The effects of menstrual cycle phases on running repeated sprint ability

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Abstract

Female participation in regular sport activities has increased in recent years, yet their representation in the sports and exercise science literature remains low. Therefore, an understanding of the effects different phases of the menstrual cycle (MC) have on exercise responses is important due to the practical and theoretical implications. The aim of this study was to compare performance, physiological and perceptual differences when performing a running repeated sprint ability (RSA) during the early-follicular (EF), and mid-luteal (ML) sub-phases of the MC. Six healthy, physically active female participants (age: 25.67 ± 2.49 years; height: 1.66 ± 0.08 m; body mass: 69.8 ± 19.3 kg; $\dot{V}O_2$peak: 46.00 ± 6.76 ml·kg^{1}·min^{-1}) took part in this study. After the initial health screening, the participants completed two familiarisation and four intervention sessions (twice during each MC sub-phase). The RSA protocol consisted of five 'all-out' sprints of six seconds on a non-motorised treadmill with 24 seconds of active recovery (walking) between the sprints. Results indicated no significant differences between MC sub-phases in mean (p = 0.998, d = 0.00) and peak power output (p = 0.14, d = 0.16), distance (p = 0.59, d = 0.07), pre-exercise (p = 0.78, d = 0.41) and post-exercise lactate (p = 0.58, d = 0.24), oxygen uptake (p = 0.10, d = 0.30), respiratory exchange ratio (p = 0.47, d = 0.13), ventilation (p = 0.42, d = 0.12), heart rate (p = 0.49, d = 0.17). However, significant differences were found in peak acceleration (EF: 4.65 ± 0.84 m·s^{-2}, ML: 5.05 ± 1.14 m·s^{-2}) (p = 0.02, d = 0.40) and rating of perceived exertion (RPE) (EF: 13.42 ± 2.15, ML: 12.60 ± 2.26) (p < 0.001, d = 0.37). In conclusion, MC phases do not appear to influence most of the chosen RSA performance indicators. However, due to the low sample size and statistical power, further studies are required to better investigate the effects of the MC on RSA.

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List of abbreviations

ACSM: American college of sports medicine

ATP: adenosine triphosphate

BBT: basal body temperature

BIA: bioelectrical impedance analysis

BMI: body mass index

EF: early-follicular

EL: early-luteal

F: follicular

FFA: free fatty acid

FI: fatigue index

FSH: follicle-stimulating hormone

GnRH: gonadotropin-releasing hormone

HR: heart rate

L: luteal

LDL: low-density lipoproteins

LEAF-Q: low energy availability in females questionnaire

LF: late-follicular

LH: luteinising hormone

LL: late-luteal

LPD: luteal phase-deficient

MC: menstrual cycle

MF: mid-follicular

ML: mid-luteal

MPO: mean power output

PPO: peak power output

REML: restricted maximum likelihood

RER: respiratory exchange ratio

RPE: rating of perceived exertion

RSA: repeated sprint ability

$\mathbf{S_{dec}}$: decrement score

$\dot{\mathbf{V}}$**e**: minute ventilation

$\dot{\mathbf{V}}\mathbf{CO_2}$: carbon dioxide production

$\dot{\mathbf{V}}\mathbf{O_2}$: oxygen uptake

$\dot{\mathbf{V}}\mathbf{O_2max}$: maximal oxygen consumption

$\dot{\mathbf{V}}\mathbf{O_2peak}$: peak oxygen consumption

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1. Introduction

1.1 Women in sport

Most studies in sports and exercise sciences have been conducted on men, with the results generalised to women, without considering how the sex differences may affect the transferability of these results (Bruinvels et al., 2017; Sims & Heather, 2018). In fact, women have been, and still are, often excluded from sport and exercise research, or are often included together with men without giving much consideration to the physiological differences (Johnson, Greaves, & Repta, 2009).

Female underrepresentation in sport and exercise research has been clearly shown by an analysis of 1382 articles from key sports and exercise science journals such as the *British Journal of Sports Medicine*, *American Journal of Sports Medicine* and *Medicine and Science in Sports and Exercise*, which indicated that only 39% of over six million participants in these studies were female (Costello, Bieuzen, & Bleakley, 2014). Out of all studies published in the *American Journal of Sports Medicine* between 2011 and 2013 only 4% of them recruited female only participants compared to 18% that had male only and 78% that included both sexes (Costello et al., 2014). Frankovich & Lebrun (2000) and Sims & Heather (2018) indicated that some of the main reasons for the exclusion of females in research studies are the complexities associated with the menstrual cycle (MC), such as the biphasic response of oestrogen and progesterone, the high variability of hormone fluctuations throughout the day and the differences of hormone concentrations between persons. Moreover, studying the MC phases can be time consuming and expensive, as the gold standard in determining these phases require laboratory-based techniques such as blood samples analysis (Glass, 2001).

Despite these challenges, sport and exercise science studies should not ignore the effects of the MC and its hormonal fluctuations on various performance and

physiological measures. As failure to do so will prevent knowledge advancement and sporting performance optimisation in female participants. For example, training programmes cannot be optimised without the knowledge of the effects of the MC on performance. Therefore, in order to reduce the existing gap in literature between males and females, more female-only studies are required whilst carefully considering the effect that the MC phases may have on the outcome measures.

1.2 Menstrual cycle

The MC is governed by cyclic hormonal fluctuations that follow an established pattern of progesterone, oestrogen, luteinising hormone (LH) and follicle-stimulating hormone (FSH) (Frankovich & Lebrun, 2000). These hormones have previously been identified to affect female physiology and, in turn, may affect performance (Birch, 2000; Constantini, Dubnov, & Lebrun, 2005; Draper et al., 2018).

For example, oestrogen affects metabolism by reducing gluconeogenesis and glycogenolysis (Bunt, 1990; D'Eon et al., 2002) and increasing fat oxidation, potentially affecting performance that relies on specific metabolic pathways to resynthesise energy (Nicklas, Hackney, & Sharp, 1989). In contrast, progesterone increases muscle glycogen utilisation (D'Eon et al., 2002), and has been recently demonstrated by Mata et al. (2019) to alter carbohydrate availability, thereby affecting performance and training adaptations. This indicates a link between hormonal fluctuations and both physiological and performance changes.

1.2.1 Effects of the menstrual cycle on performance

The physiological changes caused by oestrogen and progesterone during the MC sub-phases might also affect physical performance, as shown by Julian et al. (2017) who reported a lower maximal endurance performance during the mid-luteal sub-phase when compared with the early-follicular sub-phase. Specifically, Julian et al. (2017)

reported that the lower performance, as measured by the total distance ran, might have been caused by an increase in body temperature due to the higher concentrations of progesterone, which can limit endurance performance and increase cardiovascular strain. McNulty et al. (2020) reviewed the current literature and concluded that performance might be trivially reduced during the early-follicular when compared with any other sub-phase. However, due to limitations in the studies, it is not possible to draw any conclusion (McNulty et al., 2020).

Therefore, studying the effects of the different sub-phases of the MC on physical performance can benefit both athletic and general population by helping inform and implement an evidence-based approach, to reach a higher performance level or to improve their fitness and health. As these benefits might differ based on the type of performance chosen, and since previous research focused mostly on endurance aspects, there is a need to understand other forms of performance. Interestingly, only Giacomoni et al. (2000), Middleton & Wenger (2006) and Julian et al. (2017) focused on exercise performance shorter than 10 seconds, and no studies have analysed the running repeated sprint ability. Due to its importance in team sports but also its use as an exercise modality by general population, this study will focus on running repeated sprints performance.

1.2.2 Repeated sprint ability

Repeated Sprint Ability (RSA) is defined as the ability to perform repeated sprints with a short, incomplete recovery between repetitions (Bishop, Girard, & Mendez-Villanueva, 2011). The repeated sprint ability is considered an important factor for team sports' athletes, as being able to perform several sprints consecutively, with an incomplete rest, is a common situation in these sports (Bishop et al., 2011; Buchheit, Mendez-villanueva, Simpson, & Bourdon, 2010). Recently, the effectiveness of repeated sprint exercise has also been demonstrated in the general population. With sprint interval training shown

to increase aerobic fitness and decrease body fat in inactive overweight/obese women (Rowley, Espinoza, Akers, Wenos, & Edwards, 2017), as well as reduce low-density lipoproteins (LDL) and total cholesterol in young healthy participants (Sandvei et al., 2012).

Therefore, assessment of repeated sprint ability responses in different MC sub-phases has important practical and theoretical implications. From a practical point of view, knowing how different aspects of performance are influenced by MC sub-phases could help coaches and sport scientists tailor their schedules and programmes in order to maximise performance. From a theoretical point of view, it could benefit future researchers in this under-researched field by informing future research design and providing new data for comparison purposes.

To date there is very limited research on the effects the MC has on athletic performance, as only Middleton & Wenger (2006) studied the effects of the MC phases on RSA performance, analysing 10 sprints of six seconds each with 30 seconds recovery on a cycle ergometer. They reported the average work over a series of sprints and oxygen uptake ($\dot{V}O_2$) between sprints to be higher during the luteal phase than the follicular phase. However, Middleton & Wenger (2006) only analysed a cycling performance, whilst running RSA has never been studied before. Analysing running RSA performance is important because the results could be applied in a multitude of sport activities both in athletes and/or general population, where running modality is common.

1.3 Project's aim

Therefore, the aim of this study was to measure physiological, performance and perceptual responses during a running RSA at the early-follicular and mid-luteal sub-phases of the MC. These two MC sub-phases were chosen as they exhibit the greatest

370 hormonal differences, allowing a clearer understanding of the effects this may have on
371 any measured variable (Julian, Hecksteden, Fullagar, & Meyer, 2017).

2. Literature review

The current female-specific sport science literature, particularly in high-level athletes is scarce (Emmonds, Heyward, & Jones, 2019), and there is a clear disparity between the number of papers analysing females and males (Emmonds et al., 2019). Due to this lack of data, researchers cannot draw effective conclusions about the influence the MC has on sport performance, and practitioners cannot follow an evidence-based approach in their work (Emmonds et al., 2019). Therefore, there is a clear need to do more female-specific research, but the first step would be to analyse the current literature available to understand the present situation and recognise the limitations (Emmonds et al., 2019).

A total number of 42 papers published between 1981 and 2019 were identified and analysed for the purpose of this literature review. Papers with keywords like “menstrual cycle” and “performance”, “physical activity” or “sport” were searched on PubMed and Google Scholar, and then only papers related to “sprinting”, “endurance” or “intermittent activity” were chosen for review. Details of the chosen papers are presented in Table 1.

Table 1: details of the published papers included in this literature review.

Paper	N[b]	Activity levels	MC phases	MC determination	Test	Parameters[d]
Jurkowski et al. (1981)	9	Active	MF, ML	Blood samples	IETE (cycle)	HR, lactate[a], $\dot{V}O_2$, $\dot{V}e$
Schoene et al. (1981)	12	Active / Non active	MF, ML	Blood samples, BBT	IETE (cycle)	$\dot{V}O_2max$[a], Ve[a]
Stephenson et al. (1982)	6	Active	Days 2, 8, 14, 20, 26	Diary only	5 min at 4 submaximal intensities, 15 min recovery	RPE, HR, $\dot{V}O_2max$, $\dot{V}O_2$
De Bruyn-Prevost et al. (1984)	7	No info	Ovulation, days 1, 2	BBT	9 min cycling at increasing intensity; TTE (cycle)	HR, lactate, $\dot{V}O_2max$, $\dot{V}O_2$
Bale & Nelson (1985)	20	Active	Days 1, 8, 12-15, 21	Diary only	50 m (swim)	n/a

Lamont (1986)	9	Active / Non active	EF, ML	Blood samples	60 min at 70% $\dot{V}O_2$max (cycle)	Lactate, $\dot{V}O_2$, $\dot{V}e$, RER
Dombovy et al. (1987)	8	Non active	MF, ML	Blood samples	IETE; 4 min constant-load exercises at 33, 50, 67, 75% $\dot{V}O_2$max (cycle)	RPE, HR, lactate, $\dot{V}O_2$max, $\dot{V}e$, $\dot{V}e$, $\dot{V}O_2$
Nicklas et al. (1989)	6	Active	MF, ML	Blood samples, BBT	SETE at 70% $\dot{V}O_2$max preceded by 90 min at 60% $\dot{V}O_2$max and 4x1 min sprints (cycle)	RPE, lactate, $\dot{V}O_2$, RER
De Souza et al. (1990)	8	Active	EF, ML	Blood samples, urine	IETE; 40 min at 80% $\dot{V}O_2$max (run)	RPE, HR, lactate, $\dot{V}O_2$max, body comp, $\dot{V}e$, RER, $\dot{V}O_2$
Hackney et al. (1991)	6	Active	MF, ML, ovulation	Blood samples, urine, BBT	60 min at 70% $\dot{V}O_2$max (cycle)	RPE[a], HR, $\dot{V}O_2$, $\dot{V}e$, RER[a]
Quadagno et al. (1991)	15	Active	Pre-menses, menses, post-menses	Diary only	100 m; 200 m (swim)	n/a
Pivarnik et al. (1992)	9	Active	MF, ML	Blood samples, urine	60 min at 65-70% $\dot{V}O_2$max (cycle)	RPE[a], HR[a], body comp, $\dot{V}O_2$
McCracken et al. (1994)	9	Active	MF, ML	Urine, BBT	IETE (run)	Lactate[a]
Lebrun et al. (1995)	16	Active	EF, ML	Blood samples, BBT	IETE; AST; TTE at 90% $\dot{V}O_2$max (run)	HR, $\dot{V}O_2$max[a], body comp, RER, $\dot{V}e$
Bemben et al. (1995)	5	Active	EF, LF, ML	Blood samples, BBT	IETE (run)	HR, lactate, $\dot{V}O_2$max, $\dot{V}e$, body comp
Miskec et al. (1997)	10	Active	L, menses	Diary only	15x15 sec sprints, 2 min recovery (cycle)	RPE, lactate, body comp, power
Williams & Krahenbuhl (1997)	8	Active	EF, LF, EL, ML, LL	Blood samples, BBT	6 minutes at 44 and 80% $\dot{V}O_2$max (run)	$\dot{V}O_2$[a], $\dot{V}e$[a], fatigue[a],
Masterson (1999)	32	Active	F, L	Diary only	WAnT	Fatigue[a], power[a]
Beidleman et al. (1999)	8	Active	EF, ML	Blood samples, urine	IETE; SETE at 70% $\dot{V}O_2$max (run)	RPE, $\dot{V}O_2$max, $\dot{V}O_2$, $\dot{V}e$, HR, body comp
Giacomoni et al. (2000)	7	Unknown	MF, ML, menses	Blood samples	4x8 sec sprints, 3' recovery (cycle)	Body comp, power
Redman et al. (2003)	14	Non active	F, L	Blood samples, urine	IETE; 20 min at 25% $\dot{V}O_2$max followed by 20 min at 75% $\dot{V}O_2$max (cycle)	HR, lactate[a], $\dot{V}O_2$max, power, $\dot{V}e$, RER[a], $\dot{V}O_2$[a]

Sunderland et al. (2003)	7	Active	MF, ML	Blood samples	LIST (run)	RPE, HR, lactate
Dean et al. (2003)	8	Active	EF, MF, ML	Blood samples, BBT	IETE (cycle)	HR, lactate, $\dot{V}O_2$max, RER
Oosthuyse et al. (2005)	13	Active / Non active	EF, LF, ML	Blood samples, urine, BBT	15 or 30 km (cycle)	HR, RER
Middleton & Wenger (2006)	6	Active	MF, LL	Blood samples	10x6 sec sprints, 30 sec recovery (cycle)	Lactate, power, $\dot{V}O_2$[a], RER[a]
Bushman et al. (2006)	7	Active	L, menses	Urine, BBT	Margaria-Kalamen; WAnT	Power
Smekal et al. (2007)	19	Active	F, L	Blood samples, BBT	IETE (cycle)	HR, lactate, $\dot{V}O_2$max, power, $\dot{V}$e, RER
Gurd et al. (2007)	7	Active	MF, ML	Blood samples	Incremental three steps transition exercise (cycle)	Lactate, $\dot{V}O_2$, RER
Tsampoukos et al. (2010)	8	Active	F, L, prior to ovulation	Blood samples, urine	2x30 m sprints, 2 min recovery (run)	Lactate, power, body comp, speed, fatigue
Vaiksaar et al. (2011)	15	Active	F, L	Blood samples	IETE (row)	HR, lactate, $\dot{V}O_2$max, body comp, power, $\dot{V}$e, RER
Hooper et al. (2011)	73	Non active	EF, LF, L	Unknown	30 min at 65% $\dot{V}O_2$max (run)	RPE[a]
Shaharudin et al. (2011)	12	Active	MF, ML	Blood samples, BBT	3 sprints at 120% VO_2max, 20 min recovery (cycle)	Lactate
Lamina et al. (2011)	15	Non active	EF, LL, ovulation	BBT	20 m SRT	$\dot{V}O_2$max
Janse de Jonge et al. (2012)	8	Active	EF, ML	Blood samples, BBT	60 min at 65-70% $\dot{V}O_2$max; IETE (cycle)	RPE, HR[a], $\dot{V}O_2$max, $\dot{V}O_2$, RER
Abdollahpor et al. (2013)	14	Active	EF, ML	Blood samples	IETE (run)	RPE, lactate, $\dot{V}O_2$max, body comp, HR, fatigue
Wiecek et al. (2016)	16	Active	MF, ML	Blood samples, BBT	20 sec sprint (cycle)	Lactate, power, speed
Stefanovsky et al. (2016)	8	Active	MF, LL	Diary only	Upper limbs WAnT; SJFT	Lactate, power, fatigue
Pestana et al. (2017)	21	Active	MF, LL	Diary only	WAnT	HR[a], body comp, power

Julian et al. (2017)	9	Active	EF, ML	Blood samples	Yo-Yo IET, 3x30 m sprints, 2 min recovery (run)	Body comp
Köse (2018)	10	Active	EF, MF, L	Diary only	WAnT, IETE (walk)	HR, power
Gordon et al. (2018)	10	Active	MF, ML, menses, pre-menses	Saliva	IETE (cycle)	HR, lactate, $\dot{V}O_2max$[a], power, $\dot{V}e$, RER
Cristina-Souza et al. (2019)	12	Active	F, L, ovulation	Blood samples	n/a[c]	RPE
Mattu et al. (2019)	15	Active	MF, ML	Urine	Incremental three steps transition exercise (cycle)	RPE[a], HR, lactate, $\dot{V}O_2max$, $\dot{V}O_2$, $\dot{V}e$, power, RER

L: Luteal; F: Follicular; EL: Early-luteal; ML: Mid-luteal; LL: Late-luteal; EF: Early-follicular; MF: Mid-follicular; LF: Late-follicular; BBT: Basal Body Temperature; Ve: Ventilation; HR: Heart Rate; RPE: Rating of Perceived Exertion; RER: Respiratory Exchange Ratio; PPO: Peak Power Output; WAnT: Wingate Anaerobic Test; IETE: Incremental Exercise To Exhaustion; LIST: Loughborough Intermittent Shuttle Test; SRT: Shuttle Run Test; SJFT: Special Judo Fitness Test; IET: Intermittent Endurance Test; SETE: Submaximal Exercise To Exhaustion; TTE (Time To Exhaustion); AST (Anaerobic Speed Test)

[a] Results with a significant difference between menstrual cycle phases

[b] Sample size may differ from the original paper, excluding participants using oral contraceptives or amenorrheic

[c] A technical training session was analysed, containing multiple kind of movements

[d] Not all the parameters are shown here, but only the one also analysed in this project

2.1 Menstrual cycle

The MC is an important biological rhythm which main purpose is to prepare the uterus for possible pregnancy (Stefanovsky, Peterova, Vanderka, & Lengvarsky, 2016). In addition to regulating the reproductive function, the MC affects a wide range of physiological functions and responses, including the characteristics of the epidermis, hair growth, immune function and disease susceptibility (Farage, Neill, & MacLean, 2009). As shown in Table 2, the MC is governed and characterised by cyclic fluctuations of oestrogen and progesterone that are regulated using a negative and positive feedback system that controls the MC (Thiyagarajan, Basit, & Jeanmonod, 2020), and have been identified to lead to physical and psychological changes, which subsequently might influence sport performance (Birch, 2000; Constantini et al., 2005; Draper et al., 2018).

Table 2: typical hormones range during the menstrual cycle[a].

Phases (days on a 28-day cycle)	Oestradiol (ng/mL)	Progesterone (ng/mL)	LH (mUS/mL)
Menses (1-4)	20 – 60	<2	5 – 25
Early-follicular (4-5)	20 – 100	<2	5 – 25
Mid-follicular (5-7)	100 – 200	<2	5 – 25
Late-follicular (8-12)	>200	<2	5 – 25
Ovulation (13-15)	>200	2 – 20	25 – 100
Early-luteal (16-20)	100 – 200	2 – 20	5 – 25
Mid-luteal (21-23)	100 – 200	2 – 30	5 – 25
Late-luteal (24-28)	20 – 60	2 – 20	5 – 25

[a] (Allen et al., 2016)

A regular MC lasts between 24 and 35 days, with an average of 28 days, and it can be divided into three phases: follicular (proliferative), ovulatory and luteal (secretory) (Figure 1) (F. C. Baker & Driver, 2007; Gordon et al., 2018; Lebrun, McKenzie, Prior, & Taunton, 1995). Furthermore, the follicular and luteal phases can be divided into sub-phases: early, mid and late (Lebrun et al., 1995).

The follicular phase is the first part of the ovarian cycle, which starts with menses and ends with ovulation (Frankovich & Lebrun, 2000). During this phase, the hypothalamus releases the gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary to secrete LH and FSH (Barbieri, 2014; Thiyagarajan et al., 2020). According to the two-cell two-gonadotropin theory, LH stimulates thecal cells in order to produce androgens, whilst FSH stimulates the granulosa cells to produce oestrogen from these androgens (Enea, Boisseau, Fargeas-Gluck, Diaz, & Dugué, 2011). Even though both LH and FSH release is stimulated by the GnRH, they are released differently throughout the MC. This difference is modulated by GnRH pulse frequency, where slow frequencies favour FSH secretion and fast frequencies favour LH secretion, inhibin A and B, which inhibit FSH secretion, and activins, which stimulate FSH secretion (Barbieri, 2014).

In this period the follicles grow under the influence of FSH which upon release also increases the synthesis of oestrogen, whose role is to thicken the endometrium to prepare for the coming egg and for possible pregnancy (Constantini et al., 2005; Frankovich & Lebrun, 2000). Furthermore, the subsequent increase of oestrogen induces

the secretion of LH, which will trigger the ovulation at the end of the follicular phase (Constantini et al., 2005; Frankovich & Lebrun, 2000; Su, Yi, Wei, Chang, & Cheng, 2017).

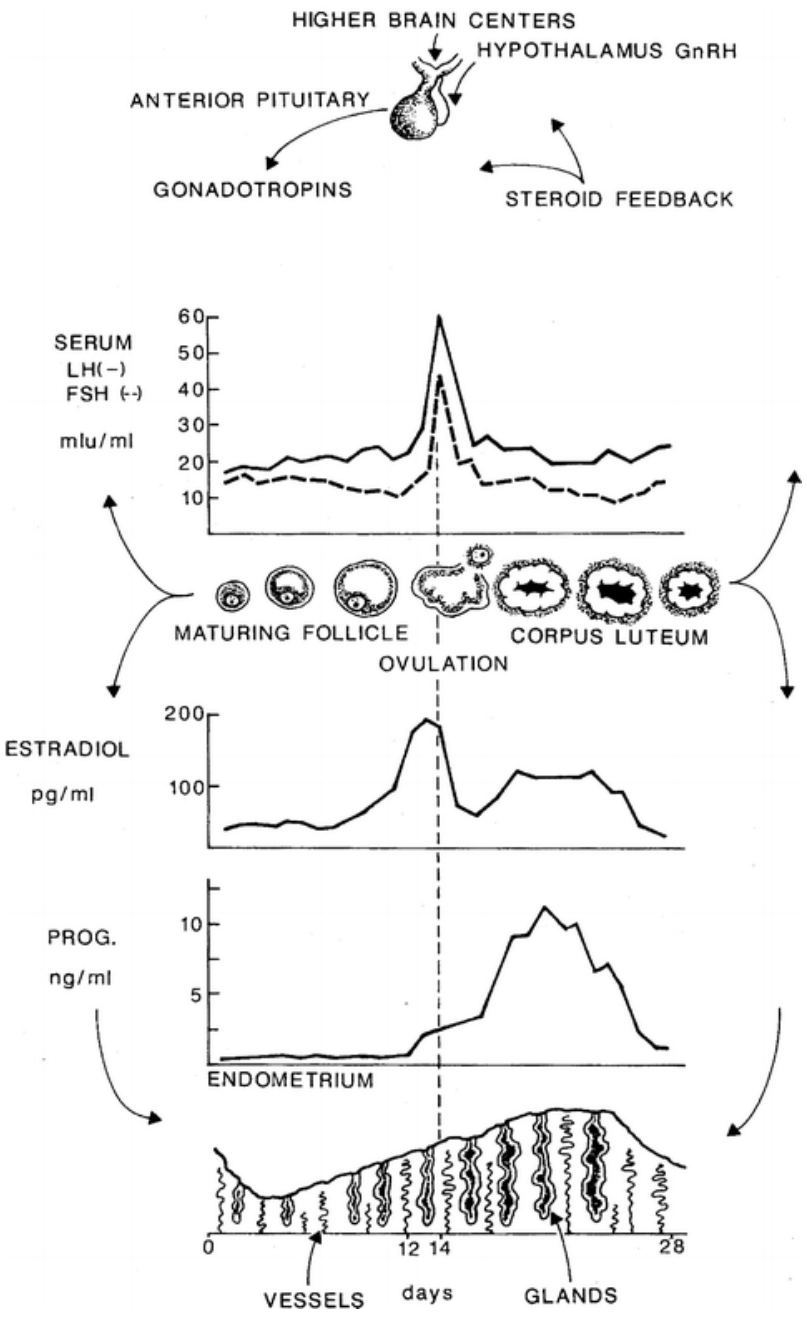
The duration of the follicular phase, on average, lasts 12-14 days and it is characterised by initial low concentrations of oestrogen and progesterone (Frankovich & Lebrun, 2000; Sims & Heather, 2018). However, the hormonal concentrations change throughout this phase. Specifically, the early-follicular sub-phase (EF) is characterised by low concentrations of both oestrogen and progesterone, the mid-follicular sub-phase (MF) being characterised by low concentrations of progesterone and raising concentrations of oestrogen, and the late-follicular sub-phase (LF) characterised by a low concentration of progesterone and high concentrations of oestrogen (Janse de Jonge, 2003).

At the end of the follicular phase, the ovulatory phase occurs. During the ovulation, an exception to the negative-feedback system occurs, where an increase in oestrogen will provide a positive feedback to produce FSH and LH, called LH surge (Stefanovsky et al., 2016; Thiyagarajan et al., 2020).The LH surge will cause the mature follicle to release the egg (ovulation) from a woman's ovary (Barbieri, 2014; Stefanovsky et al., 2016). From the ovary, the egg travels to the fallopian tubes where the potential of fertilisation may occur (Jonge, 2009).

The luteal phase is the latter phase of the MC, which begins with ovulation and ends at the start of menses (Frankovich & Lebrun, 2000). In this phase an empty follicle transforms into a structure known as corpus luteum, which acts to stabilise the endometrium for implantation of the fertilised egg (Frankovich & Lebrun, 2000). When fertilisation or implantation do not occur, the corpus luteum will begin to break down (Frankovich & Lebrun, 2000). As the secretory phase ends, the negative feedback from progesterone to the anterior pituitary will decrease FSH and LH levels, which will cause a decrease in oestrogen and progesterone (Thiyagarajan et al., 2020). This decline in

progesterone, called progesterone withdrawal, will lead to the start of another menstrual period (Frankovich & Lebrun, 2000; Stefanovsky et al., 2016).

On average, the luteal phase lasts 12-14 days, and is characterised by high concentrations of oestrogen and progesterone (Sims & Heather, 2018). Similar to the follicular phase, the luteal phase can be divided into three sub-phases. The early-luteal sub-phase (EL), which is characterised by decreasing concentrations of oestrogen and raising concentrations of progesterone. The mid-luteal sub-phase (ML), described by high concentrations of both oestrogen and progesterone. And the late-luteal (LL) sub-phase, which is characterised by a decrease of progesterone and oestrogen (Janse de Jonge, 2003).



468

469 **Figure 1**: changes in a woman's body during the menstrual cycle (Barbieri, 2014).

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2.2 Effects of hormones

Throughout the MC there is an established pattern of hormonal changes that involves progesterone, oestrogen, LH and FSH (Frankovich & Lebrun, 2000). These hormones have also been found to affect several other functions not related to the reproductive function, but that can affect aspects related to physical performance, such as the cardiovascular and respiratory systems. Whilst oestrogen and progesterone change a lot between the different phases, FSH and LH concentrations are more stable between the early-follicular and mid-luteal sub-phases (Figure 2), and therefore it can be assumed that they will be more consistent in their effects on performance during the MC.

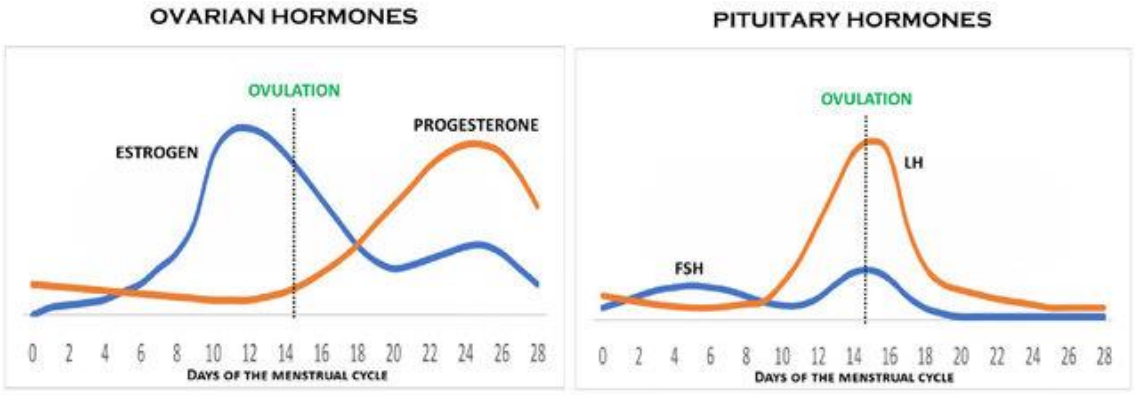

Figure 2: menstrual cycle hormonal fluctuations (Ocampo Rebollar, Menéndez Balaña, & Conde Pastor, 2017).

2.2.1 Oestrogen

When investigating the hormonal effect on physical performance, oestrogen was found to affect energy metabolism by increasing the muscle glycogen storage and repletion, as well as being direct mobilisers of adipocyte free fatty acid (FFA) (Oosthuyse, Bosch, & Jackson, 2005). As a result of the increased use of fat oxidative pathways a lower respiratory exchange ratio (RER) occurs (Nicklas et al., 1989), which subsequently results in a glycogen-sparing effect (Smekal et al., 2007). The enhanced glycogen repletion and higher fat usage particularly benefits submaximal endurance exercises (Nicklas et al., 1989). In addition, Bunt (1990) reported that oestrogen increases lipid availability and

utilisation, by increasing triglyceride synthesis and lipolysis in muscle and adipose tissue and decrease gluconeogenesis and glycogenolysis by increasing the ratio between insulin and glucagon. Furthermore, D'eon et al. (2002) reported that increased concentrations of oestrogen were found to lower carbohydrate oxidation during submaximal exercise, by reducing muscle glycogen utilisation, without affecting blood glucose uptake. However, the authors reported that this change in carbohydrate oxidation and reduced muscle glycogen usage did not seem to affect performance as measured by HR and $\dot{V}O_2$.

Oestrogen is also known to have an influence on pain by reducing some types of pain such as migraine or arthritis (Hooper, Bryan, & Eaton, 2011). However, due to the oestrogen's effects on several systems, including the nervous and the immune systems, oestrogen can produce both pro and antinociceptive effects, and the mechanisms underlying this modulation are specific to each kind of pain (Craft, 2007). For example, oestrogen alter opioid antinociceptive pathways and suppress adrenergic antinociception, with which they modulate different types of pain such as musculoskeletal pains (Craft, 2007). This is particularly important, as pain influences players ability to fully participate in training and/or competition, and also affects other aspects such as rehabilitation programmes (Kakiashvili, Tsagareli, Mjavanadze, & Kvachadze, 2016). This was further confirmed by Hooper et al. (2011) who reported higher RPE during the early-follicular sub-phase than the late-follicular and the luteal phase. Furthermore, Hooper et al. (2011) reported that changes in RPE during the MC phases might be due to changes in pain experienced whilst performing during the same phases. Thus, based on the current literature it appears that oestrogen fluctuations affect different systems and functions, such as the nervous system, energy metabolism and/or pain modulation.

2.2.2 Progesterone

Previous studies on the effects of progesterone on cardiac function suggest that progesterone increases heart rate by shortening the cardiac action potential, due to changes in myocardial L-type calcium channel current, potassium channel currents and inward rectifier current (Sedlak, Shufelt, Iribarren, & Merz, 2012). Furthermore, progesterone has been shown to stimulate ventilation by stimulating chemoreceptors (Williams & Krahenbuhl, 1997), which could lead to a lower $\dot{V}O_2max$ and therefore to a decrease in performance (Abdollahpor, Khosravi, & Zahra, 2013). Progesterone has also been found to have metabolic effects, stimulating deposition of body fat by reducing sensitivity of adipocytes to insulin-induced glucose uptake and oxidation, and having a catabolic effect on protein metabolism (Kalkhoff, 1982). As performance in prolonged exercise relies on fat oxidation consequently, a lower availability of this energy source may negatively impact performance (Maunder, Plews, & Kilding, 2018).

Therefore, it appears that these negative effects associated with progesterone are more apparent during the luteal phase, when progesterone concentrations are highest. Furthermore, it appears that oestrogen and progesterone have contrasting effects on energy metabolism, with oestrogen increasing the muscle glycogen storage and repletion, as well as being direct mobilisers of adipocyte free fatty acid (FFA), and progesterone stimulating deposition of body fat by reducing sensitivity of adipocytes to insulin-induced glucose uptake and oxidation.

2.3 Menstrual cycle phases definition

As oestrogen and progesterone cause modifications in cardiovascular and metabolic parameters this has important implications for endurance performance (Constantini et al., 2005). Considering that these hormones fluctuate greatly between different phases (Figure 2) it is therefore very important to define them accurately and consistently.

Furthermore, as oestrogen and progesterone are not constant and change throughout a single phase, the definition of sub-phases is also fundamental in defining the effects hormonal concentrations have during each sub-phase (Allen et al., 2016). However, even though defining the sub-phases is of primary importance, Masterson (1999), Redman et al. (2003), Smekal et al. (2007), Tsampoukos et al. (2010), Vaiksaar et al. (2011) and Cristina-Souza et al. (2019) did not take into consideration any sub-phases, and only focused on the differences between the main MC phases.

Furthermore, the definition of the MC sub-phases is often unclear, as when referring to a 28-day MC, different authors associated sub-phases with different days, leaving some degree of uncertainty when comparing different papers (Table 3). This inconsistency in the association between specific sub-phases and days of the MC is likely to cause methodological errors, because different papers might declare to be comparing different sub-phases whilst using the same days. Additionally, this brings a great level of confusion for sport and exercise scientists looking for specific information and should therefore be addressed in future studies. For example, the early-follicular sub-phase definitions range from 1-5 days (Hooper et al., 2011) to 3-8 days (Lebrun et al., 1995). At the same time, different authors define the mid-follicular sub-phase starting on the 5th (Mattu, Iannetta, MacInnis, Doyle-Baker, & Murias, 2019), 6th (Middleton & Wenger, 2006; Shaharudin, Ghosh, & Ismail, 2011), or 7th day (Giacomoni, Bernard, Gavarry, Altare, & Falgairette, 2000; Pestana et al., 2017), overlapping with Lebrun et al.'s (1995) definition for the early-follicular sub-phase (Table 3). Similarly, an overlap is seen in the mid-follicular sub-phase around the 9th and 10th days, where Hooper et al. (2011) labels these days as late-follicular (Table 3) whilst Pestana et al. (2017), Middleton & Wenger (2006), Shaharudin et al. (2011), and Mattu et al. (2019) all defined these days as mid-follicular. Furthermore, in one specific case the exact same days are defined as two different sub-phases, as Shaharudin et al. (2011) defined the mid-luteal sub-phase between the 20th

569 and 24th day, but the same days were used by Middleton & Wenger (2006) to define the
570 late-luteal sub-phase (Table 3).

571 **Table 3**: different definitions of each sub-phase, used by different authors.

Authors	EF	MF	LF	ML	LL
Hooper et al. (2011)	1 – 5		9 – 15		
Bemben et al. (1995)	2 – 5		12 – 15	20 – 23	
Lamont (1986)	2 – 4			20 – 22	
Janse de Jonge (2003)	3 – 6			19 – 25	
Julian et al. (2017)	5 – 7			21 – 22	
Mattu et al. (2019)		5 – 10		19 – 24	
Shaharudin et al. (2011)		6 – 10		20 – 24	
Gordon et al. (2018)		9 – 11		19 – 20	
Giacomoni et al. (2000)		7 – 9		19 – 21	
Middleton & Wenger (2006)		6 – 10			20 – 24
Pestana et al. (2017)		7 – 9	10 – 14		26 – 28
Oosthuyse et al. (2005)	2 – 7		From 2d before LH surge to LH surge	4 – 10 after LH surge	
De Souza et al. (1990)	2 – 4			6 – 8 from LH surge	
Beidlemn et al. (1999)	3 – 6			6 – 9 after LH surge	
Lebrun et al. (1995)	3 – 8			4 – 9 after ovulation	
Schoene et al. (1981)		6 – 10		4 – 9 after ovulation	
Nicklas et al. (1989)		7 – 8		7 – 8 after ovulation	
Jurkowski et al. (1981)		6 – 9		6 – 9 after ovulation	
McCracken et al. (1994)		6 – 9		6 – 9 after ovulation	
Dombovy et al. (1987)		7 – 11		3 – 11 days before expected menses	

572 EF: Early-follicular, MF: Mid-follicular, LF: Late-follicular, ML: Mid-luteal, LL: Late-luteal

573 From Table 3 it can be noted that, whilst Hooper et al. (2011), Bemben et al. (1995),
574 Lamont (1986), Janse de Jonge (2003), Julian et al. (2017), Mattu et al. (2019),
575 Shaharudin et al. (2011), Gordon et al. (2018), Giacomoni et al. (2000), Middleton &
576 Wenger (2006) and Pestana et al. (2017) used specific days to identify the sub-phases,
577 based on a 28-day cycle, Oosthuyse et al. (2005), De Souza et al. (1990), Beidlemn et al.
578 (1999), Lebrun et al. (1995), Schoene, Robertson, Pierson, & Peterson (1981), Nicklas et
579 al. (1989), Jurkowski et al. (1981) and McCracken et al. (1994) used the ovulation or the
580 LH surge as a starting point to count for the luteal phases. Using the ovulation or the LH
581 surge means that the follicular phase length might not be 14 days. However, using this
582 method requires the assumption that the luteal phase has a standard duration. In fact,

583 if ovulation occurred on two different days, the instructions (i.e. number of days) to
584 determine the luteal sub-phases would not change. Even though using the LH surge or
585 the ovulation accounts for a different follicular phase duration between different
586 participants, no solutions have been proposed to account for the luteal phase, which is
587 just assumed to be exactly the same between all participants. However, the luteal phase
588 has been demonstrated to vary significantly, as Liu et al. (2004) reported that luteal
589 phase can be shorter than 11 days or longer than 15 days.

590 Furthermore, when a MC is not 28-days long, there are no clear references on how to
591 divide the sub-phases. All the indications provided are based on an average 28-days long
592 MC, but this is often not the real length of the cycle, as shown by the different mean MC
593 length in several papers. For instance, Shaharudin et al. (2011) reported a mean length
594 of 30.3 ± 2.1 days for the MC, but still provided a division in sub-phases based on a 28-
595 day MC. Therefore, it is unknown as to how they determined specific sub-phases in all
596 individual participants with different MC lengths.

597 A recent systematic review by Schmalenberger et al. (2019) proposed a new definition
598 of the sub-phases, completely changing the way they have been described until now.
599 This reclassification, shown in Table 4, is based on hormone concentrations and
600 therefore it can only be used when hormones are collected and analysed. However, from
601 a practical point of view this might not be possible for coaches/practitioners due to the
602 high cost, the invasiveness, and the need for competent staff to collect and analyse
603 hormonal data. If a cheaper, simpler, yet accurate way to determine hormones is made
604 available by future technologies, then it could become the more convenient way to
605 describe the MC phases.

606

607

608 **Table 4**: menstrual cycle phases' reclassification proposed by Schmalenberger et al. (2019).

Phase	Hormone concentrations
Menstrual phase	Low oestrogen, low progesterone
Mid-to-late-follicular phase	Rising oestrogen, low progesterone
Ovulatory phase	Peak oestrogen, low progesterone
Early-to-mid-luteal phase	Secondary lesser peak of oestrogen, peak progesterone
Premenstrual phase	Falling oestrogen, falling progesterone

609

610 ## 2.4 Menstrual cycle determination

611 An important aspect in the determination of the MC sub-phases is the method by which
612 these sub-phases are assessed (Allen et al., 2016). Using different methods to determine
613 the MC sub-phases can result in having the same days defined as two different sub-
614 phases, and therefore creating a problem interpreting and comparing the results. This
615 may also explain the inconsistent definitions of MC sub-phases between studies as
616 described in the previous section.

617 The choice of the appropriate method to determine the MC should be based on its
618 accuracy, the cost, and the invasiveness, as shown in Table 5. All of these different
619 methods can impact on the classification of the sub-phases of the MC (Allen et al., 2016)
620 and the accuracy is certainly a fundamental aspect to consider. However, the cost and
621 invasiveness are also important when choosing the method for MC determination,
622 especially for the use of non-clinical practice including sports practitioners. As even if
623 they understand the advantages of monitoring the MC, the cost and invasiveness of the
624 methods could be a deterrent in doing so.

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Table 5: cost, invasiveness, and accuracy of each method for menstrual cycle phases' determination.

Method	Cost (1 – 3)*	Invasiveness	Sub-phase identification accuracy
Diary	1	Very low	Low
BBT	2	Low	Medium
Urinary LH	2	Low	Medium**
Salivary analysis	3	Medium	High**
Blood analysis	3	Medium	High

Adapted from (Allen et al., 2016)
* 1 is the lowest cost, 3 is the highest
** The accuracy was found to be higher with the urinary kit, when compared with salivary analysis, by Eichner & Timpe (2004)

When assessing the time of ovulation or the different sub-phases of the MC, a number of different methods are available (Su et al., 2017). Blood sample analysis is considered to be the gold standard (Frankovich & Lebrun, 2000; Janse de Jonge, 2003) as it provides specific hormonal values that can be used to confirm MC sub-phases. However, this method is expensive and invasive (Su et al., 2017), which limits its application to general use. Other methods that may be utilised include urinary kits, basal body temperature, salivary analysis and a diary-based approach (Su et al., 2017).

The urinary kits have been shown to be an accurate and reliable method to identify ovulation, and it is considered to be superior to basal body temperature, calendar-based and salivary analysis methods (Eichner & Timpe, 2004; Miller & Soules, 1996; Su et al., 2017). A study by Guida et al. (1999) compared different methods to detect ovulation such as salivary hormones, basal body temperature and urinary kits and concluded that the determination of urinary LH concentrations should be the preferred method for defining ovulation. A commonly used urinary kit to determine ovulation is the Clearblue Advanced Digital Ovulation Test, which has been recently used by Mattu et al. (2019). The manufacturer of the Clearblue Advanced Digital Ovulation Test declares an accuracy of 99% in detecting the LH surge, and can therefore be considered reliable (Mattu et al., 2019). However, even though urinary kits are considered accurate and reliable when identifying ovulation, they do not measure hormone concentrations, and therefore cannot guarantee the accurate determination of the MC sub-phases. This is because the

MC sub-phases would be counted/predicted based on the ovulation and the average MC length, instead of being directly determined.

Monitoring of basal body temperature is reported as one of the simplest and least invasive methods to detect ovulation (Allen et al., 2016). Researchers have previously identified that core body temperature usually decreases to its lowest point one day before ovulation, and then rises during the luteal phase to a higher level than the follicular phase (increase of 0.28 to 0.56°C) (Barron & Fehring, 2005). However, this method is not considered accurate, as some women have been found to ovulate without a clear rise in temperature, and therefore should not be used to predict ovulation (Barron & Fehring, 2005). The number of women experiencing ovulation without a rise in temperature has been reported to range between 20 and 22.1% (Bauman, 1981; Moghissi, 1976). Furthermore, other factors might impact the basal body temperature, such as fever, alcohol consumption, climate changes or emotional and physical stress (Su et al., 2017), thus creating difficulties in the interpretation of the basal body temperature.

Salivary analysis is another method to determine the MC by measuring hormone concentration in a saliva sample. However, this method has been previously reported to be less accurate than the use of blood samples, yet more accurate than the body temperature monitoring (Allen et al., 2016; Su et al., 2017). In addition, when comparing salivary analysis with the LH urinary kit, different results have been found. Whilst Allen et al. (2016) reported salivary analysis to be more accurate than the use of a urinary kit, Eichner & Timpe (2004) reported a higher accuracy for the urinary kit compared with the salivary analysis when determining MC sub-phases. These differences between studies might be explained by the accuracy of each method and any other discrepancies in the methodological approaches.

The accuracy of a calendar-based method has previously been analysed by Wideman et al. (2013), concluding that a self-reported history and a diary should not be used to determine ovulation because of its low accuracy. Nonetheless, Wideman et al. (2013) suggest that a urinary kit and serial blood samples would provide better results than the calendar-based method for the identification of the MC sub-phases, due to its enhanced chances to properly identify MC events.

The aforementioned methods can be used together in a combined approach, to increase the probability to identify the correct sub-phases (Schaumberg, Jenkins, Janse de Jonge, Emmerton, & Skinner, 2017). Different authors have utilised a variety of different combinations of methods to increase the accuracy in identifying the correct sub-phases, as shown in Table 6. For example, Mattu et al. (2019) utilised a urinary kit and a calendar-based approach. Whereas, Hackney et al. (1991) and Oosthuyse et al. (2005) combined four methods (blood sample analysis, urinary kit, basal body temperature monitoring, diary) to determine the MC sub-phases. Blood samples analysis have also been used in combination with a urinary kit and a diary to determine the MC sub-phases (Beidleman et al., 1999; De Souza, Maguire, Rubin, & Maresh, 1990; Pivarnik, Marichal, Spillman, & Morrow, 1992; Redman, Scroop, & Norman, 2003; Tsampoukos, Peckham, James, & Nevill, 2010). A recent review by Janse de Jonge et al. (2019) suggested that the best approach would be a combination using a calendar-based method to count the days, a urinary kit to detect ovulation and the measurement of serum oestrogen and progesterone the day of the testing, to confirm whether or not the test was done in the correct MC phase, based on expected hormonal concentrations in each one (EF: low oestrogen and progesterone; LF: oestrogen higher than EF and ML and progesterone higher than EF and lower than 16 nmol/L; ML: progesterone higher than 16 nmol/L).

Table 6: combined approaches used by different authors to determine the menstrual cycle phases.

References	Combined approach utilised
Hackney et al. (1991), Oosthuyse et al. (2005)	Blood samples + urinary kit + temperature + calendar
De Souza et al. (1990), Pivarnik et al. (1992), Tsampoukos et al. (2010), Redman et al. (2003), Beidleman et al. (1999)	Blood samples + urinary kit + calendar
Schoene et al. (1981), Shaharudin et al. (2011), Lebrun et al. (1995), Bemben et al. (1995), Nicklas et al. (1989), Wiecek et al. (2016), Williams et al. (1997), Janse de Jonge et al. (2012), Dean et al. (2003)	Blood samples + temperature + calendar
Smekal et al. (2007)	Blood samples + temperature
Bushman et al. (2006), McCracken et al. (1994)	Urinary kit + temperature + calendar
Mattu et al. (2019)	Urinary kit + calendar

As the MC phases and sub-phases are based on a regular ovulatory cycle, an important aspect to consider is the possibility of a luteal phase-deficient (LPD) or anovulatory cycle, characterised by a lower LH surge and reduced hormone production (Janse De Jonge, Thompson, & Han, 2019). A regular MC with normal bleeding is not enough to confirm ovulation, as these ovulatory disturbances can occur without showing anything abnormal nor any particular symptoms (Janse de Jonge et al., 2019; Schliep et al., 2014).

Luteal phase-deficient or anovulatory cycles are common in physically active females, with approximately 50% of exercising women experiencing LPD or anovulation (De Souza et al., 2010). It is also important to mention that over one third of menstrual cycles are anovulatory even in healthy females, and it is believed that those cycles occur in all women (Prior, Naess, Langhammer, & Forsmo, 2015). Therefore, when studying females and their MC, it is important to accurately verify if participants are experiencing these conditions or not (Janse de Jonge, 2003). LPD and anovulatory cycles are characterised by low progesterone during the luteal phase when compared to a normal ovulatory cycle. Therefore, the inclusion of participants with LPD or anovulatory cycles potentially influences the results of a study (Birch, 2000; Janse de Jonge, 2003).

Among the different methods to determine the MC phases and sub-phases, some of them can also confirm a regular ovulatory cycle (i.e. non anovulatory or LPD) (Janse De

Jonge et al., 2019). The calendar-based approach and the basal body temperature, both on their own or combined, cannot dismiss an anovulatory or LPD cycle (Schliep et al., 2014; Wideman, Montgomery, Levine, Beynnon, & Shultz, 2013). The urinary kit, however, can exclude anovulatory cycles, but cannot exclude LPD cycles (Janse De Jonge et al., 2019). Salivary hormones analysis can be used to exclude both anovulatory and LPD cycles, but due to the low concentration of hormones in saliva, tests with a very high sensitivity are needed along with multiple samples (Janse De Jonge et al., 2019). Blood sample hormone analysis have been considered the main method to verify that both LPD and anovulatory cycles are excluded (Janse de Jonge et al., 2019). Therefore, the blood sample hormone analysis should be considered the gold standard because it is, among the above-mentioned methods, the only one that can guarantee both a precise determination of the MC phases whilst also excluding LPD and anovulatory cycles (Janse de Jonge et al., 2019). However, blood sample hormone analysis is expensive, time-consuming and requires a high level of expertise, which reduces its practical applications in an applied sport environment.

2.5 Effects of hormones on participant's characteristics

Participant's characteristics such as body mass and body composition have been analysed previously, as they have been deemed by researchers to affect performance (Esco et al., 2018; Maciejczyk, Wiecek, Szymura, Szygula, & Brown, 2015). The effects of the MC on body mass and body composition have been studied because it has been hypothesised that changes could occur due to changes in fluid regulation (Giacomoni et al., 2000; Janse de Jonge, 2003). However, no previous studies that measured body mass and/or body composition between the sub-phases have found significant differences in either of those parameters.

The differences in body mass and body composition have been analysed between the early-follicular and mid-luteal sub-phases by Julian et al. (2017) that reported non-

significant differences in body mass and body fat (17.7 ± 2.5 % vs 17.8 ± 2.2 % respectively). Similar non-significant differences were reported by Lebrun et al. (1995), with a body mass of 59.5 ± 1.7 kg vs 59.5 ± 1.8 kg, and a body fat of 17.4 ± 1.2 % vs 17.3 ± 0.9 %. Furthermore, Abdollahpor et al. (2013), Beidleman et al. (1999) and De Souza et al. (1990) also reported non-significant results between the early-follicular and mid-luteal sub-phases. Lebrun et al. (1995) reported that the MC sub-phases affects fluid retention, which in turn affects body mass and composition. However, Lebrun et al. (1995) also reported that regular exercise might reduce this effect, and therefore may help explain why no differences in body mass or composition were found between MC sub-phases.

Furthermore, a number of studies made a comparison between phases, without specifying the sub-phases analysed, and reported non-significant differences in body mass and/or body composition (Giacomoni et al., 2000; Miskec, Potteiger, Nau, & Zebas, 1997; Tsampoukos et al., 2010; Vaiksaar et al., 2011). Miskec et al. (1997) reported that differences in body mass might be due to water gains associated with an increase in progesterone and oestrogen. However, no differences were reported by the authors, and therefore the effects of the hormones might not be enough to be statistically relevant.

In conclusion, from the available literature, it appears that the hormonal fluctuations during the MC do not influence body mass or body composition. This could be due to fluid regulation not being affected by the MC, as previously hypothesised by Janse de Jonge (2003), or due to the fact that the effects of the MC on fluid regulation might not be enough to impact body mass and body composition. Furthermore, it is possible that the differences in body composition and body mass occur in sub-phases that have not been compared previously, and therefore remain unknown. Further research involving different time-points might show significant differences in these parameters.

2.6 Menstrual cycle physiology

2.6.1 Heart Rate

Heart Rate (HR) is an important parameter often monitored during exercise to determine exercise intensity, the status of the autonomic nervous system and cardiovascular fitness (Pestana et al., 2017; Schneider et al., 2018). As progesterone has been found to increase heart rate, it is plausible to assume that a higher HR would be found during the luteal phase, where the concentrations of progesterone are the highest throughout the MC (Pivarnik et al., 1992; Sedlak et al., 2012). The higher HR during the luteal phase has been explained by a possible decrease in plasma volume (Pivarnik et al., 1992), an increased capillary permeability (Pivarnik et al., 1992), an enhanced sympathetic activity (Pestana et al., 2017) and/or a direct effect of temperature on the sinoatrial node (Janse de Jonge, Thompson, Chuter, Silk, & Thom, 2012).

The differences in HR have been studied between the early-follicular and mid-luteal sub-phases, with non-significant results being reported by Bemben et al. (1995) and Dean et al. (2003) during a running and cycling incremental exercise to exhaustion, respectively. Other non-significant results between the early-follicular and mid-luteal sub-phases during exercise have also been reported by Abdollahpor et al. (2013), De Souza et al. (1990), Lebrun et al. (1995), Beidleman et al. (1999), and Oosthuyse et al. (2005). However, a significant difference was found by Janse de Jonge et al. (2012), who reported a higher resting HR during the mid-luteal (72 ± 5 bpm) compared to the early-follicular sub-phase (68 ± 6 bpm). Interestingly, significant differences disappeared during an incremental cycling test to exhaustion (182 ± 16 bpm vs 182 ± 12 bpm). Furthermore, Janse de Jonge et al. (2012) did not report any significant differences during 60-minutes of submaximal exercise at 60% of $\dot{V}O_2$max, but no exact results were provided in the study. Janse de Jonge et al. (2012) suggested that the change in body temperature might explain approximately 40% of the increased HR, and therefore it is

possible that no differences were found at exhaustion because other factors might out-weigh the effect of the temperature on HR. However, it is not clear what the other 60% refers to, as the authors did not provide further explanations. Except for the study by Janse de Jonge et al. (2012) who reported a significant difference in resting HR between the early-follicular and mid-luteal sub-phases, there is a unanimous consensus that exercise HR is not influenced by these two MC sub-phases regardless of the different methodologies implemented.

The mid-luteal sub-phase has also been compared with the mid-follicular phase by Gordon et al. (2018) who reported non-significant differences during incremental cycling exercise to exhaustion. Furthermore, Hackney et al. (1991) found non-significant results during 60-minutes of cycling exercise at 70% of $\dot{V}O_2$max at different time points. Conversely, Pivarnik et al. (1992) reported opposite results, even though the testing protocol was very similar to the one used by (Hackney, Curley, & Nicklas, 1991). In fact, Pivarnik et al. (1992) reported a significantly higher HR at all time points (10, 20, 30, 40, 50 and 60 minutes) during 60-minutes of exercise at 65-70% of $\dot{V}O_2$max, with an average 10 bpm higher during the mid-luteal sub-phase. Other non-significant results between the mid-follicular and mid-luteal sub-phases have been reported by Jurkowski et al. (1981), Dombovy et al. (1987), Mattu et al. (2019), Dean et al. (2003) and Sunderland & Nevill (2003) across a range of exercise modalities and intensities in participants of varying sporting backgrounds.

While some sub-phases have been studied quite well in relation to HR differences, studies on HR comparisons between specific sub-phases, such as mid-follicular and late-luteal or late- and early-follicular sub phases are limited. For example, the differences in HR between the mid-follicular and late-luteal sub-phases have been tested only by Pestana et al. (2017), which compared Wingate anaerobic test results and reported significant differences in maximum HR (180.48 ± 11.83 bpm vs 183.90 ± 12.95 bpm

829 respectively) but not in resting HR (73.43 ± 12.37 bpm vs 76.1 ± 12.33 bpm). Non-
830 significant results were also reported by Bemben et al. (1995) during a running
831 incremental exercise to exhaustion between the late-follicular and the early-follicular
832 sub-phases and between the late-follicular and the mid-luteal one. In agreement, Dean
833 et al. (2003) also reported non-significant differences during an incremental cycling
834 exercise to exhaustion between the early-follicular sub-phase and the mid-follicular sub-
835 phase.

836 There are also a few studies that generically compared the luteal and follicular phases,
837 but did not specify the sub-phases (De Bruyn-Prevost, Masset, & Sturbois, 1984; Köse,
838 2018; Redman et al., 2003; Smekal et al., 2007; Stephenson, Kolka, & Wilkerson, 1982;
839 Vaiksaar et al., 2011). In these studies, all authors reported non-significant differences
840 between the luteal and follicular phases across different tests, including incremental
841 tests to exhaustion, submaximal exercise, and Wingate anaerobic test in both non-active
842 and active participants.

843 In conclusion, it seems that the hormonal fluctuations during the MC phases do not
844 impact the heart rate, independently of the protocol used. A possible explanation to this
845 has been provided by Janse de Jonge et al. (2012) who stated that the difference in
846 temperature between phases might be too low to significantly affect differences in HR,
847 while other factors affecting HR have not been analysed or reported previously.

848 **2.6.2 Blood lactate**

849 Blood lactate is another important parameter commonly measured during both
850 performance and clinical exercise testing to determine exercise intensity (Goodwin,
851 Harris, Hernández, & Gladden, 2007). It has been hypothesised that a higher utilisation
852 of fat during the mid-luteal sub-phase should be supported by a lower concentration of
853 lactate, due to a decreased utilisation of carbohydrates, and the sparing of glycogen

(Mattu et al., 2019; Redman et al., 2003). However, the current literature seems to contrast this hypothesis as majority of previous studies reported non-significant differences in blood lactate.

The differences in blood lactate have been tested between the mid-follicular and mid-luteal sub-phases, and the majority of the papers (9 out of 11) reported non-significant results. For example, Shaharudin et al. (2011) reported no differences between the mid-follicular and mid-luteal sub-phases at rest and after the last sprint during a set of 3 sprints at 120% $\dot{V}O_2$max, with 20 minutes of recovery. Similarly, Dean et al. (2003) reported no differences at rest and at the end of an incremental cycling exercise to exhaustion with moderately active participants. Moreover, Dombovy et al. (1987), Nicklas et al. (1989), Sunderland & Nevill (2003), Gurd et al. (2007), Wiecek et al. (2016), Gordon et al. (2018) and Mattu et al. (2019) all reported non-significant differences between the mid-follicular and mid-luteal sub-phases. Even though it has been speculated that oestrogen can increase lipid oxidation and spare glycogen, causing a decreased lactate response, the findings from Shaharudin et al. (2011) study showed no differences in lactate response to exercise during MC phases.

However, in contrast with these findings, Jurkowski et al. (1981) and McCracken et al. (1994) reported higher lactate during the mid-follicular than the mid-luteal sub-phases. Jurkowski et al. (1981) reported a higher lactate during the mid-follicular than the mid-luteal after 20-minutes of exercise at 66% of maximum power output (6.62 ± 0.8 mmol·l^{-1} vs 4.92 ± 2.5 mmol·l^{-1}), and 20-minutes of exercise at 90% of maximum power output (8.12 ± 0.9 mmol·l^{-1} vs 6.76 ± 0.6 mmol·l^{-1}), but no differences after 20-minutes of exercise at 33% of maximum power output. McCracken et al. (1994) also reported a higher lactate during the mid-follicular sub-phase than the mid-luteal when measured 3 minutes post-exercise (8.7 ± 1.8 mmol·l^{-1} vs 5.4 ± 1.2 mmol·l^{-1} respectively) as well as 30 minutes post- exercise (2.4 ± 0.4 mmol·l^{-1} vs 4 ± 1.3 mmol·l^{-1}) following a running

incremental exercise to exhaustion. However, McCracken et al. (1994) did not report any differences at rest (1.6 ± 0.2 mmol·l^{-1} vs 1.7 ± 0.3 mmol·l^{-1} respectively). Jurkowski et al. (1981) reported that these differences in lactate production may be due to factors influencing energy substrates and/or glycolytic enzymes. However, McCracken et al. (1994) reported that even though their data were not enough to provide an explanation, it is possible that the differences in lactate was due to a preferential metabolism of lipid associated with oestrogen that reduced the carbohydrate utilisation and the glycolytic activity. Even though contrasting results have been reported between the mid-luteal and mid-follicular sub-phases, the differences do not appear to be related to the participant's level, the exercise modality, the test chosen or the modality to determine the MC sub-phases.

The mid-luteal sub-phase has also been compared with the early-follicular sub-phase, but no significant results were reported. Lamont (1986) reported no differences during a 60-minutes cycling exercise at 70% of $\dot{V}O_2$max but did not provide any numeric values. In agreement with the findings by Lamont (1986), Abdollahpor et al. (2013), Dean et al. (2003), Bemben et al. (1995) and De Souza et al. (1990) also reported non-significant differences between these two sub-phases. Dean et al. (2003) concluded that the MC effects on lactate might be hidden by the exercise-related increase in lactate levels. The increase in lactate due to the exercise would be higher than any difference caused by the MC, therefore possibly hiding its effect.

Other sub-phases have been analysed only in a few studies. For example, a comparison between the early-follicular and the mid-follicular sub-phases has been made by Dean et al. (2003) who reported non-significant differences both at rest and at the end of the exercise. Similarly, no differences were reported between the early-follicular and the late-follicular sub-phases by Bemben et al. (1995) at rest and post-exercise. Bemben et al. (1995) was also the only study that compared the late-follicular with the mid-luteal

sub-phases and reported non-significant differences. Non-significant results have also been reported between the late-luteal and the mid-follicular sub-phases by Middleton & Wenger (2006) and Stefanovsky et al. (2016).

A relatively high number of papers compared the luteal and follicular phases but did not specify the sub-phases, and therefore these results should be considered with caution due to fact that different sub-phases with different hormonal concentrations might have been compared. Whilst De Bruyn-Prevost et al. (1984), Miskec et al. (1997), Smekal et al. (2007), Tsampoukos et al. (2011) and Vaiksaar et al. (2011) did not report any significant differences between those two phases, Redman et al. (2003) found significant differences between the luteal and follicular phases. Specifically, Redman et al. (2003) showed a significantly lower lactate concentration in the luteal phase during a steady state cycling exercise of 20 minutes at 25% $\dot{V}O_2$max followed by 20 minutes at 75% of $\dot{V}O_2$max, however, exact values related to lactate levels were not provided. Therefore, the different results might be explained by the lack of sub-phase determination, which can lead to testing the participants under different hormonal conditions whilst considering them in the same phase. As both progesterone and oestrogen concentrations fluctuate during a single MC phase, the determination of sub-phases is necessary to provide more certainty that the participants are being tested during the same sub-phases.

The results from Jurkowski et al. (1981), McCracken et al. (1994) and Redman et al. (2003) showed a lower lactate concentration during the luteal phase, which might suggest a reduction of carbohydrate metabolised during this phase. However, when the overall results from the current literature are considered it appears that the MC phases do not influence the lactate production, thus suggesting no differences in the contributions of adenosine triphosphate (ATP) production between follicular and luteal phases (Gurd, Scheid, Paterson, & Kowalchuk, 2007; Smekal et al., 2007).

2.6.3 Maximal oxygen consumption

Maximal oxygen consumption ($\dot{V}O_2max$) is defined as the highest rate of oxygen uptake and consumption by the body, during a maximal exercise (Poole, Wilkerson, & Jones, 2008). It is widely considered the gold standard measure of cardiovascular fitness and exercise capacity (Koutlianos et al., 2013), but it is also an important parameter for repeated sprint ability, as it affects the recovery between sprints and the ability to maintain a high performance when fatigue arises (Aguiar et al., 2016; Aziz et al., 2000; Jones et al., 2013; Sanders et al., 2017). Abdollahpor et al. (2013) hypothesised that $\dot{V}O_2max$ might be lower during the luteal phase because the minute ventilation would be higher due to progesterone's effect on ventilation ($\dot{V}e$). The increased minute ventilation, and therefore the higher oxygen cost, would utilise a higher portion of $\dot{V}O_2max$ that might limit maximal exercise performance (Vella, Marks, & Robergs, 2006). Janse de Jonge (2003) reported that $\dot{V}O_2max$ might be affected by the MC if its determinants, such as blood lactate, body mass and heart rate, would be affected by it. However, as blood lactate, body mass and heart rate are not influenced by the menstrual phases it is also speculated that MC would not affect $\dot{V}O_2max$ (Janse de Jonge, 2003).

The differences in $\dot{V}O_2max$ have been tested between the mid-luteal and the early-follicular sub-phases by different authors, and no significant results have been reported. Beidleman et al. (1999) and De Souza et al. (1990) reported non-significant differences in $\dot{V}O_2max$ during an incremental running exercise to exhaustion. Furthermore, other non-significant results were reported by Lebrun et al. (1995), Abdollahpor et al. (2013), Bemben et al. (1995), Dean et al. (2003), and Janse de Jonge et al. (2012).

The mid-luteal sub-phase was also compared with the mid-follicular sub-phase by Dean et al. (2003), Gordon et al. (2018), Mattu et al. (2019) and Dombovy et al. (1987) with all studies reporting non-significant differences in $\dot{V}O_2max$. Dean et al. (2003), Gordon et al. (2018), Mattu et al. (2019) and Dombovy et al. (1987) all found similar results, despite

using different determination methods (Table 1). In contrast, Schoene et al. (1981) reported a lower $\dot{V}O_2$max during the mid-luteal sub-phase (29.3 ± 4.2 ml·kg^{1}·min^{-1}) when compared with the mid-follicular (33.6 ± 2.7 ml·kg^{1}·min^{-1}) in non-active participants during an incremental cycling exercise to exhaustion. However, Schoene et al. (1981) could not find significant differences in the active participants group (48.6 ± 2.2 ml·kg^{1}·min^{-1} vs 48.1 ± 2.6 ml·kg^{1}·min^{-1} respectively). The authors provided an explanation stating that the active participants, due to their experience, might be able to tolerate subjective/personal factors that influence motivation and be driven to perform well in any circumstances, and therefore be less susceptible to MC effects (Schoene et al., 1981).

Other sub-phases have been compared by Bemben et al. (1995) that reported non-significant results between the late-follicular with both the early-follicular and the mid-luteal. Moreover, non-significant differences were also found between the early-follicular and the mid-follicular sub-phases (Dean, Perreault, Mazzeo, & Horton, 2003), and between the early-follicular and late-luteal ones during a 20m running shuttle test (Lamina, Hanif, & Muhammed, 2011). Furthermore, a number of studies compared the follicular and luteal phases and reported non-significant results, but failed to mention the exact sub-phases (De Bruyn-Prevost et al., 1984; Redman et al., 2003; Smekal et al., 2007; Stephenson et al., 1982; Vaiksaar et al., 2011). Based on the current literature, regardless of the exercise modality, the tests or the procedures, there seems to be a general consensus that $\dot{V}O_2$max is not affected by the MC phases (Gordon et al., 2018; Jurkowski, Jones, Toews, & Sutton, 1981).

2.6.4 Respiratory Exchange Ratio

Whilst $\dot{V}O_2$ can be analysed independently, it is also used to calculate the respiratory exchange ratio (RER). The RER is the ratio between the carbon dioxide production and the oxygen usage, and it reflects the relative contribution of carbohydrate and lipids to

the expenditure of energy (Ramos-Jiménez et al., 2008). The RER can range from 0.7, which is the ratio between carbon dioxide production and the oxygen usage during the oxidation of a molecule of fatty acid and go above 1.0, where 1.0 the ratio from the oxidation of a molecule of carbohydrate (Nilsson, Björnson, Flockhart, Larsen, & Nielsen, 2019). When the RER goes above 1.0, it means that the production of CO_2 is coming from a different source, non-related to the oxidation of fatty acids or carbohydrates, such as hyperventilation (Péronnet & Aguilaniu, 2006). The respiratory exchange ratio has been shown to be correlated with other fitness variables such as maximum HR, $\dot{V}O_2$max and lactate threshold (Ramos-Jiménez et al., 2008), and it has been hypothesised that a higher utilisation of fat during the luteal phase would be confirmed by a lower RER (Bunt, 1990; Nicklas et al., 1989; Redman et al., 2003).

The effects of the mid-luteal and early-follicular sub-phases on RER have been analysed, but no significant results have been reported by Dean et al. (2003), Lamont (1986), De Souza et al. (1990), Janse de Jonge et al. (2012), Lebrun et al. (1995) and Oosthuyse et al. (2005). As RER is a ratio between $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$), an explanation can be provided by the fact that Dean et al. (2003), De Souza et al. (1990) and Janse de Jonge et al. (2012) reported non-significant differences in $\dot{V}O_2$ and/or $\dot{V}CO_2$ between the MC phases. For this reason, it is plausible to believe that the ratio was also unchanged by the MC phases.

The RER has also been analysed between the mid-luteal and the mid-follicular sub-phases, and no significant differences have been shown by Hackney et al. (1991) and Gordon et al. (2018). Further non-significant results between the mid-luteal and mid-follicular sub-phases were also found by Dean et al. (2003), Nicklas et al. (1989), Gurd et al. (2007) and Mattu et al. (2019).

Moreover, the mid-follicular sub-phase has also been used as a comparison with the early-follicular sub-phase by Dean et al. (2003), who reported non-significant results.

Furthermore, a significantly lower RER has been found in the late-luteal sub-phase (1.17 ± 0.06) when compared with the mid-follicular one (1.19 ± 0.06) by Middleton & Wenger (2006) in the only study about the effects of the MC on RSA in literature, during a cycling RSA protocol of 10 sprints of 6 seconds each.

Oosthuyse et al. (2005) reported non-significant results between the late-follicular, early-follicular and mid-luteal sub-phases. Smekal et al. (2007) and Vaiksaar et al. (2011) compared the follicular and luteal phases, without mentioning the sub-phase, and reported non-significant differences. The findings from Smekal et al. (2007) and Vaiksaar et al. (2011) can be explained by the fact that they did not analyse specific sub-phases, and therefore they might have included data from different sub-phases, and therefore different hormonal concentrations. As their aim was to compare MC phases because of their different hormonal concentrations, by including unspecified sub-phases and therefore mixing different hormonal concentrations together, they might have affected the results. In contrast, Redman et al. (2003) reported a lower RER during the luteal phase following incremental cycling exercise to exhaustion than during the follicular one (1.05 ± 0.03 vs 1.16 ± 0.04 respectively), but no effects at rest (0.88 ± 0.03 vs 0.9 ± 0.03 respectively). The same study by Redman et al. (2003) also reported lower RER during the luteal phase during submaximal exercises at 25% and 75% $\dot{V}O_2$max but did not actually provide the results. From the current literature it appears that the MC does not affect RER, contrasting the hypothesis of a change in energy supply during exercise (Vaiksaar et al., 2011) and suggesting no changes in fat metabolism and fuel oxidation between phases (Gurd et al., 2007; Smekal et al., 2007).

2.6.5 Minute Ventilation

Minute ventilation ($\dot{V}e$) has been defined as the volume of gas inhaled or exhaled from a person's lungs per minute and it is the product between breathing frequency and tidal volume (Forman et al., 2010). Due to its close relationship with $\dot{V}O_2$ and $\dot{V}CO_2$, as

ventilation increases during exercise to meet a higher demand for O_2 uptake and CO_2 elimination, V̇e is considered an important physiological parameter to analyse (Forster, Haouzi, & Dempsey, 2012). V̇e has been shown to be stimulated by progesterone, and therefore it would be expected to see a higher V̇e during the mid-luteal sub-phase, when the progesterone is the highest throughout the MC (Beidleman et al., 1999; De Souza et al., 1990; Schoene, Robertson, Pierson, & Peterson, 1981; Williams & Krahenbuhl, 1997). However, V̇e does not appear to change significantly between MC phases. The most studied sub-phase in relation to V̇e has been the mid-luteal, which has been compared with the early-follicular. Non-significant differences in V̇e between the mid-luteal and the early-follicular sub-phase have been reported by Bemben et al. (1995), Lamont (1986), De Souza et al. (1990), Lebrun et al. (1995), and Beidleman et al. (1999). An explanation has been provided by Beidleman et al. (1999) who suggested that other factors (i.e., central motor command; reflexes from the exercising limb) might impact ventilation to a greater extent than progesterone. In contrast, Williams & Krahenbuhl (1997) reported a significantly higher V̇e during the mid-luteal sub-phase than the early-follicular at rest (12.4 0.7 l·min^{-1} vs 10.3 0.8 l·min^{-1} respectively), at 55% V̇O_2max (46.2 ± 0.9 l·min^{-1} vs 42.2 ± 1.4 l·min^{-1} respectively) and at 80% V̇O_2max (68.8 ± 3 l·min^{-1} vs 63.6 ± 2 l·min^{-1} respectively), which is in agreement with the hypothesis of progesterone affecting V̇e. Williams & Krahenbuhl (1997) also analysed and compared the late-follicular, early-luteal and late-luteal sub-phases, but no other differences were reported. As progesterone peaks during the mid-luteal sub-phase, a lack of significant differences between other phases can be explained by the fact that progesterone might have been too low to affect ventilation significantly (Williams & Krahenbuhl, 1997).

The mid-luteal sub-phase has also been compared with the mid-follicular, and the majority of the authors did not report any significant differences (Dombovy, Bonekat, Williams, & Staats, 1987; Gordon et al., 2018; Hackney et al., 1991; Jurkowski et al., 1981; Mattu et al., 2019). However, Schoene et al. (1981) reported a significantly higher V̇e at

rest in the mid-luteal sub-phase, when compared with the mid-follicular one in active participants (10.7 ± 0.7 l·min^{-1} vs 8.8 ± 0.6 l·min^{-1} respectively) and non-active participants (10 ± 0.7 l·min^{-1} vs 7.7 ± 0.9 l·min^{-1} respectively). The authors suggested that these findings are due to progesterone that, through a central mechanism, increases ventilation (Schoene et al., 1981).

The follicular and luteal phases were also compared without specifying the sub-phases, but no significant results were reported by Redman et al. (2003) during an incremental cycling exercise to exhaustion in non-active participants. Similarly, Vaiksaar et al. (2011) reported non-significant result in an incremental cycling exercise to exhaustion, but with active participants. Furthermore, Smekal et al. (2007) also reported non-significant results in an incremental rowing exercise to exhaustion, with active participants. These 3 studies found the same results regardless of the participants' level and background, and regardless the testing modality, reinforcing the conclusions that the MC hormonal fluctuations do not influence V̇e. Smekal et al. (2007) suggested that even though changes in body temperature and progesterone might affect V̇e, no significant differences have been seen between different phases of the menstrual cycle. This is consistent with what Janse de Jonge et al. (2012) reported, that the differences in temperature between different sub-phases might be too low to significantly impact physiological parameters.

In conclusion, minute ventilation does not seem to be influenced by the MC phases. However, due to the multiple possible explanations provided by different authors, more studies are required to understand how all these factors interact with the menstrual cycle and between them.

2.7 Perceptual measures

Whilst physiological factors are fundamental in understanding sport performance, perceptual measures are also important in doing so, as they can provide a measure of how the athlete feels during an exercise (Williams, 2017). The rating of perceived exertion (RPE) can be used as a simplified measure of physiological and performance indices, and to monitor exercise intensity and load (Halperin & Emanuel, 2019). There are clear advantages to using RPE, such as the easiness of use and the requirement for any material, however there are limitations that threaten the validity of RPE scales. Some of these limitations include different definitions, instructions and administration strategies (Halperin & Emanuel, 2019), and therefore the results should be taken with caution.

Due to the lack of published data available it is currently not clear whether the MC does or does not affect RPE. The current literature on the effects of MC phases on RPE also makes a limited attempt to explain the potential mechanisms behind the findings. It has been hypothesised by Abdollahpor et al. (2013) and Stephenson et al. (1982) that no differences in RPE would be found because the MC does not have effect on physiological or performances parameters, such as $\dot{V}O_2$, HR or the time to exhaustion. However, as it has been shown that RPE is influenced by circadian rhythmicity, it is plausible that RPE could be influenced by the MC rhythmicity (Florida-James, Wallymahmed, & Reilly, 1996; Vitale, La Torre, Baldassarre, Piacentini, & Bonato, 2017). As RPE might change during the day, comparing two testing sessions during different moments of the day might show a difference in RPE that is only due to the daily variations and not due to the menstrual cycle. Furthermore, progesterone and oestrogen are also affected by the circadian rhythm, and their concentrations fluctuate during the same day (Janse de Jonge, 2003; Vaiksaar et al., 2011). The complexity generated by having multiple biological rhythms potentially affecting RPE might justify why contrasting results have been found, and why

more data are needed to better understand the relationship between RPE, the menstrual cycle and circadian rhythms.

In the few papers that analysed RPE responses, Hackney et al. (1991), Sunderland & Nevill (2003), Dombovy et al. (1987) and Nicklas et al. (1989) reported no significant differences between mid-luteal and mid-follicular sub-phases. In contrast, significant differences were reported between the mid-follicular (6.2 ± 1.5) and mid-luteal (5.3 ± 1.4) sub-phases by Mattu et al. (2019), using a 0-10 Borg scale, during a 30 minute constant load exercise at maximal lactate steady-state power. Conversely, Pivarnik et al. (1992) reported a higher RPE during the mid-luteal sub-phase than during the mid-follicular, but only after 50 minutes of exercise. Pivarnik et al. (1992) concluded that the higher RPE was related to the inability to achieve thermal equilibrium when exercising during the luteal phase. In fact, during the luteal phase Pivarnik et al. (1992) reported an increase in rectal temperature, but not in sweat loss.

However, Mattu et al. (2019) reported opposite conclusions and explained the lower RPE during the luteal phase by a decreased perception in pain typical of the luteal phase, known as “luteal analgesia effect”. These conclusions from Mattu et al. (2019) link with hypothesis of a decrease in pain modulated by oestrogen, as reported by Hooper et al. (2011). However, as oestrogen peaks at the end of the follicular phase, but also in the middle of the luteal phase, it is not clear which phase would be influenced the most. In order to better understand the effects of oestrogen, pain modulation and RPE, the two sub-phases where the oestrogen is at its top (late-follicular and mid-luteal) should be analysed and compared with every other sub-phase.

Non-significant differences were also reported between the mid-luteal and early-follicular sub-phases by Beidleman et al. (1999), Abdollahpor et al. (2013), and De Souza et al. (1990). Moreover, Janse de Jonge et al. (2012) reported no differences between

1139 the early-follicular and mid-luteal sub-phases at different times of a 60-minutes exercise
1140 at 60% of $\dot{V}O_2$max.

1141 Significant differences were also found by Hooper et al. (2011) that reported a higher
1142 RPE during the early-follicular (13.46 ± 1.46) when compared with both the late-follicular
1143 (13.03 ± 1.32) and the luteal phase (12.62 ± 1.5). Hooper et al. (2011) reported that the
1144 higher RPE occurred when a higher pain score was reported, that might have influenced
1145 the perceived exertion. Other papers focused on unspecified sub-phases, but did not
1146 provide the values (Cristina-Souza et al., 2019; Miskec et al., 1997; Stephenson et al.,
1147 1982).

1148 Unfortunately, Dombovy et al. (1987), Nicklas et al. (1989), De Souza et al. (1990),
1149 Hackney et al. (1991), Beidleman et al. (1999) and Sunderland & Nevill (2003) did not
1150 provide any attempt to explain why they could or could not find differences in RPE
1151 between sub-phases, or were uncertain/unable to provide one. The literature is not clear
1152 and is not in agreement on how RPE is affected by the menstrual cycle phases, and
1153 different authors provided different explanations to justify their results. It appears that
1154 both a lower and a higher RPE during the luteal phase, compared with the follicular
1155 phase can be justified by a number of different mechanisms (i.e. pain, luteal analgesia
1156 effect, thermal equilibrium), but the exact mechanism behind the findings remains
1157 largely unknown. However, the lack of reported differences in RPE scores between the
1158 MC phases may be explained through its strong correlation with the previously
1159 mentioned physiological parameters and their lack of differences between MC phases
1160 (Abdollahpor et al., 2013; Janse de Jonge et al., 2012; Stephenson et al., 1982).

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2.8 Effects of hormones on sports performance

Interestingly, the majority of aforementioned studies (32 out of 43) were conducted using aerobic exercises, with very little focus on anaerobic exercise. Currently, only Giacomoni et al. (2000), Middleton & Wenger (2006) and Julian et al. (2017) analysed a performance shorter than 10 seconds, showing a lack of available data in short duration, high intensity exercise that can be associated with team sport performance (Bishop & Girard, 2013; Middleton & Wenger, 2006). These three papers all analysed multiple sprints, but with different protocols and different modalities, reducing the possibility of a direct comparison of results.

Giacomoni et al. (2000) analysed the differences between the mid-follicular and mid-luteal sub-phases when performing 4 cycling sprints of 8 seconds each, with 3 minutes recovery. Giacomoni et al. (2000) reported no differences in maximal cycling power, but no explanation was provided. Middleton & Wenger (2006) compared the mid-follicular and late-luteal sub-phases in active participants performing 10 sprints 6 seconds each, with 30 seconds recovery. Similar to Giacomoni et al. (2000), no differences in peak power were reported. However, the average work during the sprints was higher during the late-luteal sub-phase than the mid-follicular. Middleton & Wenger (2006) explained the higher work during the late-luteal sub-phase was due to higher PCr and ATP stores, caused by high oestrogen, which sustains the performance throughout subsequent sprints. Furthermore, the authors calculated that the difference in work between the two phases would translate in approximately 1 meter difference during a 6-seconds sprint, which could make the difference during a performance (Middleton & Wenger, 2006). More recently, Julian et al. (2017) recruited active participants to perform 3 sprints of 30 meters each, with 2 minutes recovery, and compared the results between the early-follicular and mid-luteal sub-phases. They reported non-significant differences in sprinting times at 5, 10 and 30 meters marks between both MC sub-phases. Julian et

al. (2017) suggested that a higher basal body temperature might improve sprinting performance, but also reported that this hypothesis has found contrasting results and opinions in previous literature. If this hypothesis were to be true, and considering that basal body temperature changes throughout the cycle, a higher sprinting performance would be expected during the mid-luteal sub-phase than the early-follicular because of its higher basal body temperature. However, as no differences were reported, the hypothesis does not seem to hold, and it could be because the changes in basal body temperature between the two sub-phases of the MC are too small to significantly affect sprinting performance.

In conclusion, it appears that the MC sub-phases do not influence repeated sprints performance as measured by peak power or time, but may influence the average work throughout the sprints. However, due to the lack of studies on this kind of performance, drawing conclusions would be premature.

2.9 Repeated Sprint Ability

After defining RSA as the ability to perform repeated sprints with a short, incomplete recovery between repetitions, Bishop et al. (2011) further described specific characteristics of RSA exercises. Sprints have to last less than 10 seconds each, where maximal workout can be nearly maintained until the end of the exercise, and the recovery has to be less than 60 seconds long (Bishop et al., 2011). In order to study and analyse an RSA performance, is important to determine physiological and metabolic factors affecting it.

2.9.1 Aerobic and anaerobic factors

The contributing factors to RSA have been studied by different authors that tried to identify aerobic and anaerobic parameters that might be important for RSA performance (Aguiar et al., 2016; Aziz, Chia, & Teh, 2000; Bishop & Spencer, 2004; Gharbi, Dardouri,

Haj-Sassi, Chamari, & Souissi, 2015; Jones et al., 2013; Pyne, Saunders, Montgomery, Hewitt, & Sheehan, 2008; Sanders et al., 2017; Thébault, Léger, & Passelergue, 2011). When trying to determine the importance of aerobic fitness for RSA, a number of studies were analysed and provided evidence showing a significant relationship between $\dot{V}O_2$max and parameters that reflect RSA performance such as total sprint time (Aziz et al., 2000; Jones et al., 2013; Sanders et al., 2017), average sprint time (Jones et al., 2013; Sanders et al., 2017), and fatigue indexes (Aguiar et al., 2016).

$\dot{V}O_2$max does not directly affect the single sprinting performance, but rather helps with the recovery between sprints, as has been clearly shown for $\dot{V}O_2$ kinetics (Buchheit, 2012; Dupont, McCall, Prieur, Millet, & Berthoin, 2010) allowing to maintain a higher performance throughout subsequent sprints (Aguiar et al., 2016; Aziz et al., 2000; Jones et al., 2013; Sanders et al., 2017). The advantages of a better recovery seem to be related to the ability to restore PCr and ATP, and tolerate, remove and buffer hydrogen ions from the active muscles (Aguiar et al., 2016; Aziz et al., 2000; Jones et al., 2013; Sanders et al., 2017).

However, some contrasting results have been shown by other authors in regards of the importance of $\dot{V}O_2$max, reporting no significant relationship with total sprint time (Castagna et al., 2007; Pyne et al., 2008), power output (Bishop, Lawrence, & Spencer, 2003), power decrement (Bishop et al., 2003), total work (Bishop et al., 2003) or fatigue indexes (Castagna et al., 2007). The contrasting results by Pyne et al. (2008) could be explained by the fact that $\dot{V}O_2$max was estimated and not measured directly, thus leaving the possibility of a wrong estimation and therefore the likelihood of different results if a direct measurement was used instead. Bishop et al. (2003) and Castagna et al. (2007) explained the lack of significance between $\dot{V}O_2$max and RSA performance due to the short duration of the protocol and the recovery time, stating that $\dot{V}O_2$max might be more relevant with longer protocols or longer recoveries that could give more time

to the organism to recover better, as a short recovery might not be enough to influence PCr resynthesis.

Whilst the aerobic fitness contribution seems to be important, anaerobic parameters and their relationship with RSA have also been analysed (Aguiar et al., 2016; Pyne et al., 2008). As a RSA performance indicator, total sprint time has been studied and has been found to significantly correlate with anaerobic parameters such as the best sprint time (Aguiar et al., 2016; Dupont et al., 2010), speed and acceleration (Pyne et al., 2008).

Aguiar et al. (2016) and Pyne et al. (2008) concluded that the strong correlation between the best time, and therefore maximal velocity, and the RSA performance suggests that one of the fundamental mechanisms responsible for RSA is the maximum ATP production rate. Dupont et al. (2010), Aguiar et al. (2016) and Pyne et al. (2008) suggested that, even though aerobic fitness might play a role for RSA performance, it is the anaerobic components that influences the ability to repeat sprints the most.

Not every author agrees with the magnitude of the importance of aerobic or anaerobic parameters for RSA, showing contradictory results (Aguiar et al., 2016; Gharbi et al., 2015; Sanders et al., 2017). These discrepancies could be due to different types of RSA tests used (Sanders et al., 2017; Thébault et al., 2011), or the test used to assess aerobic and anaerobic capacity (Gharbi et al., 2015; Thébault et al., 2011), that might have influenced the outcome of a study. Nonetheless, based on the current literature it appears that RSA performance is influenced by both aerobic and anaerobic fitness parameters. Specifically, aerobic fitness seems to be more important to maintain the performance throughout multiple sprints (Aguiar et al., 2016; Aziz et al., 2000; Jones et al., 2013; Sanders et al., 2017), when the athlete is subjected to higher levels of fatigue, and its importance appears to gradually increase with the number of repeated sprints. Anaerobic parameters, instead, seems to be important to perform faster sprints, and

therefore achieving better results (total time, best sprint time) (Aguiar et al., 2016; Dupont et al., 2010; Pyne et al., 2008).

From the current literature it appears that the hormonal fluctuations during the MC do not affect $\dot{V}O_2$max and/or speed and acceleration. This suggests that the influence from both the aerobic and anaerobic parameters taken into consideration might not change between different sub-phases of the MC, and therefore RSA performance may not be influenced by the different phases.

2.9.2 Energy metabolism

As both aerobic and anaerobic factors related to RSA performance are dependent on energy availability, it is important to understand the energy metabolism during a single high-intensity sprint, but also changes in energy contribution as sprints are repeated (Aguiar et al., 2016; A. Aziz et al., 2000; Gharbi et al., 2015).

2.9.2.1 Single sprint

PCr availability, anaerobic glycolysis and oxidative metabolism all have a role in providing the energy necessary to perform a maximal sprint, but they contribute in different proportions and their contribution changes throughout the sprint (Baker, McCormick, & Robergs, 2010). At the beginning of an intense short exercise, all the energy mechanisms are activated (Hargreaves & Spriet, 2020). However, PCr and anaerobic glycolysis can provide ATP at a faster rate in comparison to aerobic pathways (Hargreaves & Spriet, 2020). In fact, PCr availability and anaerobic glycolysis seem to be the two most important factors in a single maximal 6-s sprint, contributing ~45% and ~40% of the total energy, respectively (Bishop, 2012; Dawson et al., 2007). A much smaller contribution during a single short sprint is provided by the oxidative metabolism, which supplies 10% of the total energy (Bishop, 2012). The relative energetic contribution during a maximal sprint also depends on the duration of it (Hargreaves & Spriet, 2020; Spencer, Bishop,

Dawson, & Goodman, 2005). Longer sprints will result in a higher PCr depletion, as shown by Spencer et al. (2005) that reported 40-70% depletion of PCr stores during a 10-12.5 seconds sprint, and 35-55% depletion during a 6 seconds sprint.

2.9.2.2 RSA

Energy contribution changes do not only occur during a single short sprint, but also with repeated consecutive sprints. The contribution of different energy systems during a RSA exercise appears to be largely influenced by sprint intensity, sprint duration, number of sprints and recovery duration between sprints (Spencer et al., 2005). When the sprints are repeated with a short recovery time that does not allow the replenishment of PCr and ATP, it consequently changes the contribution of the energetic systems. It appears that the contribution from glycolysis diminishes much more than the PCr, which becomes predominant. Although the PCr absolute contribution decreases, when compared with the glycolysis contribution, PCr raises to 80% of the total anaerobic ATP production (Gaitanos, Williams, Boobis, & Brooks, 1993). This shows that the reliance on PCr to produce ATP, compared to the glycolysis, increases with the number of subsequent sprints. Similar conclusions have been drawn by Bishop (2012), who demonstrated a link between PCr availability and RSA performance (Bishop, 2012).

Furthermore, Gaitanos et al. (1993) suggested that with repeated 6-s maximal sprints cycling sprints the contribution of PCr and oxidative metabolism increases during the latter sprints, whilst the anaerobic glycogenolysis becomes increasingly inhibited. In fact, the contribution from the aerobic metabolism may increase to as much as 40% of the total energy supply during the final sprints of repeated sprints protocol (Bishop, 2012).

Even though it has been demonstrated that oestrogen and progesterone can affect energy metabolism, most of the studies were not able to report these differences, and therefore it is plausible to think that there will be no differences between phases during

a RSA exercise. However, as running RSA in relation to the MC has never been tested before, this is only an assumption based on previous studies on other tests and performances.

2.9.3 Fatigue

Fatigue during RSA exercises is shown as a decrease in sprint speed, both maximal and mean, and/or a decrease in peak power or total work over sprint repetitions (Girard, Mendez-Villanueva, & Bishop, 2011; Sanders et al., 2017). A typical performance decrement has been reported by Aziz et al. (2000), which reported a decrease in performance (sprinting time) of 5.4% that confirms a previous report by Wadley & Le Rossignol (1998) (5.5%). The fatigue develops after the first sprint, and it is caused by different factors such as sprinting duration and recovery time, that contribute to its timing and magnitude (Girard et al., 2011). Another aspect affecting fatigue is the limitation in energy supply. Since during a RSA performance the recovery is incomplete, the PCr cannot be completely restored before the following sprint, resulting in a lower availability of PCr to restore the ATP for subsequent sprints, resulting in a lower performance (Dawson et al., 2007; Gaitanos et al., 1993). Furthermore, hydrogen ion accumulation occurring during RSA exercises may affect the sprinting performance because of the inhibition on glycogen phosphorylase activity, causing a lower ATP production from glycogenolysis (Girard et al., 2011; Spriet, Lindinger, McKelvie, Heigenhauser, & Jones, 1989). Moreover, another important factor is the neural drive, as the inability to fully activate the musculature can reduce the force production, and therefore reduce the RSA performance (Girard et al., 2011). This mechanism has been shown especially when the fatigue level is high (Fatigue Index or Sprint Decrement Score higher than 10%) (Girard et al., 2011).

The rate at which fatigue develops seems to be mostly related to aerobic fitness, and especially to $\dot{V}O_2max$ (Aguiar et al., 2016; Gharbi et al., 2015), as a higher $\dot{V}O_2max$ has

been correlated with the ability to clear hydrogen ions, delaying the fatigue and allowing a better performance through subsequent sprints (Aguiar et al., 2016; Aziz et al., 2000; Jones et al., 2013; Sanders et al., 2017). Girard et al. (2011) and Spriet et al. (1989) explained that the mechanism by which hydrogen ions accumulate during RSA may affect sprinting performance through the inhibition of ATP derived from glycolysis, and adverse effects on the contractile apparatus. Bishop et al. (2003) also analysed the role of $\dot{V}O_2$max in RSA performance, and were not able to report any significant relationship between $\dot{V}O_2$max and fatigue. However, they reported a significant correlation between power decrement and muscle pH, reporting that hydrogen ion accumulation may impair RSA performance via inhibition of glycolysis, or negative effects with the contractile process (Bishop et al., 2003). However, even though Aguiar et al. (2016), Gharbi et al. (2015) and Bishop et al. (2003) reported that aerobic fitness is the major factor to attenuate fatigue, Castagna et al. (2007) could not report the same conclusions. The lack of relationship between fatigue and $\dot{V}O_2$max was justified by short recovery time, which did not give enough time to the aerobic system to be relevant and influence recovery (Castagna et al., 2007).

Other aspects have been studied and correlated with performance decrement during RSA tests. For example, a higher performance in the initial sprint brings a higher decrement in performance of the consecutive bouts, and this happens because a greater initial sprint will have a greater effect in muscle metabolites (Bishop et al., 2003; Girard et al., 2011; Hamilton, Nevill, Brooks, & Williams, 1991). Furthermore, as mentioned earlier, one of the effects of hydrogen ion accumulation is the inhibition of glycolysis, and therefore an important aspect to consider about fatigue is the limitation in energy supply. Since during a RSA performance the recovery is incomplete, PCr cannot be completely restored before the following sprint, resulting in a lower availability of PCr to restore the ATP for subsequent sprints, causing a reduction in performance (Dawson et al., 2007; Gaitanos et al., 1993). As the parameters that can affect fatigue, such as

$\dot{V}O_2$max, might not be affected by the MC, it appears that fatigue might also not be affected by the MC and the hormonal fluctuations between sub-phases.

2.9.4 Fatigue indexes

Fatigue can be quantified by the use of a specific index called the Fatigue Index (FI), which is calculated using the best and the worst sprinting times during the RSA exercise (equation 1) (Girard et al., 2011).

$$FI = 100\ x\ \left(\frac{S_{best} - S_{worst}}{S_{best}}\right) \text{ (Eq. 1)}$$

When looking at equation 1, S is used to indicate the sprint performance, and can be used for speed, work or power based on the parameter and the type of performance you are considering. However, this method is not considered as reliable and valid as the percentage decrement score (S_{dec}) (Glaister, Howatson, Pattison, & McInnes, 2008). This second method takes into consideration all the sprints, instead of using only the best and worst performance (equation 2). It is considered better because it is not dependent on only two sprints, but on all of them, and therefore it is more reliable and accurate (Glaister et al., 2008).

$$S_{dec} = \left\{\frac{(S_1 + S_2 + S_3 + \cdots + S_{final}}{S_{best}\ x\ Number\ of\ sprints} - 1\right\}\ x\ 100 \text{ (Eq. 2)}$$

It is important to consider that fatigue cannot be the only index taken into consideration when analysing RSA performance, as an increase in the best sprinting performance will bring a higher fatigue index, but will also increase the mean/peak performance parameters. For this reason, other aspects such as the energy metabolism, aerobic and anaerobic factors, or the perceptual responses should be considered.

2.9.5 Perceptual responses during RSA

As fatigue is an important factor during RSA, and considering that RPE has been linked to fatigue (Guo, Sun, & Zhang, 2017; Madueno, Guy, Dalbo, & Scanlan, 2018), it is plausible to expect RPE to increase as fatigue increases in subsequent bouts. Furthermore, it has also been shown that RPE increases following an increase of heart rate (Coutts, Rampinini, Marcora, Castagna, & Impellizzeri, 2009), further showing that RPE is expected to increase between consecutive sprints during a RSA exercise, and therefore when the recovery is incomplete. This hypothesis has been confirmed by Laurent et al. (2010), Buck et al. (2015) and Halperin et al. (2018), as no differences between MC phases have been found in the parameters that can influence RPE. Therefore, it might be possible that no differences exist in RPE between the MC phases.

2.9.6 Effects of hormones on RSA

Based on the definition of repeated sprint ability, only Middleton & Wenger (2006) analysed the effects of the MC phases on RSA performance in female participants. The authors reported a higher average work over the series of repeated sprints during the late-luteal sub-phase when compared with the mid-follicular, and concluded that higher PCr and ATP stores during the luteal phase would help sustain the work levels over the last sprints (Gaitanos et al., 1993; Middleton & Wenger, 2006). The higher PCr and ATP availability during the luteal phase might also be explained by the higher $\dot{V}O_2$ during the recovery between sprints that was found in the luteal phase, allowing the participants to replenish more PCr and ATP (Aguiar et al., 2016; Aziz et al., 2000; Jones et al., 2013; Sanders et al., 2017). Furthermore, in support to the higher usage of PCr and ATP, a lower RER was found during the luteal phase, showing that less glycogen was used, in favour of a higher usage of PCr and ATP (Middleton & Wenger, 2006). Due to the higher work and recovery $\dot{V}O_2$, and therefore the enhanced ability to maintain a higher performance, a lower drop off in power during the same phase was also expected

(Middleton & Wenger, 2006). However, no significant differences were reported between the two phases, but no explanation was provided by Middleton & Wenger (2006). The author concluded that the difference in work could be translated in approximately 1m difference in a 6-seconds sprint, which could be a huge difference in a sporting action (reaching the ball, catching your opponent, etc.) (Middleton & Wenger, 2006).

To conclude, there is a lack of studies directly measuring the effects of MC on RSA performance. To date, only Middleton & Wenger (2006) reported significant physiological differences between the MC sub-phases in two variables ($\dot{V}O_2$ and RER). Therefore, due to the multi-factorial aspect of RSA performance, and lack of data, it is currently not possible to draw clear conclusions about the effects of the MC on RSA performance.

2.10 Hypotheses

It was hypothesised that the MC sub-phases will affect physiological and perceptual parameters, but not the performance measures. Specifically, it is hypothesised that heart rate, minute ventilation and RPE will be lower during the mid-follicular sub-phase. In contrast, lactate and RER values will be lower during the mid-luteal sub-phase compared to the mid-follicular sub-phase, with no changes in $\dot{V}O_2$, power output, acceleration, or distance between the sub-phases.

3. Methods

3.1 Participants

Eight physically active females initially agreed to take part in this study, however, only six participants were included in the analysis. Two participants were excluded as they started to use hormonal and contraceptive medications, respectively during testing. Out of the six participants included in the analysis, only four of them completed all the testing sessions. One participant missed the second testing session during the mid-luteal sub-phase due to personal problems and could not come again before the mid-luteal sub-phase ended (i.e., 86% compliance with the study). Another participant missed both sessions during the early-follicular sub-phase because she started using hormonal contraceptives due to medical issues (i.e., 71% compliance with the study). However, the medical issues did not interfere with the results as they did not affect her hormones concentrations and production before starting to use the contraceptive, and therefore the participant was included in the study. These two participants were still included in the study because their data could be analysed with the correct statistical approach in case of missing data. The sample size was determined to be 20 by a power calculation (G*Power 3.1), but the number was not reached due to difficulties in recruiting (e.g. strict inclusion criteria, participants drop out) and due to the limited time available to complete the project.

From the population assessed four participants were team sport players (one badminton, one ice hockey, one football and one rugby union), whilst the fifth participant performed ballet and yoga, and the sixth participant did resistance training and running. Participants' baseline parameters and anthropometric measures are presented in Table 7. The participation in this study was subject to specific inclusion criteria. All participants were training at least three times per week for a full year (any form of training). Participants had to be nonusers of any hormonal contraceptives for at

least four months, they had to have regular (24 to 35 days) eumenorrheic MCs for no less than one year, and menstruating for at least three years at the time of the study (Giacomoni et al., 2000; Middleton & Wenger, 2006; Tsampoukos et al., 2010). Participants had to be healthy, non-smokers, and not currently under medication or treatments that could influence hormones or performance (Stefanovsky et al., 2016). The participants were asked to abstain from caffeine, alcohol, and heavy exercises during the 24h before each of the sessions, and were instructed to keep their normal dietary habits (Casazza, Suh, Miller, Navazio, & Brooks, 2002). Participants were also instructed to drink 500ml of water 1h prior to each session, to ensure hydration (Meckel, Gottlieb, & Eliakim, 2009).

Participants were recruited using posters placed around Edinburgh Napier University, online posts on social media (Facebook, Twitter, and LinkedIn) or other websites (Reddit) and directly contacting female teams in Edinburgh. All participants were asked to complete the American College of Sports Medicine (ACSM) Exercise Preparticipation Health Screening Questionnaire for Exercise Professionals and provide written consent to verify eligibility for the study (Riebe et al., 2015). If the ACSM questionnaire highlighted any contraindications to exercising, the participant was excluded from the study and informed to contact their GP to get clearance to participate, if they wanted to. The research and procedures were approved by the University Ethics Committee of Edinburgh Napier University and in regulation with the Declaration of Helsinki for Medical Research involving human participants.

Table 7: baseline participant characteristics collected at the first visit.

Parameter	Participants (n=6)
Age (years)	25.67 ± 2.49
Height (m)	1.66 ± 0.08
Body Mass (kg)	69.8 ± 19.3
BMI ($kg \cdot m^{-2}$)	25 ± 6
Waist circumference (cm)	78.12 ± 12.55
Hips circumference (cm)	101.42 ± 14.28
Waist-to-hip ratio	0.77 ± 0.02
Fat mass (%)	26.72 ± 11.25
$\dot{V}O_2peak$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	46 ± 6.76

All data are presented as mean ± standard deviation (SD).

3.2 First visit

3.2.1 Documentation

On the arrival to the Sports and Exercise Science laboratory, the participants had to read and complete the Low Energy Availability in Females Questionnaire (LEAF-Q) and ACSM questionnaires to verify their eligibility in the study. If they were deemed eligible, they were then asked to sign the consent form to participant in the project, allowing data collection.

The ACSM questionnaire was used as a pre-participation health screening questionnaire, to identify individuals who may be at risk for exercise practice (Riebe et al., 2015). The LEAF-Q is a questionnaire that has been used before to identify female athletes at risk for the female athlete triad, and can collect MC related information such as the date of your last period, the regularity of your period and the average bleeding length (Melin et al., 2014). The LEAF-Q was used to determine the eligibility of the participant in the study.

3.2.2 Baseline measures and anthropometric measures

Once the questionnaires were completed, blood pressure was measured using a Homron Professional Blood Pressure Monitor HEM-907-E7 (Omron, Japan). Participants were placed in the supine position, with the cuff placed on the participant's left arm, with the

blood pressure being measured after five minutes of seated rest (Raffalt, Hovgaard-Hansen, & Jensen, 2013). If the blood pressure was lower than 140/90 mmHg (Mancia et al., 2013), participants and researcher signed the consent form to engage in physical activity. If the reported blood pressure was higher than 140/90 mmHg (Mancia et al., 2013), participants were excluded from the study and informed to contact their GP.

A first measurement of blood lactate at rest was done using the Lactate Pro 2 LT-1730 (Arkray Factory Inc., Kyoto, Japan). Blood was collected by puncturing the fingertip of the participants with a lancing device. The first drop of blood was wiped, with the second drop being collected using a lactate strip that absorbed 0.3µL of blood. The strip was then inserted in the lactate analyser to provide a blood lactate value after 15 seconds.

Following the blood lactate measurement, the participant's height was measured to the nearest 1 cm using a stadiometer (Harpenden Portable, Holtain Limited, Dyfed, UK), whilst body mass was measured to the nearest 0.1 kg using a mechanical column scale (SECA 711, Hamburg, Germany). During the collection of anthropometric data, participants were asked to dress as light as possible, and to remove their shoes to measure their body mass. Furthermore, participants were asked if they needed to void their bladder prior to the beginning of the measurements, to ensure replicability, but whether they voided their bladder or not was not confirmed.

Waist and hip circumferences were also measured using a body tape (SECA, Hamburg, Germany), and a Bioelectrical Impedance Analysis (BIA) (Quadscan 4000, Bodystat, UK) was used to measure body composition. Waist circumference was measured at the narrowest point of the waist, whilst the hip measurement was made at the largest part of the buttocks, and both the measurements were made keeping the tape horizontal to the ground (Wang et al., 2003; WHO, 2008). The measurement points were assessed visually by the same researcher.

To use the BIA, the participants were required to lie on a bed. Two adhesive electrodes were placed upon the right wrist and right ankle, following the manufacturer's instructions. The device requested to insert information about body mass, height, age, circumferences, and activity levels, which we collected previously, and after the analysis it provided data about body composition such as lean mass, fat mass and hydration. After the collection of the participants' anthropometric measures, participants were required to perform a $\dot{V}O_2$peak test, as indicated in Figure 3.

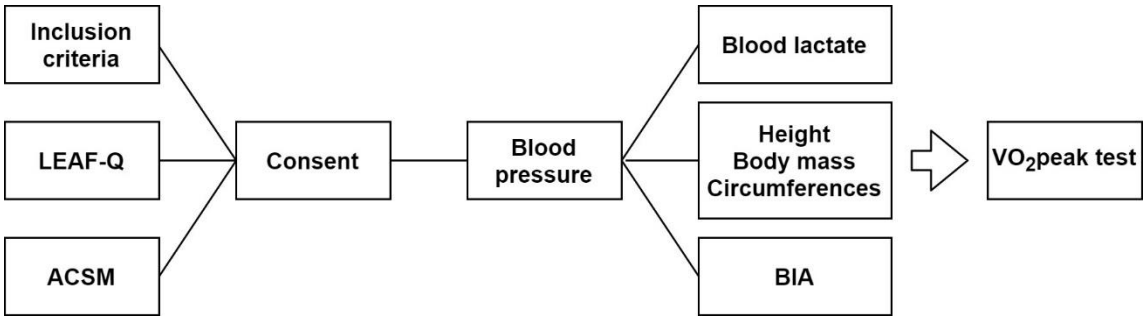

Figure 3: overview of the steps taken during the first visit, leading to the $\dot{V}O_2$peak test.

3.2.3 Peak oxygen uptake test

Participants' $\dot{V}O_2$peak was determined using an incremental test to exhaustion on a motorised treadmill (Ergo ELG-55, Woodway, Germany), performed to volitional exhaustion. Following a five minute standardised warm-up, running at a constant speed of 9.6 km/h at 0% gradient, the speed was increased to 11.2 km/h were the set speed remained, and every two minutes the gradient was increased by 2.5% (Figure 4) (Kavaliauskas, Aspe, & Babraj, 2015; Taylor, Buskirk, & Henschel, 1955).

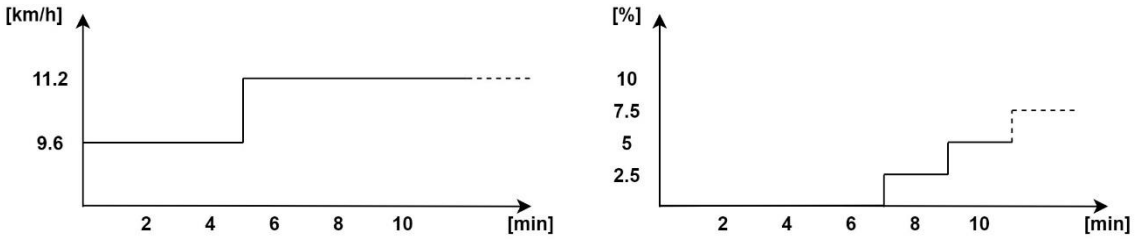

Figure 4: $\dot{V}O_2$peak protocol. On the left, the figure shows the speed in relation to the time. On the right, the figure shows the gradient in relation to the time.

During the test, exchanged air was analysed using a breath-by-breath gas analyser (Metalyzer 3B, Cortex, Leipzig, Germany). Expired gases were collected through a mask that participants had to wear, that allowed the Cortex to analyse the airflow through the mask. The Cortex was prepared following the manufacturer's instructions, performing the calibration approximately 30 minutes before every use. The calibration included three steps: the gas calibration of O_2 and CO_2 analyser, the calibration of the volume transducer and the calibration of the pressure analysed. Heart Rate (HR) was monitored using an H7 Bluetooth Polar HR monitor (Polar, Kempele, Finland), which was linked with the Cortex Metalyzer.

Immediately after the test, the Rating of Perceived Exertion (RPE) data were collected using the 6-20 Borg scale, by verbally asking the participant to give a number between 6 and 20, where 6 corresponded to the lowest exertion (rest) and 20 being the highest exertion possible (Borg, 1982). The first of three post-exercise measures of blood lactate were collected immediately after finishing the peak oxygen uptake test. The second blood lactate measure was done three minutes after the exercise, and the last one was done five minutes after the exercise completion, following the procedures explained earlier.

3.3 Menstrual cycle phases determination

The procedure to predict and determine the MC phases started immediately after the first visit. A combined approach adapted from Beatriz et al. (2019) using an electronic

diary (FitrWoman, https://www.fitrwoman.com/, Orreco Limited, Ireland) and a urinary kit (Clearblue Advanced Digital Ovulation Test, SPD Swiss Precision Diagnostics, Geneva, Switzerland) was chosen for this project, in order to increase the chances to detect ovulation (Mattu et al., 2019; Wideman et al., 2013). The participants started to use the electronic diary immediately after the first visit, and the urinary kit after the first menstruation following the third visit, as shown in Figure 5. Participants had to communicate with the researcher through an online survey (Novi Survey, USA) the first day of bleeding, the last day of bleeding, and the results from the urinary kit to detect ovulation. The data from the survey allowed for the determination of the MC phases, with the determination of the early-follicular sub-phase being two days from the beginning of bleeding until the seventh day (Janse de Jonge, 2003). Once ovulation was indicated, the mid-luteal sub-phase was classified as four days post ovulation to ten days post (Janse de Jonge, 2003; Köse, 2018; Pestana et al., 2017; Stefanovsky et al., 2016). The ovulation was considered to have occurred 24h after the LH surge was detected.

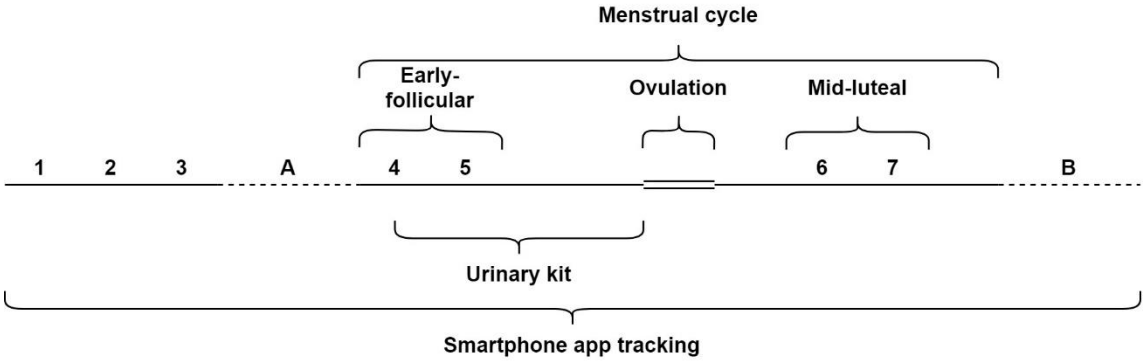

Figure 5: visual representation of the phases of the project. Each number corresponds to a session (1: first visit, 2-3: familiarisation sessions, 4-5: early-follicular sessions, 6-7: mid-luteal sessions). The letter A is the time spent waiting from the second familiarisation to the beginning of the first new MC. The letter B is the time used to gather the last MC data with the smartphone application. Below the main line, it is indicated when the urinary kit was used and when the smartphone app was used (from the beginning to the end of the project).

The main purpose of FitrWoman app was to collect data about the length of the MC, to help the identification of the menstrual phases, and to collect symptoms during the

cycle. This application was previously reviewed by Brown (2018) and was found to be interactive and easy to use.

To determine the LH surge, the Clearblue Advanced Digital Ovulation Test was used. This test determines when to indicate high fertility by looking for a marked increase in oestrogen concentration (Clearblue Technical Support, personal communication, 1st August 2019). In order to measure an increase, the test initially establishes a baseline concentration of the hormone before it becomes elevated at the start of the fertile period (Clearblue Technical Support, personal communication, 1st August 2019). The baseline concentration is set with the first test of a new cycle of use and then adjusted with every subsequent test until high fertility is detected and indicated (Clearblue Technical Support, personal communication, 1st August 2019). The holder will then continue to display High Fertility after each test until it detects the LH surge to 40 mIU/ml and will then display Peak Fertility (Clearblue Technical Support, personal communication, 1st August 2019). The holder can detect an LH surge as low as 22 mIU/ml depending on the hormone concentrations of previous results as it can adjust its sensitivity (Clearblue Technical Support, personal communication, 1st August 2019).

Following the manufacturer instructions, the test was done once per day after sleeping, using the first urine of the day. Based on the MC length information that was collected using a questionnaire and referring to a table provided by the manufacturer (Table 8), the starting day of the test was decided.

Table 8: starting day for the utilisation of the urinary kit

Your cycle length in days	≤21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	≥41
Start testing on the day next to your cycle length	5	6	6	6	7	7	7	8	9	10	11	12	13	14	15	16	17	18	19	20	20 days before next period

Information provided on the Clearblue Advanced Digital Ovulation Test leaflet

To take the test the participants had to place the absorbent tip pointing downwards to the urine stream for two seconds. Alternatively, the participant was able to collect a sample of urine in a clean and dry container and dip the absorbent tip in the urine for 15 seconds. At this point, after waiting for five minutes, the results would appear on the screen, showing one of the three possible results (Figure 6). If a circle was shown, it indicated low fertility. If a flashing smiley face was shown, the test detected a rise in oestrogen concentrations. In this case, the participant had to continue to test daily to find the LH surge. In this phase, testing more than once per day was allowed. If a static smiley face was shown, the test detected the LH surge.

Figure 6: visual results from the Clearblue Advanced Digital Ovulation Test.

3.4 Repeated Sprint Ability intervention (4th - 7th visits)

After the first visit, all the participants were required to complete two familiarisation trials before doing the four intervention sessions. All the sessions were successfully completed by every participant, and were conducted in the Sport and Exercise Science laboratory in a controlled environment (temperature: 20.92 ± 0.97 °C, humidity: 32.79 ± 9.54 %). During the familiarisation and intervention sessions, participants performed the same protocol with at least 24h of rest between consecutive sessions. Physiological, perceptual and performance data were collected to check repeatability of measures, to know that changes between the interventions were not due to the learning effect.

3.4.1 Baseline measures and anthropometric measures

At the arrival at the laboratory, the participants were asked to lie down, and the blood pressure was measured, followed by a blood lactate measurement at rest. After this, anthropometric measures were collected (body mass, height, circumferences, BIA) following the procedures explained in section 3.2.1.

3.4.2 RSA protocol

After these measurements, the participants performed a standardised warm up of five minutes running at a self-selected speed on a non-motorised Force treadmill (Force 3.0, Woodway, Germany), before performing the RSA intervention. The RSA protocol consisted of five 'all-out' sprints of six seconds from a standing start on the non-motorised treadmill with 24 seconds of active recovery (walking) between the sprints (McGawley & Bishop, 2006). The participants were verbally encouraged throughout the exercise, and they were given verbal instructions by the researcher about what to do in each phase.

During the test, a breath-by-breath gas analyser (Metalyzer 3B, Cortex, Leipzig, Germany) was used to measure exchanged air continuously, and the heart rate was continuously monitored using an H7 Bluetooth Polar HR monitor (Polar, Kempele, Finland), following the same procedures utilised during the peak oxygen uptake test, and using the same instruments. For every parameter, the mean value during each sprint without including rest periods was used for analysis. Moreover, during the recovery time between the sprints the Rating of Perceived Exertion (RPE) data were collected using the 6-20 Borg scale, by asking the participant to verbally give a number between six and 20 (Borg, 1982).

The peak values of the physiological parameters during each sprint were not analysed due to synchronisation issues: as the breath-by-breath analyser registered data during

each breath and the breaths were not synchronised with the sprinting times, the data collected could not be perfectly synchronised with the sprints. For this reason, the data in each sprint have been manually selected trying to be as accurate as possible (i.e. selecting only the data collected during the sprint, as close as possible). However, as the peak values were found to be at the end of the sprint and therefore near to where we selected the data manually, a manual mistake could have affected the results. Averaging the data during the selected interval, instead, allowed us to avoid this problem.

Performance data were collected by the treadmill that provided power, acceleration and distance every 0.005 seconds. Mean values for power output during each sprint, peak values for power output and acceleration during each sprint, and distance were used for analysis. The fatigue index was calculated for mean and peak power output and peak acceleration, using the formula presented in section 2.9.4 (Sdec).

After the intervention, three additional measures of blood lactate were done following the same procedures and using the same materials explained previously. The first measure was done immediately after the exercise, the second one after three minutes and the last one after five minutes after the exercise completion (Figure 7).

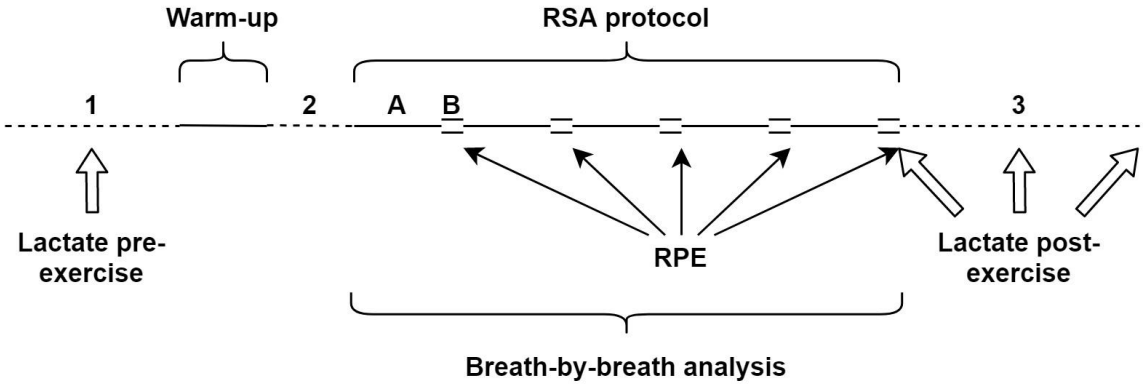

Figure 7: RSA protocol, starting from the first measure of pre-exercise lactate. **1**: pre-exercise lactate measurement; **2**: rest between the warm-up and the RSA protocol; **3**: post-exercise lactate measurement; **A**: 24 seconds walking; **B**: 6 seconds 'all-out' sprint

3.5 Statistical analysis

Normal distribution was assessed using the Shapiro-Wilk test. To analyse the differences between the familiarisation, the early-follicular and the mid-luteal sub-phases, a mixed-effects model (restricted maximum likelihood (REML)) was used for performance parameters (distance, peak acceleration, mean power output, peak power output), physiological parameters (post-exercise lactate, heart rate, $\dot{V}O_2$, RER, Ve) and perceptual parameters (RPE). A one-way ANOVA was used to analyse body composition parameters (BMI, fat mass, hips-to-waist ratio), fatigue indexes and pre-exercise lactate because these parameters were collected only once, and not after every sprint. A Geisser-Greenhouse correction for violation of sphericity was used. Non-normally distributed data (fatigue index for mean power output) were analysed with a Friedman test. If significance was found between the familiarisation, the early-follicular and the mid-luteal sub-phase, a Tukey's multiple comparison test was used to further analyse the data and see which phases were significantly different.

To analyse the differences between two sessions of the same phase, a two-way ANOVA with repeated measures, or a mixed-effects model (REML) in case of missing data, were used for performance parameters (distance, peak acceleration, mean power output, peak power output), physiological parameters (post-exercise lactate, heart rate, $\dot{V}O_2$, RER, Ve) and perceptual parameters (RPE). A Geisser-Greenhouse correction for violation of sphericity was used. A Student's paired t-test was used to analyse the differences in body composition parameters (BMI, fat mass, hips-to-waist ratio), fatigue indexes and pre-exercise lactate between two sessions of the same phase. Non-normally distributed data ($\dot{V}O_2$, mean power output, HR, $\dot{V}e$, fatigue index for mean power output) were analysed with a Wilcoxon matched-pairs signed-rank test.

All statistical analyses were carried out using GraphPad Prism (version 8.4.0) for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com) with

statistical significance being set at $p \leq 0.05$. Effect sizes were reported using Cohen's d, described as trivial (0.00 – 0.19), small (0.20 – 0.59), moderate (0.60 – 1.19), large (1.20 – 1.99), and very large (2.0 – 4.0) (Hopkins, Marshall, Batterham, & Hanin, 2009). Furthermore, the difference between means was reported as a measure of effect size when a comparison between two groups was done. All data were reported as group means ± standard deviation.

4. Results

4.1 Participant's characteristics

Participants' BMI did not significantly differ between the familiarisation (23 ± 5 $kg \cdot m^{-2}$), EF (23 ± 5 $kg \cdot m^{-2}$) and ML (24 ± 5 $kg \cdot m^{-2}$) (p = 0.29) (Table 9). Furthermore, no significant differences were found between EF and ML (p = 0.35, d = 0.2, difference between means = -0.4 $kg \cdot m^{-2}$ (1.71 %)) (Table 9). No significant differences were also found when comparing BMI measures between the two sessions of F (25 ± 6 $kg \cdot m^{-2}$ vs 25 ± 6 $kg \cdot m^{-2}$) (p = 0.18, d = 0.65, difference between means = 0.3 $kg \cdot m^{-2}$ (-1.19 %)), EF (23 ± 5 $kg \cdot m^{-2}$ vs 24 ± 5 $kg \cdot m^{-2}$) (p = 0.37, d = 0.45, difference between means = -0.2 $kg \cdot m^{-2}$ (0.85 %)) or ML (26 ± 6 $kg \cdot m^{-2}$ vs 26 ± 6 $kg \cdot m^{-2}$) (p = 0.18, d = 0.73, difference between means = 0.4 $kg \cdot m^{-2}$ (-1.53 %)).

No significant differences were found in fat mass between the familiarisation (23.71 ± 10.74 %), EF (24.47 ± 8.94 %) and ML (23.93 ± 10.53 %) (p = 0.92) (Table 9). A comparison between EF and ML showed non-significant differences (p = 0.93, d = 0.23, difference between means = 0.54 % (-2.21 %)) (Table 9). Moreover, fat mass did not significantly differ between the two sessions of the familiarisation (27.43 ± 15.52 % vs 27.50 ± 15.72 %) (p = 0.94, d = 0.00 difference between means = -0.07 % (0.26 %)), EF (24.98 ± 8.94 % vs 23.96 ± 9.07 %) (p = 0.36, d = 0.11, difference between means = 1.02 % (-4.08 %)), or ML (28.63 ± 14.41 % vs 28.20 ± 13.59 %) (p = 0.76, d = 0.03, difference between means = 0.43 % (-1.50 %)).

Hips-to-waist ratio among participants did not significantly differ between the familiarisation (0.76 ± 0.03), EF (0.76 ± 0.03) and ML (0.77 ± 0.03) (p = 0.98) (Table 9). There were no significant differences between EF and ML (p = 0.98, d = 0.33, difference between means = -0.01 (1.32 %)) (Table 9). No significant differences were found in hips-to-waist ratio between the two sessions of the familiarisation (0.76 ± 0.02 vs 0.76 ± 0.03)

(p = 0.80, d = 0.00), EF (0.76 ± 0.03 vs 0.76 ± 0.03) (p > 0.99, d = 0.11), or ML (0.76 ± 0.03 vs 0.77 ± 0.03) (p = 0.48, d = 0.33, difference between means = -0.01 (1.32 %)).

Table 9: comparison of body composition parameter during the familiarisation, early-follicular and mid-luteal sub-phases.

Parameter	Familiarisation	Early-follicular	Mid-luteal
BMI	23 ± 5	23 ± 5	24 ± 5
Fat mass (%)	23.71 ± 10.74	24.47 ± 8.94	23.93 ± 10.53
Hips-to-waist ratio	0.76 ± 0.03	0.76 ± 0.03	0.77 ± 0.03

4.2 HR

HR was not significantly different between the familiarisation (159.04 ± 21.52 bpm), EF (159.08 ± 20.36 bpm) and ML (155.55 ± 20.43 bpm) (p = 0.40) (Figure 8). No significant differences were found between EF and ML (p = 0.49, d = 0.17, difference between means = 3.23 bpm (-2.22 %)) (Figure 8). Furthermore, HR was not found to differ significantly between the two sessions of the familiarisation (158.66 ± 22.05 bpm vs 156.93 ± 18.47 bpm) (p = 0.81, d = 0.09, difference between means = 1.73 bpm (-1.09 %)), EF (158.25 ± 20.13 bpm vs 159.92 ± 20.93 bpm) (p = 0.13, d = 0.08, difference between means = -1.67 bpm (1.06 %)) and ML (156.28 ± 19.56 bpm vs 162.28 ± 19.36 bpm) (p = 0.06, d = 0.31, difference between means = -6 bpm (3.84 %)).

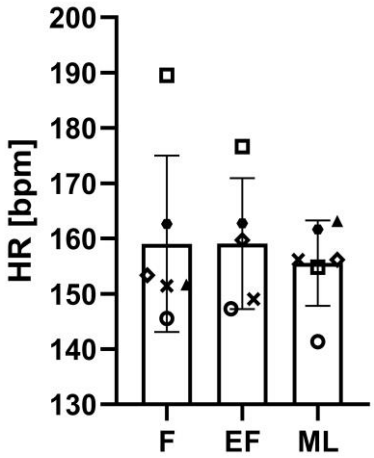

Figure 8: comparison of HR during the familiarisation (F), early-follicular (EF) and mid-luteal (ML) sub-phases of the MC (Mean ± SD). ○: participant 1; □: participant 2; ▲: participant 3; ◇: participant 4; ⬣: participant 5; ✕: participant 6.

4.3 Lactate

The differences in pre-exercise baseline lactate levels were not found to be significant between the familiarisation (1.30 ± 0.50 mmol·l^{-1}), EF (1.43 ± 0.46 mmol·l^{-1}) and ML (1.63 ± 0.51 mmol·l^{-1}) (p = 0.51). Moreover, pre-exercise lactate levels did not differ between EF and ML (p = 0.78, d = 0.41, difference between means = -0.20 mmol·l^{-1} (13.99 %)) (Figure 9). No significant differences were found in pre-exercise lactate between the two sessions of the familiarisation (1.26 ± 0.30 mmol·l^{-1} vs 1.38 ± 0.81 mmol·l^{-1}) (p = 0.59, d = 0.20, difference between means = -0.12 mmol·l^{-1} (9.52 %)), EF (1.20 ± 0.56 mmol·l^{-1} vs 1.62 ± 0.44 mmol·l^{-1}) (p = 0.17, d = 0.83, difference between means = -0.42 mmol·l^{-1} (35%)), or ML (1.60 ± 0.52 mmol·l^{-1} vs 1.58 ± 0.53 mmol·l^{-1}) (p = 0.46, d = 0.04, difference between means = 0.02 mmol·l^{-1} (-1.25 %)).

Post-exercise lactate levels were non-significant between the familiarisation (12.19 ± 2.80 mmol·l^{-1}), EF (12.66 ± 3.11 mmol·l^{-1}) and ML (12.01 ± 2.32 mmol·l^{-1}) (p = 0.31) (Figure 9). Furthermore, post-exercise lactate did not significantly between EF and ML (p = 0.58, d = 0.24, difference between means = 0.65 mmol·l^{-1} (-5.13 %)) (Figure 9). The differences

in post-exercise lactate were also not significant between the two sessions of the familiarisation (12.34 ± 3.55 mmol·l^{-1} vs 12.04 ± 2.61 mmol·l^{-1}) (p = 0.67, d = 0.09, difference between means = 0.3 mmol·l^{-1} (-2.43 %)), EF (12.32 ± 3.34 mmol·l^{-1} vs 12.85 ± 3.08 mmol·l^{-1}) (p = 0.17, d = 0.17, difference between means = -0.53 mmol·l^{-1} (4.30 %)), or ML (12.14 ± 2.56 mmol·l^{-1} vs 11.78 ± 2.45 mmol·l^{-1}) (p = 0.46, d = 0.14, difference between means = 0.36 mmol·l^{-1} (-2.97 %)).

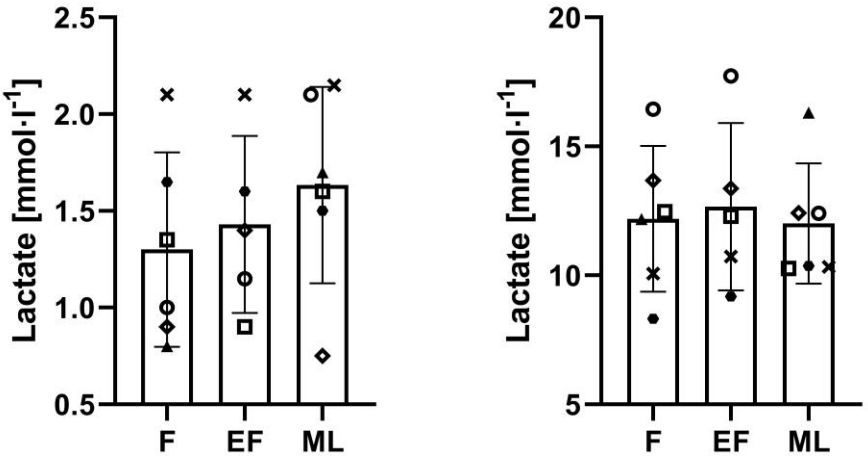

Figure 9: comparison of pre-exercise lactate (left) and post-exercise lactate (right) during the familiarisation (F), early-follicular (EF) and mid-luteal (ML) sub-phases of the MC (Mean ± SD). ○: participant 1; □: participant 2; ▲: participant 3; ◇: participant 4; ⬣: participant 5; ✕: participant 6.

4.4 $\dot{V}O_2$

No significant differences were found in $\dot{V}O_2$ between the familiarisation (34.10 ± 9.50 ml·kg^{-1}·min^{-1}), EF (35.65 ± 8.68 ml·kg^{-1}·min^{-1}) and ML (32.97 ± 8.97 ml·kg^{1}·min^{-1}) (p = 0.33) (Figure 10). A comparison between EF and ML showed non-significant differences (p = 0.10, d = 0.30, difference between means = 2.68 ml·kg^{-1}·min^{-1} (-7.52 %)) (Figure 10). Furthermore, $\dot{V}O_2$ was not found to differ between the two sessions of the familiarisation (32.13 ± 8.92 ml·kg^{-1}·min^{-1} vs 33.07 ± 11.05 ml·kg^{-1}·min^{-1}) (p = 0.06, d = 0.09, difference between means = -0.94 ml·kg^{-1}·min^{-1} (2.93 %)), EF (36.33 ± 8.29 ml·kg^{-1}·min^{-1} ± 34.97 ± 10.14 ml·kg^{-1}·min^{-1}) (p = 0.63, d = 0.15, difference between means =

1.36 ml·kg^{-1}·min^{-1} (-3.74 %)) and ML (33.00 ± 8.81 ml·kg^{-1}·min^{-1} vs 31.39 ± 8.80 ml·kg^{-1}·min^{-1}) (p = 0.06, d = 0.18, difference between means = 1.61 ml·kg^{-1}·min^{-1} (-4.88 %)).

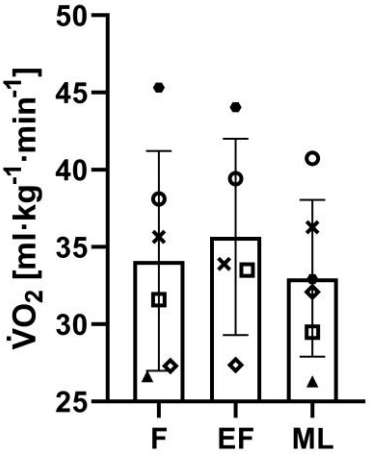

Figure 10: comparison of $\dot{V}O_2$ during the familiarisation (F), early-follicular (EF) and mid-luteal (ML) sub-phases of the MC (Mean ± SD). ○: participant 1; □: participant 2; ▲: participant 3; ◇: participant 4; ⬢: participant 5; ✕: participant 6.

4.5 RER

RER was not significantly different between the familiarisation (1.09 ± 0.16), EF (1.11 ± 0.15) and ML (1.09 ± 0.16) (p = 0.75) (Figure 11). A comparison between EF and ML showed non-significant differences (p = 0.47, d = 0.13, difference between means = 0.02 (- 1.80%)) (Figure 11). No significant differences were found in RER between the two sessions of the familiarisation (1.06 ± 0.19 vs 1.12 ± 0.18) (p = 0.13, d = 0.32, difference between means = -0.06 (5.66 %)), EF (1.11 ± 0.18 vs 1.10 ± 0.14) (p = 0.61, d = 0.06, difference between means = 0.01 (-0.9 %)) and ML (1.07 ± 0.16 vs 1.10 ± 0.17) (p = 0.21, d = 0.18, difference between means = -0.03 (2.80 %)).

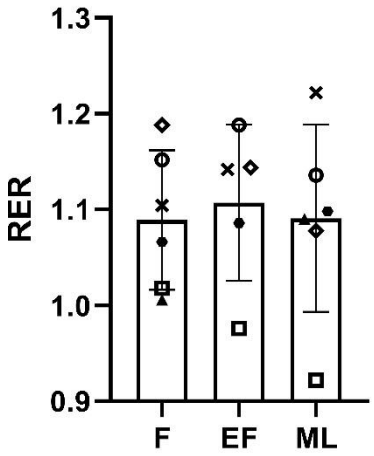

Figure 11: comparison of RER during the familiarisation (F), early-follicular (EF) and mid-luteal (ML) sub-phases of the MC (Mean ± SD). ○: participant 1; □: participant 2; ▲: participant 3; ◇: participant 4; ⬢: participant 5; ✕: participant 6.

4.6 V̇e

The differences in V̇e were not found to be significant between the familiarisation (84.97 ± 21.41 l·min^{-1}), EF (91.73 ± 19.82 l·min^{-1}) and ML (89.10 ± 25.36 l·min^{-1}) (p = 0.08) (Figure 12). Moreover, V̇e did not significantly differ between EF and ML (p = 0.42, d = 0.12, difference between means = 2.63 l·min^{-1} (-2.87 %)) (Figure 12). V̇e was not found to significantly differ between the two session of the familiarisation (85.49 ± 22.00 l·min^{-1} vs 84.45 ± 24.25 l·min^{-1}) (p = 0.63, d = 0.04, difference between means = 1.04 l·min^{-1} (-1.22 %)), EF (94.14 ± 21.09 l·min^{-1} vs 89.32 ± 19.54 l·min^{-1}) (p = 0.13, d = 0.24, difference between means = 4.82 l·min^{-1} (-5.12 %)) and ML (86.90 ± 25.43 l·min^{-1} vs 89.39 ± 27.60 l·min^{-1}) (p = 0.06, d = 0.09, difference between means = -2.49 l·min^{-1} (2.87 %)).

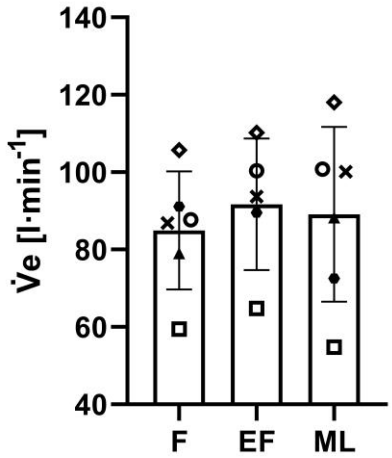

Figure 12: comparison of V̇e during the familiarisation (F), early-follicular (EF) and mid-luteal (ML) sub-phases of the MC (Mean ± SD). ○: participant 1; □: participant 2; ▲: participant 3; ◇: participant 4; ⬢: participant 5; ✕: participant 6.

4.7 RPE

Significant differences were found in RPE between the familiarisation (13.82 ± 1.91), EF (13.42 ± 2.15) and ML (12.60 ± 2.26) (p = 0.001) (Figure 13). The adjusted P value after a Tukey's multiple comparison test showed a significant difference between EF and ML (p < 0.001, d = 0.37, difference between means = 0.82 (-6.11 %)), and between the familiarisation and ML (p < 0.001, d = 0.58, difference between means = 1.22 (-8.83%)) (Figure 13). Comparison within sub-phases also showed significant differences between the two sessions of the familiarisation (14.21 ± 1.97 vs 13.37 ± 1.96) (p < 0.001, d = 0.43, difference between means = 0.74 (-5.91 %)). However, no significant differences were found within the two sessions of EF (13.48 ± 1.98 vs 13.36 ± 2.43) (p = 0.59, d = 0.05, difference between means = 0.12 (-0.89 %)) or ML (12.57 ± 2.33 vs 12.58 ± 2.47) (p = 0.57, d = 0.00, difference between means = -0.01 (0.08 %)).

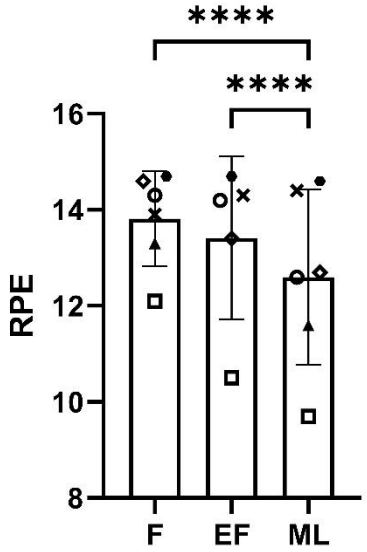

Figure 13: comparison of RPE during the familiarisation (F), early-follicular (EF) and mid-luteal (ML) sub-phases of the MC (Mean ± SD). ○: participant 1; □: participant 2; ▲: participant 3; ◇: participant 4; ⬢: participant 5; ✕: participant 6. ****: $p < 0.0001$.

4.8 Power output

MPO was not found to significantly differ between the familiarisation (1375.15 ± 205.19 W), EF (1406.33 W ± 208.97 W) and ML (1405.71 ± 212.86 W) ($p = 0.17$) (Figure 14). Non-significant differences were also found between EF and ML ($p = 0.998$, $d = 0.00$, difference between means = 0.62 W (-0.4 %)) (Figure 14). No significant differences were found in MPO between the two sessions of the familiarisation (1379.77 ± 218.14 W vs 1370.54 ± 204.24 W) ($p = 0.63$, $d = 0.04$, difference between means = 9.23 W (-0.67 %)), EF (1413.23 ± 197.44 W vs 1399.44 ± 226.32 W) ($p = 0.31$, $d = 0.06$, difference between means = 13.79 W (-0.98 %)) or ML (1400.95 ± 211.09 W vs 1407.39 ± 237.50 W) ($p = 0.44$, $d = 0.03$, difference between means = -6.44 W (0.46 %)). The fatigue index for MPO did not significantly differ between the familiarisation (-6.64 ± 2.28), EF (-7.59 ± 1.72) and ML (-5.69 ± 1.48) ($p = 0.37$). Non-significant differences were found between EF and ML ($p = 0.25$, $d = 1.18$, difference between means = -1.9 (-25.03 %)). Furthermore, no significant differences were also found in the fatigue index for MPO between the two

familiarisation sessions (-7.86 ± 3.34 vs -5.44 ± 2.66) (p = 0.16, d = 0.80, difference between means = -2.42 (-30.79 %)), EF (-7.56 ± 1.29 vs -7.63 ± 2.74) (p = 0.81, d = 0.03, difference between means = 0.07 (0.93 %)), or ML (-5.24 ± 3.05 vs -5.79 ± 0.43) (p > 0.99, d = 0.25, difference between means = 0.55 (10.50 %)).

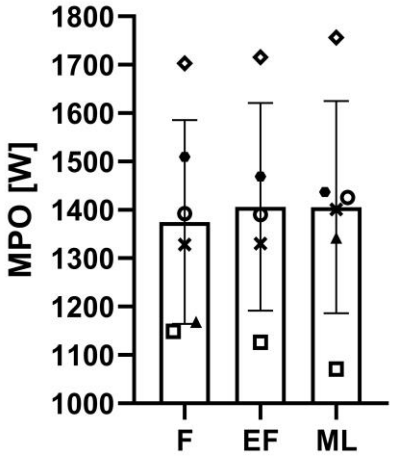

Figure 14: comparison of MPO during the familiarisation (F), early-follicular (EF) and mid-luteal (ML) sub-phases of the MC (Mean ± SD). ○: participant 1; □: participant 2; ▲: participant 3; ◇: participant 4; ⬣: participant 5; ✕: participant 6.

Similar to MPO, no significant differences were found in PPO between the familiarisation (2431.06 ± 402.78 W), EF (2498.32 ± 383.56 W) and ML (2432.75 ± 417.10 W) (p = 0.97) (Figure 15). A comparison between EF and ML indicated non-significant differences (p = 0.14, d = 0.16, difference between means = 65.57 W (0.07 %)) (Figure 15). No significant differences were found in PPO between the session of the familiarisation (2455.38 ± 421.65 W vs 2406.73 ± 403.85 W) (p = 0.17, d = 0.12, difference between means = 48.65 W (-1.98 %)), EF (2509.60 ± 425.65 W vs 2487.04 ± 357.41 W) (p = 0.52, d = 0.06, difference between means = 22.56 W (-0.90 %)) or ML (2442.19 ± 441.90 W vs 2409.43 ± 445.11 W) (p = 0.55, d = 0.07, difference between means = 32.76 W (-1.34 %)). The fatigue index for PPO was not found to significantly differ between the familiarisation (-6.98 ± 3.03), EF (-6.91 ± 2.13) and ML (-4.83 ± 0.96) (p = 0.20). A comparison between EF

and ML showed non-significant differences (p = 0.30, d = 1.26, difference between means = -2.08 (-30.10 %)). However, significant differences were found in the fatigue index for PPO between the two familiarisation sessions (-5.86 ± 3.28 vs -8.11 ± 3.05) (p = 0.03, d = 0.71, difference between means = 2.25 (38.40 %)). Yet, no significant differences were found between the two sessions of EF (-7.04 ± 3.95 vs -6.78 ± 3.97) (p = 0.93, d = 0.07, difference between means = -0.26 (-3.69 %)), or ML (-5.15 ± 1.27 vs -5.06 ± 1.44) (p = 0.93, d = 0.07, difference between means = -0.09 (-1.75 %)).

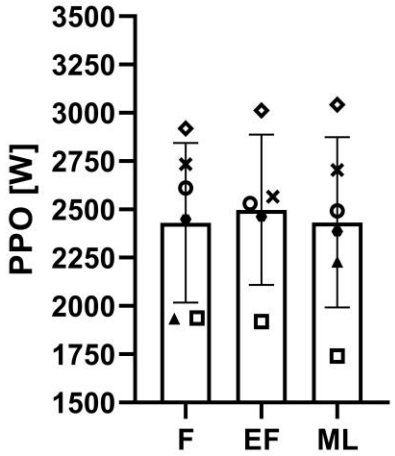

Figure 15: comparison of PPO during the familiarisation (F), early-follicular (EF) and mid-luteal (ML) sub-phases of the MC (Mean ± SD). ○: participant 1; □: participant 2; ▲: participant 3; ◇: participant 4; ⬣: participant 5; ✕: participant 6.

4.9 Peak acceleration

Significant differences were found in peak acceleration between the familiarisation (4.66 ± 0.77 $m \cdot s^{-2}$), EF (4.65 ± 0.84 $m \cdot s^{-2}$) and ML (5.05 ± 1.14 $m \cdot s^{-2}$) (p = 0.03) (Figure 16). The adjusted P value after a Tukey's multiple comparison test showed significant differences between EF and ML (p = 0.02, d = 0.40, difference between means = -0.4 $m \cdot s^{-2}$ (8.60%)), and between the familiarisation and ML (p = 0.04, d = 0.40, difference between means = -0.39 $m \cdot s^{-2}$ (8.37 %)) (Figure 16). However, no significant differences were found in the comparison of the two sessions within the same phase during the familiarisation (4.54 ±

0.85 m·s^{-2} vs 4.79 ± 0.87 m·s^{-2}) (p = 0.08, d = 0.29, difference between means = -0.25 m·s^{-2} (5.51%)), EF (4.67 ± 0.87 m·s^{-2} vs 4.65 ± 0.95 m·s^{-2}) (p = 0.88, d = 0.02, difference between means = 0.02 m·s^{-2} (-0.43%)) or ML (5.02 ± 1.11 m·s^{-2} vs 4.96 ± 1.32 m·s^{-2}) (p = 0.71, d = 0.05, difference between means = 0.06 m·s^{-2} (-1.20%)). Significant differences were also found in the fatigue index for acceleration (Sdec) between the familiarisation (-16.07 ± 3.45), EF (-12.39 ± 3.44) and ML (-9.91 ± 3.89) (p = 0.03). Specifically, there was a significant difference between the familiarisation and ML (p = 0.03, d = 1.68, difference between means = -6.16 (-38.33 %)). Non-significant differences were found between EF and ML (p = 0.51, d = 0.68, difference between means = -2.48 (-20.02 %)). The fatigue index for acceleration did not significantly differ between the two sessions of the familiarisation (-16.46 ± 4.45 vs -15.69 ± 4.14) (p = 0.69, d = 0.18, difference between means = -0.77 (-4.68 %)), EF (-10.89 ± 4.90 vs -13.88 ± 2.97) (p = 0.20, d = 0.74, difference between means = 2.99 (27.46%)), or ML (-9.04 ± 3.63 vs -11.46 ± 7.13) (p = 0.51, d = 0.43, difference between means = 2.42 (26.77%)).

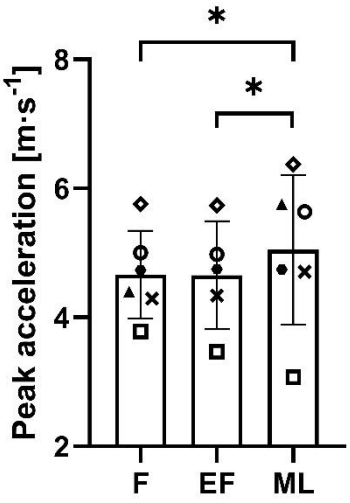

Figure 16: comparison of peak acceleration during the familiarisation (F), early-follicular (EF) and mid-luteal (ML) sub-phases of the MC (Mean ± SD). ○: participant 1; □: participant 2; ▲: participant 3; ◇: participant 4; ⬣: participant 5; ✕: participant 6. *: p < 0.05.

4.10 Distance

Distance was not found to be significantly different between the familiarisation (20.14 ± 2.56 m), EF (20.47 ± 2.44 m) and ML (20.31 ± 2.47 m) (p = 0.49) (Figure 17). Moreover, a multiple comparison specifically showed non-significant differences between EF and ML (p = 0.59, d = 0.07, difference between means = 0.16 m (-0.78 %)) (Figure 17). No significant differences were also found in distance between the two sessions of the familiarisation (20.27 ± 2.78 m vs 20.01 ± 2.54 m) (p = 0.35, d = 0.10, difference between means = -0.26 m (-1.28 %)), EF (20.57 ± 2.36 m vs 20.37 ± 2.63 m) (p = 0.38, d = 0.08, difference between means = 0.20 m (-0.97 %)) or ML (20.29 ± 2.45 m vs 20.25 ± 2.76 m) (p = 0.82, d = 0.02, difference between means = 0.04 m (-0.20 %)).

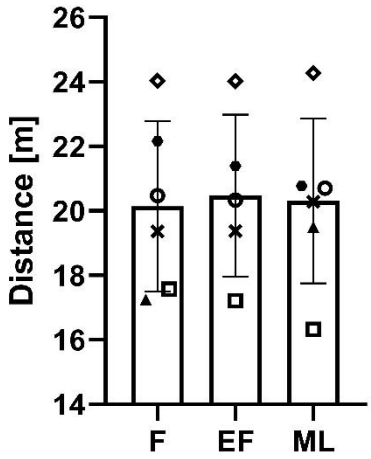

Figure 17: comparison of mean distance across all five sprints during the familiarisation (F), early-follicular (EF) and mid-luteal (ML) sub-phases of the MC (Mean ± SD). ○: participant 1; □: participant 2; ▲: participant 3; ◇: participant 4; ⬣: participant 5; ✕: participant 6.

5. Discussion

The aim of this study was to measure physiological, performance and perceptual responses during repeated running sprint ability induced by the early-follicular and mid-luteal sub-phases of the MC. It was hypothesised that the MC phases would affect physiological and perceptual parameters, but not the performance (peak power output, mean power output, distance, peak acceleration). Data suggest that the MC phases might not influence anthropometric parameters (BMI (p = 0.35)), hips-to-waist ratio (p = 0.98), fat mass (p = 0.93)), physiological parameters ($\dot{V}O_2$ (p = 0.10), HR (p = 0.49), Ve (p = 0.42), RER (p = 0.47), pre- (p = 0.78) and post-exercise lactate (p = 0.58)) and performance parameters (mean power (p = 0.998), peak power (p = 0.14), distance (p = 0.59)). No significant differences between EF and ML in any of the above-mentioned parameters are the major findings of the current study. However, results from the present study also found that the MC phases appear to influence RPE and peak acceleration. RPE was found to be significantly lower during ML when compared with EF (p < 0.001). Whereas, peak acceleration was found to be significantly higher during ML when compared with EF (p = 0.02). Furthermore, trivial or small effect sizes were identified for all the variables analysed between EF and ML, except for the fatigue indexes, showing that the magnitude of the effects of the MC on fatigue indexes is bigger than the other parameters analysed.

5.1 Participant's characteristics

The results from this study show non-significant difference in any of the body composition parameters analysed (BMI, fat mass, hips-to-waist circumference). Janse de Jonge (2003) and Giacomoni et al. (2000) suggested that body composition could be affected by the MC through fluid regulation. However, Janse de Jonge (2003) and Giacomoni et al. (2000) also reported that the MC effect on fluid regulation might not be enough to significantly affect body composition, which is also confirmed by the small

effect sizes reported in BMI (d = 0.2), fat mass (d = 0.23) and hips-to-waist circumference (d = 0.33) in the present study (Table 9). The findings agree with previous studies that did not find any differences in body composition between EF and ML (Beidleman et al., 1999; Bemben et al., 1995; De Souza et al., 1990; Julian et al., 2017; Lebrun et al., 1995). However, whilst specific instructions were given about fluid intake prior to testing, it was not directly controlled in this study and in any of the studies reviewed, and therefore further studies should be done to assess the influence of the MC on fluid regulation and its effects on body composition.

Fat mass was not found to differ between EF and ML (EF: 24.47 ± 8.94 %, ML: 23.93 ± 10.53 %, p = 0.93), as also confirmed by previous studies from Lebrun et al. (1995), De Souza et al. (1990) and Julian et al. (2017). Hips-to-waist circumference differences between EF and ML were also found non-significant (EF: 0.76 ± 0.03, ML: 0.77 ± 0.03, p = 0.98). However, as the effects of the MC on hips-to-waist circumference were never analysed previously, it is not possible to compare the results from this study with previous literature. The BMI differences between EF and ML were also found to be non-significant (EF: 23 ± 5 $kg \cdot m^{-2}$, ML: 24 ± 5 $kg \cdot m^{-2}$, p = 0.35). BMI has been previously analysed, but the differences between sub-phases were not reported, and therefore a comparison between the results of this study and previous literature is not possible. However, in agreement with our results, Vaiksaar et al. (2011) reported non-significant differences in BMI between the luteal and follicular phases, and Pestana et al. (2017) reported non-significant differences in BMI between MF and the late-luteal sub-phase. Even though the number of the studies reporting BMI is low, the results agree with the ones from this study, further confirming that the MC might not influence BMI.

As the MC does not seem to affect body composition, parameters that are influenced by it such as acceleration, power and $\dot{V}O_2$ (Esco et al., 2018; Janse de Jonge, 2003; Maciejczyk et al., 2015), should not be affected by changes in body composition during

different sub-phases of the MC. In fact, as body mass and BMI did not significantly change throughout the MC (Table 9), which helps to explain no differences in $\dot{V}O_2$, mean power output and peak power output found between EF and ML. In contrast, peak acceleration was found to be higher during ML than EF, but the body composition should not be the reason behind the significant changes seen.

In this study no significant differences in BMI, fat mass or hips-to-waist circumference were also found within the two sessions of familiarisation, EF or ML. In conclusion, it appears that the hormonal fluctuations during the MC might not influence body composition in any of the parameters analysed.

5.2 Physiological data

The findings of this study show that physiological parameters do not appear to be influenced by the MC sub-phases. In fact, no significant differences between EF and ML were found in any of the physiological parameters analysed. Further within-phase comparison between two sessions during familiarisation, EF and ML also showed no significant differences in any of the parameters. This could mean that the variability in the hormone concentrations during the same sub-phase might not be enough to have a real effect on these physiological variables. This was expected though, as the hormonal differences during two days of the same sub-phase are reported to be lower than the differences between EF and ML. As no effects were shown between EF and ML, where the hormonal differences are at their greatest magnitude, it is not surprising that no differences were found between two sessions of the same phase.

5.2.1 HR

The HR results from this study (EF: 159.08 ± 20.36 bpm, ML: 155.55 ± 20.43 bpm, p = 0.49) contrast with the hypothesis of a lower heart rate during EF when compared with ML. However, the results agree with the previous studies that did not report significant

differences between the EF and ML sub-phases (Abdollahpor et al., 2013; Beidleman et al., 1999; Bemben et al., 1995; De Souza et al., 1990; Dean et al., 2003; Lebrun et al., 1995; Oosthuyse et al., 2005). Even though a number of studies previously reported non-significant differences, significant differences were expected because it has been shown that high concentrations of progesterone can increase HR, thus possibly causing a higher HR during ML than EF. Janse de Jonge et al. (2012) have suggested that the HR would be affected by changes in body temperature between the follicular and luteal phases. However, the average increase in body temperature during the luteal phase (up to 0.6 °C) might be too low to significantly affect HR. The small effect size (d = 0.17), which showed a trivial effect between EF and ML, further supports the conclusion that MC sub-phases do not significantly and meaningfully affect HR when performing a running RSA (Figure 8). Basal body temperature was not monitored in this study, so it was not possible to quantify the temperature difference between EF and ML. Nonetheless, it is likely that the differences in temperature between the two sub-phases were consistent with previous literature, and therefore did not significantly affect HR.

As HR has been shown to be correlated with RER and $\dot{V}O_2$ (Bot & Hollander, 2000; Freedson & Miller, 2000; Habibi, Dehghan, Moghiseh, & Hasanzadeh, 2014; Ramos-Jiménez et al., 2008) it was expected to see that all of these parameters were found to be non-significant. Furthermore, HR is one of the physiological determinants of RPE (Hooper et al., 2011). Even though a significantly lower RPE (p < 0.001, d = 0.37) was found during ML than EF, the role of HR in affecting RPE can be excluded due to its non-significant difference and the small effect size.

It is also important to consider that even though this study and the above-mentioned studies found similar results, there were differences in participants' fitness levels and testing modalities. In fact, researchers used different exercise modalities (cycling, running) across a range of intensities varying from submaximal to incremental exercises

to exhaustion. Moreover, participants' fitness levels across the studies were wide-ranging from being non-active (Lamont, 1986; Oosthuyse et al., 2005), moderately active (Bemben et al., 1995; Dean et al., 2003) or having a $\dot{V}O_2$max higher than 50 ml/kg/min (Lebrun et al., 1995). Another fundamental aspect to consider is the method used to determine the MC sub-phases. Whilst all of the studies that compared EF and ML used blood sample to determine the sub-phases, the present study used a combined approach of a urinary kit and a diary. However, similar results suggest that this combined approach may be reliable enough to be used to determine MC sub-phases, particularly in an applied sport setting. In conclusion, it appears that regardless the methodological differences, HR does not appear to be significantly influenced by the MC during a running RSA performance.

5.2.2 Lactate

The hypothesis that the lactate would be significantly lower during ML than EF was not supported by our data, that showed no differences in pre-exercise lactate (EF: 1.43 ± 0.46 mmol·l^{-1}, ML: 1.63 ± 0.51 mmol·l^{-1}, p = 0.78) and post-exercise lactate (EF: 12.66 ± 3.11 mmol·l^{-1}, ML: 12.01 ± 2.32 mmol·l^{-1}, p = 0.58) (Figure 9). However, these results agree with Lamont (1986), De Souza et al. (1990), Bemben et al. (1995), Abdollahpor et al. (2013) and Dean et al. (2003) that reported non-significant differences between EF and ML. As oestrogen and progesterone have been shown to affect energy metabolism and substrate utilisation, and therefore lactate levels, this result was unexpected. A possible explanation is that, even though oestrogen and progesterone can affect lactate levels, the hormones difference between EF and ML were not big enough to show any significant difference. The lack of differences between sub-phases suggests that no metabolic changes (higher fat utilisation) occurred between EF and ML. This is further confirmed by the fact that no significant differences were found in RER (EF: 1.11 ± 0.15, ML: 1.09 ± 0.16, p = 0.47), which shows that the ratio between fat and carbohydrates

utilisation did not change between sub-phases. To confirm the conclusion that the MC sub-phases do not significantly and meaningfully affect pre- or post-exercise lactate, the effect size showed a small effect in pre- and post-exercise lactate (d = 0.41 and d = 0.24, respectively). Even though the present study and the above-mentioned studies found non-significant lactate levels between EF and ML, participants with different fitness levels were recruited to perform a range of exercises such as maximal exercises to exhaustion and submaximal exercises during running and cycling. Thus, showing that the results and conclusions reached do not seem to depend on the testing modalities and participant's fitness levels. Furthermore, different MC determination methods were used. Whilst all the above-mentioned papers used blood samples to determine the sub-phases, in this study a different approach was used. This heterogeneity and the fact that similar results were found suggest that the MC may not influence post-exercise lactate regardless of the exercise modality, exercise choice, participant's fitness level and MC determination method.

5.2.3 $\dot{V}O_2$

In agreement with our hypothesis, no differences in $\dot{V}O_2$ between EF and ML were found (EF: 35.65 ± 8.68 ml·kg^{-1}·min^{-1}, ML: 32.97 ± 8.97 ml·kg^{1}·min^{-1}, p = 0.10). Janse de Jonge et al. (2003) offered an explanation by hypothesising that $\dot{V}O_2$max would be affected by the MC only if the determinants of $\dot{V}O_2$max such as HR are also affected. Even though the author was specifically referring to $\dot{V}O_2$max, a link between HR and $\dot{V}O_2$ is strong (Bot & Hollander, 2000; Freedson & Miller, 2000; Habibi et al., 2014), which is demonstrated by our findings as no significant differences in HR (Figure 8) are also reflected in no significant changes in $\dot{V}O_2$ (Figure 10). This finding suggests that potential cardiovascular changes related to MC hormonal fluctuations are not associated with changes in oxygen uptake during repeated sprints (Gurd et al., 2007). Moreover, Figure 11 shows that RER was not significantly different between EF and ML (EF: 1.11 ± 0.15, ML: 1.09 ± 0.16, p =

0.47). In fact, there is a strong association between RER and $\dot{V}O_2$ measures and it is very likely that any changes in both of these parameters due to the effects of the MC sub-phases should follow similar pattern (Gurd et al., 2007; Ramos-Jiménez et al., 2008). Moreover, the effect size between these two sub-phases was characterised as small (*d* = 0.30), further providing evidence that the MC does not affect $\dot{V}O_2$ during RSA.

Similar results have been reported previously by Lamont (1986), De Souza et al. (1990), Beidleman et al. (1999) and Janse de Jonge et al. (2012). These authors recruited participants with different physical levels, including non-active participants that took part in the study by Lamont (1986). Furthermore, all of those studies performed sub-maximal exercises at different intensities (60/70/80% $\dot{V}O_2$max) during running or cycling. This is in contrasts to the active participants that were recruited in the current study to perform a high-intensity repeated sprint exercise. This heterogeneity of participants and exercises, considering that similar results and conclusion were reached, shows that the effects of the MC on $\dot{V}O_2$ might not depend on the participants' level or the exercise tested. Further confirmation is found in a study by Middleton & Wenger (2006), which analysed $\dot{V}O_2$ between the mid-follicular and late-luteal sub-phases during a RSA exercise, and reported non-significant differences in $\dot{V}O_2$ during the sprints (mid-follicular: 24.3 ± 2.4 $ml \cdot kg^{1} \cdot min^{-1}$, late-luteal: 23.7 ± 1.5 $ml \cdot kg^{1} \cdot min^{-1}$). Even though different sub-phases were compared, similar conclusions were reached, strengthening the hypothesis that the MC might not affect $\dot{V}O_2$.

5.2.4 RER

Our hypothesis that a lower RER during ML compared with EF would be found was not confirmed. The data from this study indicated no significant difference in RER between EF and ML (EF: 1.11 ± 0.15, ML: 1.09 ± 0.16, *p* = 0.47), which suggests no differences in fat utilisation between the two sub-phases occurred (Figure 11). Similar to lactate levels, this result was unexpected because oestrogen and progesterone have been shown to

affect energy metabolism and substrate utilisation. Moreover, the same explanation provided to explain the lack of significant differences in lactate levels can be used for RER, as even though oestrogen and progesterone can affect RER, the hormones difference between EF and ML might not big enough to show any significant difference. The effect size was found to be trivial (d = 0.13), further confirming that the MC hormonal fluctuations throughout sub-phases might not be enough to affect RER.

Non-significant differences in RER between EF and ML were also found by De Souza et al. (1990), Dean et al. (2003), Janse de Jonge (2012), Lamont (1986), Lebrun et al. (1995), and Oosthuyse et al. (2005). As there were methodological differences between the current study and the others that reported similar conclusions, such as the test choice, exercise modality and/or participants' fitness level, it is possible that these differences do not affect the results. A study by Middleton & Wenger (2006) is the only study that analysed the effect of the MC on a RSA performance. The authors reported a significantly lower (p = 0.04) RER during the late-luteal (1.17 ± 0.06) than the mid-follicular (1.19 ± 0.06) sub-phases, in contrast with the majority of the literature that reported non-significant differences. However, the difference (0.2) is quite small and is identical to the absolute difference found in the present study (Figure 11). Albeit significant, the effect size was not provided but the difference might be too small to be meaningful. In contrast with the present study, Middleton & Wenger (2006) compared the mid-follicular and the late-luteal sub-phases, instead of the early-follicular and mid-luteal one. Even though a comparison of different sub-phases can lead to different results due to different hormone concentrations, the biggest effect is expected when comparing EF and ML, as they have the biggest difference in both oestrogen and progesterone among all the sub-phases. Because significant results were reported by Middleton & Wenger (2006) between two sub-phases with a lower hormones difference, whilst all the studies that compared EF and ML did not report any significant difference, it is possible that the

contrasting results were due to another reason, unrelated to the hormonal fluctuations during the MC.

As this study and all the papers mentioned included participants with different fitness levels, to perform a variety of exercises, it does not appear that any of these differences might have been the reason to explain the contrasting results from Middleton & Wenger (2006). More studies about RSA are required to better understand the effects of the MC on RER, during this specific performance. From the current literature and the present study, it appears that the MC does not affect RER, regardless the methodological differences among the studies.

5.2.5 V̇e

The hypothesis of a higher ventilation during ML when compared with EF was not confirmed by our data, that showed non-significant differences (EF: 91.73 ± 19.82 l·min^{-1}, ML: 89.10 ± 25.36 l·min^{-1}, p = 0.42). However, in agreement with previous studies, V̇e did not significantly differ between EF and ML (Beidleman et al., 1999; Bemben et al., 1995; De Souza et al., 1990; Lamont, 1986; Lebrun et al., 1995). The results were not expected, as the hypothesis of a higher V̇e during ML than EF was formulated based on previous studies showing that high concentrations of progesterone increase V̇e (Beidleman et al., 1999; De Souza et al., 1990; Schoene, Robertson, Pierson, & Peterson, 1981; Williams & Krahenbuhl, 1997). A significantly higher V̇e was expected during ML because of the highest concentration of progesterone throughout the MC is reached in this sub-phase. Smekal et al. (2007) and Janse de Jonge et al. (2012) suggested that, other than progesterone concentrations, factors like body temperature might affect V̇e, but the differences in these factors between sub-phases might be too low to be statistically significant. However, in the current study body temperature and progesterone concentrations were not measured, and therefore the influence of these factor could not be properly determined. Furthermore, Beidleman et al. (1999),

suggested that other factors such as the central motor command might influence V̇e to a greater extent thereby masking the effects of progesterone on V̇e. The trivial effect size found between EF and ML (*d* = 0.12) enforces that conclusion that the MC does not meaningfully affect V̇e.

In this study a non-significant higher V̇e was found in EF than ML, with a difference of 2.63 l·min^{-1}. In contrast with our findings, Williams et al. (1997) reported a significantly higher V̇e during ML than EF at 55% (5.2 l·min^{-1} difference) and 80% (4.0 l·min^{-1} difference) of V̇O_2max. A RSA protocol was used in the current study, whereas Williams et al. (1997) recruited participants to perform a submaximal running exercise. The difference in testing protocol could explain the contrasting results. However, De Souza et al. (1990), Lamont (1986) and Beidleman et al. (1999) all analysed similar protocols (i.e. submaximal exercises at 70/80% V̇O_2max) and reported non-significant results, similar to the results of this study. It is also important to consider that whilst 5 studies out of 6 reported non-significant differences in V̇e between EF and ML, the participants' fitness level, the exercise choice and modality were different. Thus, showing that these methodological differences might not be relevant to determine whether the MC influences V̇e.

From these findings, it might appear that progesterone does not stimulate ventilation during the luteal phase or does not stimulate it enough to be significantly different. It is recommended that future research studies look into the possible mechanisms as more studies are required to understand how all the factors interact with the menstrual cycle influence V̇e. However, from our data and the current literature it appears that MC does not affect V̇e.

5.3 Perceptual data (RPE)

The hypothesis of a lower RPE during EF than ML was not supported by our data that showed the opposite effect, as a significantly lower RPE was found during ML when compared with EF (EF: 13.42 ± 2.15, ML: 12.60 ± 2.26, $p < 0.001$). The findings from this study contrasts with previous findings that did not report differences in RPE between EF and ML (Beidleman et al., 1999; De Souza et al., 1990; Janse de Jonge et al., 2012).

To explain the significant differences in RPE in this study, the most likely explanation would be the learning effect, where the participants became more familiar with the test the more they performed it. The two familiarisation sessions may have not been enough for the participants to feel comfortable with the protocol and the non-motorised treadmill, and it is possible that the participants needed more than two sessions to be ready to perform without seeing the influence of the learning effect (Figure 13). If more familiarisation sessions were used, it is possible that the differences between EF and ML would decrease and become non-significant. Even though there was no significant difference in RPE between the familiarisation and EF, the RPE during the familiarisation was higher (13.82 ± 1.91 vs 13.42 ± 2.15). This trend shows that the RPE values were lower throughout the phases, showing the possibility that the athletes might have become more familiar with the testing protocol, instead of meaning that the MC phases actually affected the RPE score. This learning effect is further demonstrated by the fact that the only participant that performed the test during ML first, showed a higher RPE during this phase, and a lower RPE during EF (Figure 13). Moreover, significant differences were also found between the two familiarisation sessions, with a higher value during the first one (Figure 13). This difference can be again explained by the fact that the participants became more familiar with the protocol and especially the non-motorised treadmill, which is designed so that participants move the belt themselves.

However, it was reported to be rather unnatural running motion upon first use by every participant.

In agreement with the hypothesis of a learning effect, a significantly lower RPE was also found during ML than the familiarisation sessions (12.60 ± 2.26 vs 13.82 ± 1.91, $p < 0.001$). Furthermore, it is also important to consider that even though the differences between EF and ML were significant, the effect size found was small ($d = 0.37$), showing that the magnitude of the effect was little and strengthening the conclusion that the MC might not affect RPE during RSA.

5.4 Performance data

Whilst mean power output, peak power output and distance were not affected by the MC sub-phases, peak acceleration appeared to be influenced by it. The fact that power output and distance were not affected by the MC might have important effects on sport performance, as any sport that relies on short sprints might not be affected by the MC. Furthermore, even though peak acceleration was found to be significantly different between the two sub-phases (Figure 16), the distance did not significantly change. This means that the changes in peak acceleration did not translate in a practical advantage (distance). As peak acceleration captures a single instant during a sprint, it is possible that this fraction of performance was not enough to significantly affect the distance outcome (Figure 17). Therefore, RSA performance outcomes do not appear to be influenced by the MC.

When comparing two sessions of the same sub-phase, no differences in peak/mean power, peak acceleration or distance were found during the familiarisation, EF or ML. This strengthens the previous point that the variability within hormonal concentrations during the same sub-phase might be not enough to affect these parameters. This was

expected, as the hormonal differences during two days of the same sub-phase are reported to be lower than the differences between EF and ML.

5.4.1 Power output

The findings from this study confirms our hypothesis, as no differences were found between EF and ML in mean (EF: 1406.33 ± 208.97 W, ML: 1405.71 ± 212.86 W, p = 0.998) and peak power output (EF: 2498.32 ± 383.56 W, ML: 2432.75 ± 417.10 W, p = 0.14). It has been hypothesised by Pestana et al. (2017) that power output might be affected by changes in body composition. However, the lack of significant differences between EF and ML in fat mass and BMI (Table 9) can explain the power output results (Figures 14 and 15), as no changes in body mass and muscle mass were found. Furthermore, the effect size for mean and peak power output was found to be trivial (d = 0.00 and d = 0.16 respectively) and is similar to the small effect sizes found in BMI and fat mass, showing that the effect of the MC on power output might be meaningless.

The findings agree with those by Kose et al. (2018), who reported non-significant differences between the same sub-phases during a Wingate anaerobic test, in mean and peak power output. Even though the Wingate anaerobic test and running RSA do not appear to be correlated (Aziz & Chuan, 2004), both tests measure anaerobic power output, and therefore the results can be used for comparison purposes. However, it has to be taken into consideration that the two protocols were different, and therefore this comparison has to be taken with caution. This difference can be seen from the data, as the MPO found in this study (EF: 1406.33 ± 208.97 W, ML: 1405.71± 212.86 W) highly differs from Kose et al.'s (2018) results (EF: 379.43 ± 69.23 W, ML: 377.75 ± 66.86 W). However, the mean difference in MPO between the two sub-phases in the present study was 0.62 W, which is similar to a 1.68 W difference reported by Kose et al. (2018). A big difference was also found in PPO between the study (EF: 2498.32 ± 383.56 W, ML: 2432.75 ± 417.10 W) and Kose et al. (2018) (EF: 554.56 ± 109.00 W, ML: 555.50 ± 107.29

W). The differences in PPO between the two studies were higher, with a 65.57 W difference found in this study, and a 0.94 W reported by Kose et al. (2018).

The study by Middleton & Wenger (2006) analysed a RSA performance a reported non-significant differences in peak power between the mid-follicular (6.8 ± 0.6 $W \cdot kg^{-1}$) and late-luteal (6.9 ± 0.6$\cdot kg^{-1}$) sub-phases, reaching the same conclusions as the present study. Even though different sub-phases were analysed, the results reported by Middleton & Wenger (2006) strengthen the conclusion that the MC might not influence power output.

No significant differences were found in the fatigue index for MPO and PPO between EF and ML, and similar results reported by Tsampoukos et al. (2010) between the follicular and the luteal phase. However, a significant difference in the fatigue index for PPO was found between the two sessions of the familiarisation, whilst no differences were found between the sessions of EF or ML. This shows the importance of the familiarisation sessions prior to the data collection, to reduce variability and avoid any learning effect. Future studies should take into consideration these results and include at least two familiarisation sessions to avoid possible unreliable results.

5.4.2 Peak acceleration

In contrast with our hypothesis, a significant higher peak acceleration was found during ML when compared with EF (EF: 4.65 ± 0.84 $m \cdot s^{-2}$, ML: 5.05 ± 1.14 $m \cdot s^{-2}$, $p = 0.02$). This result was not expected, because even though acceleration have never been studied before and no results are available for a comparison, there is an overall consensus that the MC does not affect different types of performances such as incremental test to exhaustion, submaximal tests or the Wingate's tests. Without having previous data on the effects of the MC on RSA, we extended the hypothesis that the MC would not affect

performance to peak acceleration, and therefore no significant differences were expected.

Albeit significant, the effect size calculated showed a small effect (d = 0.40), indicating that the difference in peak acceleration might not be meaningful. Moreover, looking at individual data it can be seen that only 3 participants had a higher peak acceleration during ML, whilst 2 of them showed the opposite result. This split in the individual results highlights the need to conduct more research and gather more data. Peak acceleration was also found to significantly differ between the familiarisation (4.66 ± 0.77 $m \cdot s^{-2}$) and ML (5.05 ± 1.14 $m \cdot s^{-2}$) (p = 0.04, d = 0.40).

A possible explanation to justify the higher peak acceleration during ML would be the learning effect, where the participants had a better performance during ML because they had the time to get more comfortable with the non-motorised treadmill and the protocol, allowing them to perform better. However, if the learning effect was the cause, we should have seen a difference between the familiarisation and EF too and a more constant improvement. Instead, a non-significant and very small difference was found between the familiarisation and EF (0.01 $m \cdot s^{-2}$), showing that the learning effect might not be the explanation for the significant difference between the familiarisation and ML, and between EF and ML. This is also confirmed by the peak acceleration values between all the 6 sessions that did not have a stable increase in value. If the learning effect were the cause for a higher performance, the peak acceleration would probably have a more stable increase in values, and not a decrease from the second session of the familiarisation to the first one during EF (4.79 ± 0.87 $m \cdot s^{-}2$ vs 4.66 ± 0.87 $m \cdot s^{-2}$).

A significant difference was also found in the fatigue index for acceleration between the familiarisation and ML. The highest value in the score decrement was found during the familiarisation, and decreased during both EF and ML. The decrease between the familiarisation and the other two sub-phases was expected, and it is the reason why the

familiarisation sessions were performed. A lower score decrement means that the performance was more stable between each of the consecutive sprints, which is a consequence of being more comfortable with an exercise such as repeated sprints. This result shows the importance and the usefulness of the familiarisation sessions.

5.4.3 Distance

In agreement with our hypothesis, no differences were found in distance between EF and ML (EF: 20.47 ± 2.44 m, ML: 20.31 ± 2.47 m, $p = 0.59$). This lack of significance can be explained by the fact that power output was also non-significant. In fact, a strong relationship between power output and sprint performance exists, especially for shorter sprints (Haugen, Seiler, Sandbakk, & Tønnessen, 2019). The conclusion that the MC might not affect the distance finds further confirmation in the effect size ($d = 0.07$), which showed trivial differences between the two sub-phases.

These findings are in agreement with Julian et al. (2017), that also reported no significant differences between EF and ML (3289 ± 801 m vs 2822 ± 896 m respectively) during a Yo-Yo intermittent endurance test (Yo-Yo IET). However, Julian et al. (2017) tested the participants during a long-distance aerobic performance, whilst there are no studies that tested a short performance and reported the distance as a parameter. The findings from Julian et al. (2017) can be explained by the fact that distance during a Yo-Yo IET has been linked with $\dot{V}O_2max$, which is not influenced by the menstrual cycle phases. As previously stated, $\dot{V}O_2$ is also an important factor for RSA performance, and we did not find any differences in $\dot{V}O_2$ during the two sub-phases analysed (Figure 10), this could also justify the lack of significant differences in distance between EF and ML.

5.5 Limitations

There are a few major limitations in this study that could be addressed in future research. First, the small sample size does not guarantee a sufficient statistical power to

draw definitive conclusions, and it can influence the research findings as a small sample size can undermine the internal and external validity of a study (Faber & Fonseca, 2014). This limitation in participant numbers is also evident in most previous studies looking at the effects of the MC on sport performance, and it should be addressed in the future.

As previously explained, the low sample size was due to difficulties in recruiting and due to the limited time available to complete the project. Therefore, when planning similar studies researchers should be aware of the time-commitment as well as other challenges, including recruitment, inclusion/exclusion criteria and dropout rates. Most of the criteria are necessary to study the effects of the menstrual cycle on performance and cannot be removed. I would suggest that a collaboration between multiple researchers and universities might be the best bet to achieve a large sample size and more meaningful results.

However, to strengthen this study each test was repeated twice during each MC sub-phase and during the familiarisation. Even though this does not make up for the low sample size, it helps in reducing the variability, and therefore strengthening the results.

Alongside a general female underrepresentation in sport and exercise research as stated in the Introduction, another possible reason for small sample sizes in similar studies is that 49.5% of athletes are reported to use hormonal contraceptives, and 69.8% used one at some point (Martin, Sale, Cooper, & Elliott-Sale, 2018), showing that the available athletic population not using any hormonal contraceptives is greatly reduced. If we combine this data with the fact that only 61% of women are active (Sport England, 2019), this presents some clear challenges in recruiting female participants, because it shows that the available population that meets the inclusion criteria of being active and not using oral contraceptives is low.

Furthermore, other inclusion criteria had to be met to participate in this project, further reducing the available population. All the participants had to be training at least three times per week for a full year (any form of training) and had to be healthy, non-smokers and not under any kind medication or treatment that could influence hormones or performance. Furthermore, the menstrual cycle had to be regular and with an average length between 24 and 35 days. Another reason is the drop out from the experiment, as 2 participants were excluded because they started to use hormonal and contraceptive medications, and one participant could not finish all the sessions due to medical issues. All these factors are important to consider, when trying to understand the difficulties in recruiting, as they lead to low sample sizes, and are the reason why researchers tend not to recruit females in their studies, as the high number of criteria and limitations would create practical problems with the recruitment. Furthermore, this challenge is amplified when the objective of the study is directly related to the MC, where more extensive criteria are applied.

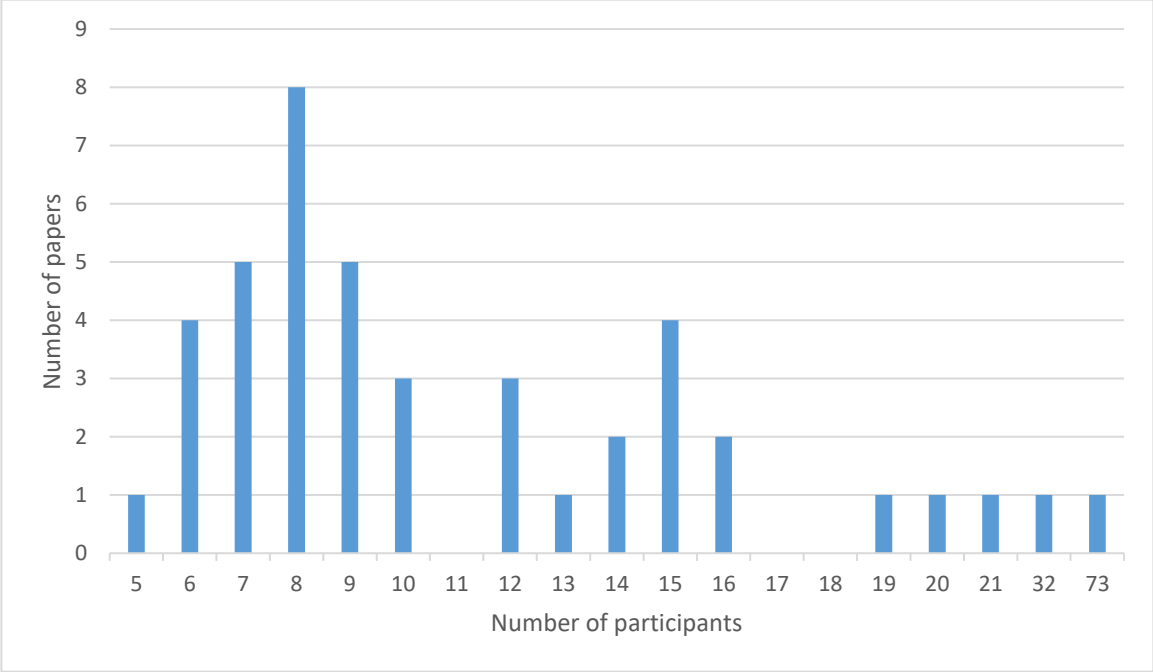

Figure 18: Sample size in the 43 papers analysed in this study

The second major limitation is the determination of the MC sub-phases, as the combined approach chosen did not guarantee the exclusion of LPD participants and did not provide hormonal values to be used to confirm the MC sub-phases. This approach was used because of its practicality and low cost, that allow it to be used by practitioners, whilst also being considered accurate. This aspect is fundamental, because the possibility to measure hormonal concentrations would allow a better comprehension of the results. In fact, differences in results might be due to different ranges of hormones among participants in the studies (Beidleman et al., 1999), or high intra-individual variability of hormone concentration (Beidleman et al., 1999).

Another limitation of this study is that the sessions were not done at the same time of the day, exposing the results to circadian variations in RPE and hormonal fluctuations and potentially affecting the results. The participants were asked to come to the lab at the same time on each session, but it has not been always possible due to work, study or personal reasons. Future studies should take this factor into consideration and test the participants at the same time of the day.

Prior to the intervention, participants were asked if they needed to void their bladder and encouraged to do so. However, whether they voided their bladder or not was not confirmed and should be taken in consideration for future studies in order to avoid its effect on body mass measurements.

Finally, the fitness level criteria used in the current study were based on training time and frequency, which does not really reflect the training status of a person. This choice was made because of recruitment problems, as using more strict criteria could have reduced the sample size even further. Therefore, future studies using female athletes should use an objective measure, such as $\dot{V}O_2max$, in their inclusion criteria.

5.6 Recommendations

Due to the limitations of this study and of the previous studies that analysed the effects of the MC phases on performance, the main recommendation would be to start by addressing the small sample size and the lack of a standardised way to determine the MC sub-phases. Furthermore, future studies should consider all the sub-phases of the MC and their effect on RSA performance, as this study only considered EF and ML whereas comparisons between other sub-phases may lead to different findings.

Additionally, it would be interesting to also look at participants using hormonal contraceptives. Sims & Heather, 2018, explained how oral contraceptives could be used in future experimental designs by helping to examine the effects of downregulated hormones concentrations on performance, and to investigate the differences between exogenous and endogenous hormones on performance.

Moreover, participants with different fitness levels should be included in future studies. Both non-active participants and professional athletes should be tested, and their results analysed. To do that, it is imperative to use objective fitness criteria in order to determine participants' fitness levels.

Another possible suggestion for future research would be to include other hormones, instead of focusing on oestrogens and progesterone only. For example, androgens could be included due to their influence on performance (Bermon, 2017). Not looking at other hormones and their interaction with the menstrual cycle might be considered a limitation.

The findings of the current study show the importance of familiarisation in order to minimise the learning effect in any future studies. In the current study, RPE results were probably affected by learning effect, and therefore the effects of the MC were not

visible. We recommend a minimum of two familiarisation sessions before starting any testing as failure to do so may lead to the inaccurate interpretation of the findings.

5.7 Practical applications

From a practical point of view, these results indicate that coaches and athletes might not have to tailor RSA training and testing based on the MC sub-phases. However, due to the low statistical power of this study, more data are required to address the research questions of this project with more certainty. This could mean that, even though during the MC a participant might report pain or discomfort (Giacomoni et al., 2000; Hooper et al., 2011; Kishali, Imamoglu, Katkat, Atan, & Akyol, 2006), this might not affect the results/performance. The current findings also highlight inter-individual variation across all reported measures, which shows that individual responses should be closely monitored.

However, this interpretation of the results is strictly related to the outcome of the performance (i.e. distance in each sprint) and does not properly consider the psychological aspects behind the performance and trainings and therefore might be limited. In fact, athlete's feelings and sensations should not be discarded just because they do not directly result in a lower performance. A recent study by Findlay et al. (2020) reported that 93% of the participants reported negative MC related symptoms such as worry, distraction and lack of motivation, and 67% of the participants considered that those symptoms impaired their performance. Furthermore, two participants reported that they were not able to complete a training session due to pain or dysmenorrhea (Findlay, Macrae, Whyte, Easton, & Forrest, 2020). This might negatively impact athletes' performance level, especially if this occurs at multiple times during the competitive season.

Even though from the current study and the existing literature it appears there are no differences in physiological parameters, Findlay et al. (2020) outlined that several athletes are still reporting negative perceptions. Therefore, practitioners should take these perceptions in consideration when working with female athletes. The relationship between an athlete and the coaching staff is fundamental and addressing what an athlete reports might help build a relationship of trust as well as optimise their training programme. In contrast, ignoring how they feel might lead to sub-optimal performance. The coaching staff should follow an evidence-based approach by monitoring each athlete's MC, openly discussing the possible effects of MC on performance, and where required adapting their training programmes based on the athletes' subjective feelings. A personalised approach based on each athlete's responses to the menstrual cycle and performance is therefore suggested as the best option with the current evidence available.

6. Conclusions

The findings of the current study demonstrate that RSA performance is not influenced by the menstrual cycle sub-phases. Even though the sample size limits the statistical power of this study, as there were no previous studies about the effects of the MC on repeated sprint ability these results provide a first insight of the effects of early-follicular and mid-luteal sub-phases on RSA. The results from this project could benefit other research in this field, by informing about the methodology used and the limitations found, and by providing data and results to be used. Furthermore, it can provide useful information for practitioners about how to organise their training sessions around the menstrual cycle. However, further studies are required to be able to fully understand if and how the MC phases may affect RSA performance.

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