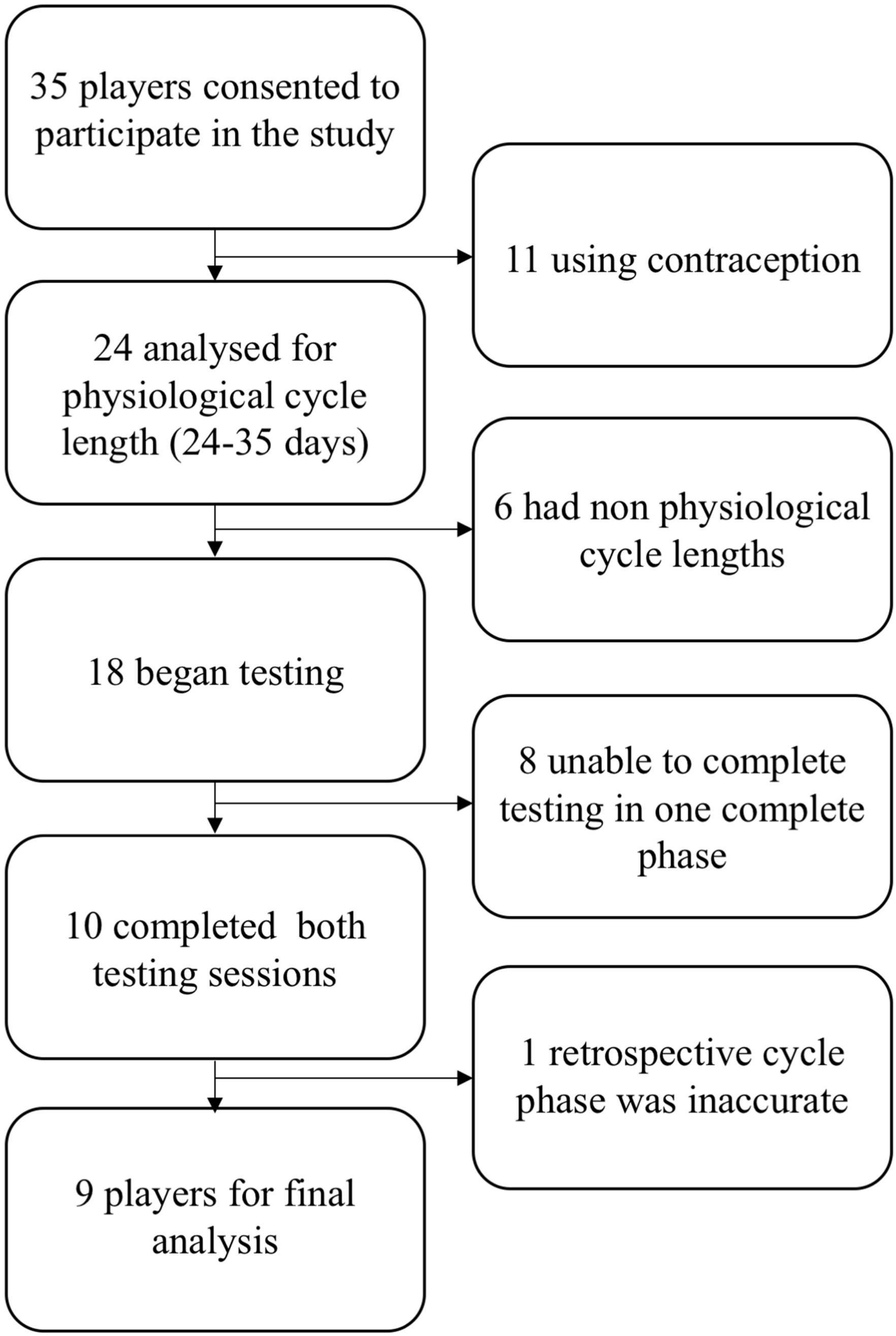
35 players consented to participate in the study
11 using contraception
24 analysed for physiological cycle length (24-35 days)
6 had non physiological cycle lengths
18 began testing
8 unable to complete testing in one complete phase
10 completed both testing sessions
1 retrospective cycle phase was inaccurate
9 players for final analysis

Fig 1. Details of the participants' timeline throughout the study.

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counter movement jump (five trials, mean of the best three) was implemented as an indicator of lower-limb power[21], 2) 30 m (recording set at 5, 10 and 30 m) sprint time was employed as a parameters of sprint capability (fastest of three trials)[22], 3) running distance in the Yo-Yo IET was used as a measure of football-specific endurance capacity[23].

Counter movement jump (CMJ). Characteristics of the CMJ were determined using a force platform (Quattro Jump, Type 9290AD, Kistler Instrument AG, Winterthur, Switzerland) and analysed using professional motion analysis software (Contemplas Bewegungsanalyse, Contemplas Gmbh, Kempten, Germany). Jump height was determined as the centre of mass displacement, calculated from the recorded force and body mass. The CMJ began from an upright position, making a downward movement to a knee angle of approximately 90˚ and simultaneously beginning to push-off, whilst hands are placed upon their hips, with a rest period of 30 s between efforts.

3x30m sprints. Players were asked to stand with their toe behind a white line (touchline of the soccer pitch), and ran maximally straight ahead for 30m. 30 m sprint recording times at 5 m, 10 m and 30 m was employed and measured by light gates and a hand-held monitor (Brower Timing System, Utah, USA). Once a sprint was completed the players had 2 mins of recovery and then repeated the test following the same procedure for all three trials.

Yo-Yo Intermittent endurance test (Yo-Yo IET). The test was performed outdoors on artificial turf similarly described by Bangsbo et al[24]. Concisely, the test consisted of repeated 2 x 20 m runs with an 180˚ turn in between at a progressively increasing speed controlled by audio beeps. Between each running bout, the players had a 5 s rest period, during the active break in the form of a 2.5 m walk. Termination of the test occurred when a player had twice failed to reach the finish line, in time. Total distance of completed shuttles was recorded as the test result. All players had been familiarised with the test procedures previously. HR was measured before the warm-up period (rest) and was continuously measured using commercial heart rate monitors (Polar, Kempele, Finland) until exercise cessation (HR_{peak}). Blood lactate (BLa) was collected in 25μl samples of capillary blood, withdrawn from the earlobe and were subsequently measured using an enzymatic method (Super GL, Rolf Grenier, Biochemica, Flacht, Germany). BLa was collected prior to exercise (rest value), then further collected immediately after, 1 min, 3 min and 5 min post-test. HR ($\geq$ 220 –age (years)), BLa concentration ($\geq$ 8 mmol/l) and RPE were used as an objective criteria of exhaustion[25].

Blood collection. Venous blood samples were collected on the day of testing prior to any form of activity. These samples were obtained from the anti-cubital vein by a standard protocol, following 10–15 min of seated rest. The participants did not perform any vigorous exercise on the day of blood sampling. Blood samples were transported immediately to the laboratory for appropriate procedures. Serum tubes were centrifuged at 4000 revolutions per min for 5

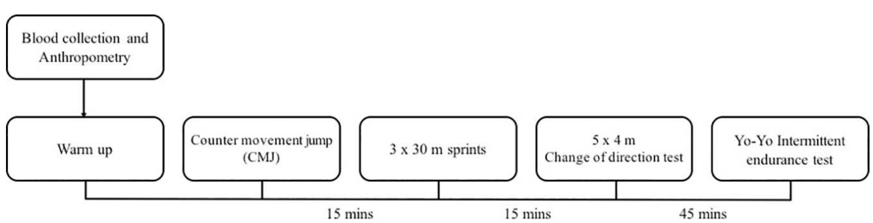

Fig 2. Timeline of the testing protocol.

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min, aliquoted and then stored frozen at -80˚C within 2 h of sampling. Hormones were measured in an accredited medical laboratory. Oestrogen was measured using an Estradiol II electrochemiluminescence immunoassay ECLIA (IBL International GMBH, Hamburg, Germany) with intra- and inter-assay coefficients of 4.13–6.81% and 6.72–9.39%, respectively. Progesterone was measured using a 17-OH-Progesterone enzyme immunoassay ELISA (IBL International GMBH, Hamburg, Germany) with intra- and inter-assay coefficients of 2.8–4.9% and 5.8–9.2%, respectively.

Statistics

Data analysis was conducted using the statistical software package SPSS v.21 (SPSS Inc., Chicago, IL, USA). All data were normally distributed (Kolmogorov-Smirnov) and expressed as means ± standard deviations (SD). Students paired *t*-tests were conducted to determine the difference in mean response between phases for all variables. The magnitude of the mean differences between phases were expressed as effect sizes (ES) [26]. The extent of the ES are classified as follows: trivial (<0.2), small (>0.2–0.6), moderate (>0.6–1.2), large (>1.2–2.0), very large (>2.0), based on updated guidelines from Batterham and Hopkins[27]. Magnitude-based inferences (MBI) were conducted to determine the possible benefit, in terms of beneficial or harmful effects, of the cycle phase. This approach represents a contemporary method of data analysis that uses confidence intervals to calculate the probability that a difference is practically meaningful. For assessing the difference in team performance smallest worthwhile change was set at Cohen effect size of 0.2[27]. When clear interpretation was possible, a qualitative descriptor was assigned to the following quantitative chances of performance effect: 0.5% to 5%, very unlikely; 5% to 25%, unlikely; 25% to 75%, possibly; 75% to 95%, likely; 95% to 99.5%, very likely; and >99.5%, most likely[27].

Results

The participants body mass and sum of four skin folds were not significantly different between menstrual phases (Table 1). Oestrogen and progesterone levels of the players were significantly higher in the mid LP compared to the early FP.

In Yo-Yo IET performance, MBI intimated the qualitative inference that there was a possibly harmful effect for maximal performance in the LP, whereas, all other performance variables were found to be unclear. Moreover, the ES for the Yo-Yo IET was small to moderate, whereas all other variables were trivial (Table 2). For all performance variables, there were no significant differences revealed, although a trend towards significance was observed in the Yo-Yo IET between phases (p = 0.07) (Fig 3).

Table 1. Anthropometric and hormone characteristics of females involved in the study. Comparison between Follicular and Luteal phase.

Variable		Follicular Phase	Luteal Phase
	Means ± SD	Means ± SD	Means ± SD
Age (y)	19 ± 4		
Height (cm)	161.3 ± 6.6		
Body mass (kg)		59.1 ± 7.7	58.8 ± 7.5
Body fat (%)		17.7 ± 2.5	17.8 ± 2.2
Oestrogen (pg/ml)		26.8 ± 22.1	109.8 ± 55.6*
Progesterone (nmol/l)		2.2 ± 0.6	6.5 ± 2.0*

Note: Body fat calculated by the sum of four skinfolds (biceps, triceps, subscapular, suprailiac).

* indicates a significant difference from early FP to LP P < 0.05.

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Table 2. Performance outcomes for all parameters measured during the follicular phase and luteal phase. Results expressed as means ± standard deviations, statistical significance, effect sizes and magnitude based inferences.

Variables	FP	LP	*P*	ES	% Chance +/trivial/-	Qualitative Inference
0-5m (s)	1.1 ± 0.1	1.1 ± 0.1	1.00	0.00	50/0/50	Unclear
0-10m (s)	1.9 ± 0.1	1.9 ± 0.1	0.25	0.18	10/7/83	Unclear
0-30m (s)	4.7 ± 0.1	4.7 ± 0.1	0.96	0.00	48/0/52	Unclear
CMJ (cm)	29.0 ± 3.9	29.6 ± 3.0	0.33	0.16	8/24/68	Unclear
Yo-Yo IET (m)	3289 ± 801	2822 ± 896	0.07	0.56	0/61/39	Possibly harmful

Note: FP = Early follicular Phase; LP = Mid luteal Phase; ES = Effects Size; MBI = Magnitude Based Inference.

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There was a likely beneficial inference for HR pre values tending towards the early FP, whereas the HR post values were deemed unclear. RPE was also deemed unclear. Lactate values were inferred to be unclear pre-and 1 min post the Yo-Yo test. However, at 3 and 5 min post, the MBIs suggested that it was very likely harmful during the early FP, where lactate values were higher. The effect sizes for all variables ranged from trivial to moderate (Table 3). There were significant differences between phases for HR prior to exercise and lactate values at minutes 1 and 3 post Yo-Yo test ($p = 0.04$, $p = 0.01$ and $p = 0.03$, respectively). For HR post, lactate pre-and lactate 1 min post Yo-Yo test and RPE there were no significant differences observed ($p > 0.05$).

Discussion

The purpose of the study was to investigate whether menstrual cycle phase influences different facets of physical performance in a group of high level soccer players. The main finding of the

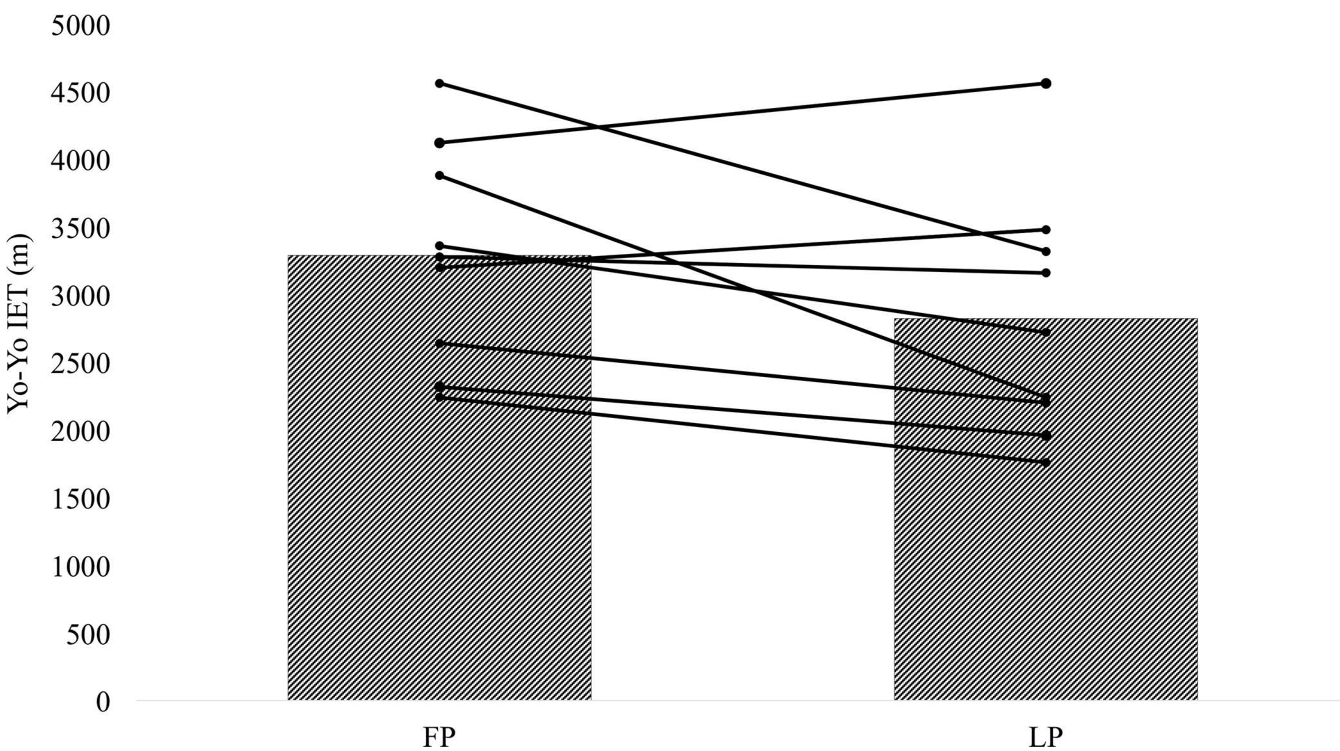

Fig 3. Mean meters of completion during the Yo-Yo IET during the two phases of the menstrual cycle. The data for each individual has been superimposed onto each group mean. The menstrual phases are abbreviated as FP (early follicular phase) and LP (mid luteal phase).

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Table 3. Internal load measures during the follicular phase and luteal phase. Results expressed as means ± standard deviations, statistical significance, effect sizes and magnitude based inferences.

Variables	FP	LP	*P*	ES	% Chance +/trivial/-	Qualitative Inference
HR Pre (bpm)	97 ± 16	105 ± 12	0.04	0.45	91/9/0	Likely beneficial
HR post (bpm)	194 ± 4	193 ± 5	0.90	0.05	27/37/36	Unclear
RPE (AU)	18.7 ± 0.7	18.6 ± 0.9	0.76	0.14	23/31/46	Unclear
Lactate pre (mmol L^{-1})	2.3 ± 0.8	2.1 ± 0.8	0.65	0.21	17/25/58	Unclear
Lactate 1min post (mmol L^{-1})	9.3 ± 1.4	8.6 ± 0.7	0.20	0.41	6/28/66	Unclear
Lactate 3min post (mmol L^{-1})	8.8 ± 2.2	7.6 ± 1.6	0.01	0.75	0/1/99	Very likely harmful
Lactate 5min post (mmol L^{-1})	8.7 ± 2.2	6.9 ± 1.9	0.03	0.93	1/4/95	Very likely harmful

Note: FP = Early follicular Phase; LP = Mid luteal Phase; ES = Effects Size; MBI = Magnitude Based Inference; HR = Heart rate; RPE = Record of perceived exertion.

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study suggested that Yo-Yo IET performance was affected during the LP of the menstrual cycle. However, in all other aspect of measured performance were not different between phases.

To the authors knowledge this is the first study to investigate the effects of menstrual cycle phase on different sport specific physical parameters in female soccer players. In the nine players, menstrual cycle phase appeared to have an effect on maximal endurance capabilities, as demonstrated through the Yo-Yo IET. It has been suggested that physical performance in elite female soccer is highly related to their trained status and maximal capacities[28], therefore, it may be postulated that the maintenance of these high levels throughout the entire cycle is important for success. In 78% of our population, a reduction of meters completed was observed in the mid LP (Fig 3). The results of the current study are in line with the extensive work of Lebrun et al[8]. Lebrun and colleagues used a cohort of 16 trained athletes (age 27.6 ± 3.8 y, height 167.9 ± 5.3 cm, mass 59.6 ± 6.7 kg, VO_{2max} 53.7 ± 0.9 $ml \cdot kg^{-1} \cdot min^{-1}$), who participated in a variety of sports including running, cycling, triathlon, squash, cross-country skiing, ultimate frisbee and rowing. This study contained a strict phase confirmation comparable to our trial. Through the use of a progressive and continuous treadmill running test, the results indicated that maximal endurance capacity was reduced in the LP.

Differences in endurance performance during various phases of the menstrual cycle have been postulated to be due to differences in heat regulation, substrate availability, and metabolism[6]. It is well documented that a female's basal body temperature can differ between phases in the region of 0.3–0.5˚C[19]. This elevation in temperature has been attributed to the surge of progesterone exhibited during the LP. The associated increase in body temperature has been suggested to limit prolonged exercise capabilities and increase cardiovascular strain[19]. This has been seen in previous studies, presenting higher VO_2 values, accompanied with higher heart rates and RPE values during exercise at given percentages of VO_{2max}[29–31]. Although basal body temperature was not measured in the present study, cardiovascular strain may have been present in our study and thus could influence performance. Conversely, it has been shown in previous studies, that exercise time to exhaustion is improved during the LP. This has been speculated that the role oestrogen plays on enhanced lipid metabolism, with spared glycogen and usually a supressed lactate response to exercise in the LP[32]. As seen in the present study, significant differences were found in lactate concentration between phases at 3 and 5 min post exercise. Therefore, there is the potential that the energy pathways may have been taxed differently between phase, for example it has been implicated previously that during the LP there is a lower contribution of anaerobic glycolysis and hence reduced La values[32]. A conclusive statement about this, however, is beyond the current scope of this study and perhaps requires deeper investigation with more sophisticated methodology (e. g. indirect calorimetry). Moreover, the present data, however, did not indicate any differences in lactate production at rest or at maximal lactate concentration, which was similarly found in female rowers completing a one-hour row at 70% VO_{2max}[33].

It is important to consider the specific type of sports to validly assess if and how menstrual cycle phase potentially affects physical performance. However, in soccer (which is intermittent in nature), there is a special challenge to evaluate sport-specific physical performance, this is due to the current difficulty to establish valid and reliable tests sensitive to the sport, unlike in endurance sports. The Yo-Yo IET has been suggested to replicate physical patterns in female soccer[34] well, however, factors such as motivation have been suggested to cause differing results when being assessed at multiple time points, however, the exhaustion criteria was achieved in all players. In the present study, a reduction during the LP was observed in the clear majority of the players, with similar maximal HR, La and RPE values. Therefore, it can be postulated that although maximal effort was elicited in both phases, there was an observed reduction in maximal performance during the LP.

Sprinting performance in the present study did not differ between cycle phases at either split or full 30 m distance (Table 2). The absence of the menstrual cycle affecting sprinting performance in the present study concurs with previously published literature[35]. It had, however, been suggested previously that sprinting performance may differ between phases. An improvement in sprinting performance being attributed to the increase in basal body temperature during the LP[36]. However, a study from Somboonwong and colleagues[36] suggested that, although basal body temperature was significantly greater in the LP, sprinting performance over 40-yd (36.6m) was not affected. Unlike the present study, Somboonwong et al., [36] only measured 40-yd sprinting time and did not investigate differing split times or acceleration. From the present study's results, it could be postulated that cycle phase does not influence sprinting ability at many various aspects of acceleration and velocities. This was seen as 5 m, 10 m and 30 m sprint time was not affected by cycle phase. In soccer, a players' ability to perform multiple high intensity actions and sprinting ability has been considered to be more important for overall performance than endurance capacity [37], therefore, the understanding that menstrual cycle phase does not affect sprinting behaviour is important for players and practitioners. This being said, in the present study and in the work from Somboonwong et al., sprinting performance was measured from singular sprints and not repeated sprint ability (RSA). RSA is another facet of soccer which has been suggested to be important for soccer performance. The only study to assess a repeated sprint and the effects of menstrual cycle phase, was conducted by Tsampoukos et al (2010). In the aforementioned study, eight females conducted two 30 s sprints with a 2 min passive recovery in between; the results indicated no effect of menstrual cycle phase on repeated sprint performance. However, this study, only employed sport science participants, not those of a sport specific population and, furthermore, only completed two sprints. Therefore, for future research, due to the lack of understanding and studies conducted in soccer, the investigation of menstrual cycle phase on RSA is warranted.

The menstrual cycle has been suggested to alter motor control and muscular strength. However, similarly to the present study de Jonge et al.,[38] found no differences between phases in 19 normally menstruating females for any strength parameter, including maximal isometric quadriceps strength with superimposed electrical stimulation, isokinetic knee flexion, and handgrip strength. It has been previously proposed that oestrogen, may have a strengthening action on skeletal muscle[39], although the mechanisms of this effect has not become clear. During the present study, the time points of measurements, were at minimal oestrogen (early FP) and when both progesterone and oestrogen were elevated (mid LP).

Therefore, it can be speculated that the levels of oestrogen were not elevated enough to cause such an effect during early FP. Moreover, the antagonistic effect of progesterone, may have restricted the effects of oestrogen on muscular strength during LP[40]. Altogether, the current results suggest that CMJ was not effected by cycle phase, however, due to the assumptions of the effect of oestrogen on strength, future investigations should target other time points within the menstrual cycle, where oestrogen is independently elevated, i.e. in the rise prior to ovulation.

A limitation to the current study is that it did not deploy a randomised counter balanced design, whereby all participants first completed the testing in the early FP followed by the mid LP, this was due to the current method of cycle phase determination. Although the participants were well accustomed to this form of testing, confounding factors that may influence the current findings need to be considered. As recommended in previous literature, solely conducting menstruation calendars does not provide all relevant information regarding menstrual cycle phase[41]. Therefore, hormone values of oestrogen and progesterone were measured. However, concentrations were found to be lower than those previously described in the general population. In regards to research a progesterone limit has been recommended conservatively at 16 nmol/L[19]. It must be noted, that unfortunately at present reference values for athletic populations have yet to be defined and are not available. Furthermore, although the levels were not at the expected magnitude, changes in hormone values were observed; thus, the associated change in phase could be verified (Table 2). Moreover, the current data have been taken from the early FP and the mid LP; the associated hormones also differ just before ovulation in the late LP, where oestrogen is elevated without a pronounced presence of progesterone. Therefore, the independent effects of oestrogen have not been assessed where these results could also differ. Finally, although the subject number is in the range of previous studies[15–17], the results may not accurately represent a wider female soccer population.

Conclusion

The current study indicates that there is potentially a reduction in maximal endurance performance during the LP of the menstrual cycle. However, this reduction in performance was not observed for jumping and sprint performance. Therefore, due to the findings of the current study, practitioners should keep menstrual cycle phase constant when completing routine physical assessments with their players, to ensure that changes in performance are consistent with the outcome and not due to the effects of the menstrual cycle. Alternatively, the cycle phase should at least be recorded and taken into account when interpreting the results.

Supporting information

S1 Data Set. Full data set including: Anthropometrics, football specific physical performance and hormonal concentration values.
(PDF)

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Methodology: RJ AH HF TM.

Supervision: AH TM.

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Writing – review & editing: RJ AH HF TM.

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Exercise Physiology of Football: Factors Related to Performance and Health

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