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“Effects of the menstrual cycle on sports-physiological performance in female team handball players”

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Abbreviations

CMJ – countermovement jump

EFP – early follicular phase

HC – hormonal contraceptive

HIG – hormonal implants group

LEA - low energy availability

LH - luteinizing hormone

LFP – late follicular phase

MLP – mid luteal phase

MC – menstrual cycle

NMG – normal menstruating group

OCG – oral contraceptive group

OCP – oral contraceptive pills

OV – ovulation

OVP – ovulation phase

SJ – squat jump

1 RM – 1 repetition maximum

Foreword

I want to thank Dr. Erik Andersson and Dr. John Osbourne for believing in me with this project and helping me through the process of planning and fulfilling the project. Dr. Erik Andersson has helped me countless times. Thanks to Sigurd Pedersen who has also contributed to the testing.

This project did not come without challenges, with Covid restrictions from early December 2021 until the end of February 2022 allowing no team training sessions or games, which affected the handball players in completing their season, and caused a delay in this project. Despite this delay, I was able to get a minimum of participants to complete all the four testing sessions.

I have learned a lot in this process with having a big responsibility not only by planning and coordinating such a comprehensive project, but also the lack of knowledge there still is about this theme. I am satisfied that I have contributed to such an important and growing research field.

Abstract

Objective: Team handball is a physically demanding sport. Research has not kept up with the increasing number of women participating in exercise and sports. The menstrual cycle (MC) is complex and should be included when conducting sport and exercise research on female athletes. Therefore, the main aim of this study was to investigate the potential effects of three different phases of the menstrual cycle phases (early follicular phase, ovulation, and mid luteal phase) on handball performance-related factors.

Materials and methods: A total of 22 female handball players (age: 20 ± 3.6 years, body fat percentage: 27.7 ± 3.3) were recruited and completed the baseline testing, and 10 (age: 19.9 ± 2.9, body fat percentage: 27.6 ± 3.1) completed the MC- phase specific testing. The testing protocol included 1RM back squat in a Smith machine, maximal handgrip strength (in Ibf), maximal vertical jumping ability (squat jumps [SJ] and countermovement jump [CMJ], a modified agility T-test, and a maximal 15-m sprint. Subjects were both users of oral contraceptives (*n* = 2), hormonal implants (*n* = 3), mini pills (*n* = 1), hormonal IUD (*n* = 1) and naturally menstruating (*n* = 3). The MC phases were confirmed through venous serum blood samples, salivary testing, urinary ovulation test, and calendar-based counting. Data were analyzed using one-way repeated measures ANOVA**.**

Results: There were no statistically significant changes for the three different phases of the MC, in terms of almost all the physical performance for the whole group: maximal handgrip strength (Ibf): EFP (109.9 ± 11.2), the OV (109.2 ± 11.7) and MLP (108.2 ± 11.6) for the left hand, and for the right hand for EFP (118.4 ± 11.1), for OV (119.2 ± 9.1) and for MLP (119.2 ± 9.1). Maximal CMJ (cm): EFP (26.0 ± 4.4), for OV (24.9 ± 3.5) and for MLP (25.8 ± 3.0). Repeated CMJ (average height of five jumps [cm]): EFP (23.5 ± 3.0), OV (23.2 ± 2.5) and for MLP (23.4 ± 3.4). Maximal SJ (cm): EFP (24.5 ± 3.8), the OV (23.6 ± 2.5) and for the MLP (24.3 ± 3.5). 15-m sprint (s): EFP (2.70 ± 0.15), the OV (2.71 ± 0.15) and for the MLP (2.68 ± 0.13). Agility T-test (s): EFP (6.63 ± 0.52), the OV (6.68 ± 0.46) and for the MLP (6.64 ± 0.49). A statistically significant change was observed in the 1RM back squat: EFP (99.9 ± 14.6), the OV (110.9 ± 13.7) and the MLP (113.9 ± 14.8).

Conclusion: No statistically significant changes for maximal handgrip, CMJ repeated, CMJ max, SJ, agility T-test and 15-m sprint were found for the three different phases of the MC. A statistically significant change was found in the 1RM back squat test during one MC (EFP: 99.9 ± 14.6, OV: 110.9 ± 13.7, MLP: 113.9 ± 14.8), probably caused by a learning effect.

1 Introduction

In recent decades, the numbers of women participating in recreational exercise and elite athletic training and competition have increased dramatically (Greydanus & Patel, 2002; Goodman & Warren, 2005); (Javed et al., 2013); Costello et al., 2014). Despite the decreasing gap between men and women in sport and exercise participation, women continue to be highly under-represented in sport and exercise research (Costello et al., 2014); (Bruinvels et al, 2016); (Sheel, 2016). It is unlikely that such research can be transferred directly to women when referring to the anatomical, physiological, and endocrine differences between women and men. Arguably one of the most important factors when conducting research with female athletes is the potential influence that the menstrual cycle (MC) may have on athletic performance (Julian et al., 2021). The MC is an important biological rhythm, whereby large cyclic fluctuations in endogenous sex hormones, such as estrogen and progesterone that create significantly different transient hormonal profiles, which are used to differentiate between MC phases (De Jonge, 2003; Mihm et al., 2011). Other than reproductive function, these hormones influence many other physiological systems, and their action during exercise may have implications for exercise performance, including cardiovascular, respiratory, metabolic, and neuromuscular parameters (Oosthuyse & Bosch, 2010; Davis & Hackney, 2017; Chrousos et al., 1998). When estrogen rises during the late follicular phase (LFP) and the ovulatory phase (OVP) and remains elevated in the mid-luteal phase (MLP), it is plausible that this influences muscular performance (Baltgalvis et al., 2010; Lowe et al., 2010). Progesterone, on the other hand, has anti-estrogenic effects (Oosthuyse & Bosch, 2010). Therefore, the MC can theoretically affect sporting performance in different ways and should be in consideration for optimizing training and performance as well as and maintaining the general health and well-being of female athletes.

The knowledge of the effects of the MC is not fully understood. A recently published systematic review with meta-analysis examined the effects of the MC phases on exercise performance in eumenorrheic (non-users of hormonal contraceptives) women (McNulty et al., 2020). These data provide information that exercise performance might, on an average level, be reduced by a trivial amount during the early follicular phase (EFP) of the MC, compared with all other MC phases. They also identified large between-study variance in the effect of the MC on exercise performance, influenced by a range of methodological factors, small participant numbers (average n = 14), participants characteristics and exercise history.

Several authors have attempted to investigate the effect of different menstrual phases on physical performance. Romero-Moraleda et al. (2019) investigated the fluctuations of muscle performance in the 1RM Smith machine half-squat exercise in resistance trained eumenorrheic women (n = 13) during three different phases of the MC (confirmed by logging via calendar app, measurement of tympanic body temperature, body mass changes and urinary luteinizing hormone measurement). Overall, they found no statistically significant changes in force, velocity, and power output in all MC phases (Romero-Moraleda et al., 2019). These findings are in line with other studies that did not find any differences in physical performance in muscle strength and power during the MC (Lebrun et al., 1995; Gür, 1997); Fridén et al., 2003; Montgomery & Schultz, 2010; Ansdell et al., 2019; Romero-Parra et al., 2020).

Moreover, the eumenorrheic MC is exposed to internal (e.g., amenorrhea, oligomenorrhea and menorrhagia) and external (e.g., hormonal contraceptives) disturbances, highlighting the diversity in ovarian hormone profiles between women (Elliott-Sale et al., 2020). Hormonal contraceptives (HC) are widespread among female athletes (De Jonge et al., 2019) and among endurance and team sports athletes in Norway, Denmark, Ireland, and the United Kingdom, approximately half of the female athletes choose to suppress the production of endogenous sex hormones using HC (Engeseth et al., 2022; Brynhildsen et al., 1997; Solli et al., 2020; Martin et al., 2018). A review with meta-analysis by Martin et al. (2018) showed that out of 430 elite female athletes, 213 of them were HC users, meaning that almost 50% of the population surveyed did not have a eumenorrheic MC (Martin et al., 2018), even though a review with meta-analysis observed that oral contraceptives (OCP) might on average result in slightly reduced exercise performance throughout the MC compared with a naturally MC (Elliot-Sale et al., 2020).

Furthermore, hormone status in physically active women is highly individual and commonly influenced by hormonal contraceptive use, anovulation, luteal phase deficiency, or menstrual disturbances such as amenorrhea (i.e., absence of menstruation) (Schaumberg et al., 2017).

Over the last 30 years, considerable research has been undertaken to understand the cause(s) of menstrual dysfunction and low bone mineral density, both of which are frequently observed amongst high-performance female athletes. It is widely acknowledged that low energy availability (LEA) is the main factor underpinning these unfortunate health outcomes (Logue et al., 2018).

Team handball is a very strenuous body-contact sport with many high-intensity actions such as accelerations, decelerations, rapid changes of direction, sprints, and jumps (Michalsik et al., 2012). Handball is a physically demanding sport where success is determined by a complex interplay between many factors such as muscle strength and power, aerobic and anaerobic capacities, technical and tactical skills, throwing velocity as well as psychological factors (Hansen et al, 2018).

To date, there is strongly limited research to our knowledge on the influence of the menstrual cycle on female handball players' performance abilities. Therefore, the primary aim of this project was to analyze the effect of different hormonal phases across the menstrual cycle on physiological sports-specific capabilities of handball athletes, such as, maximal strength in lower extremities, maximal handgrip strength, maximal vertical jump ability, maximal sprinting speed and agility. The subjects were divided into a) natural menstruating group (NMG), b) hormonal oral contraceptive group (OCG) and c) hormonal implants group (HIG). The subjects were tested during the three 'critical' phases of the menstrual cycle: early follicular phase (EFP), ovulation (OV), and mid luteal phase (MLP).

2 Theory

2.1 Earlier research

As previously mentioned above, contradictory results from studies investigating the effect of the MC on physical performance could be caused by the different methods used for MC phase determination. Isokinetic test measurements have often interested investigators to study the physical performance of women during the different phases of the MC (Montgomery & Schultz, 2010; Gür 1997, Lebrun et al., 1997; Fridén et al, 2003). Montgomery & Schultz (2010) compared maximal voluntary isometric contraction (MVIC) torque of the knee flexors and extensors in recreationally active women (*n* = 71), at the early EFP, early luteal phase (ELP) and the MLP that was confirmed by serum hormone concentrations. The maximal isometric thigh strength did not change during the three different phases of the MC in this study (Montgomery & Schultz, 2010). A similar study by Gür (1997) investigated the concentric and eccentric isokinetic measurements in knee muscles using the Cybex 6000b computer-controlled isokinetic dynamometer during the MC (MC Day 1-3, day 8-10 and day 19-21) in eumenorrheic sedentary women (*n* = 16). The MC was verified with blood samples that were analyzed for sex hormones in the follicular phase (MC Day 8-10) and luteal phase (MC Day 19-21. No statistically significant changes were found in the concentric and eccentric peak torques and total works, and their reciprocal ratios in dominant knee muscle during the MC (Gür, 1997). Lebrun et al. (1995) also measured isokinetic muscle strength of knee flexion and extension during the EFP (cycle day 3–8) and the luteal phase (4–9 days after OV) and did not find any significant variation in performance between these phases (Lebrun et al., 1995). Fridén et al. (2003) also included measurement of isokinetic muscle strength together with maximal handgrip strength and a one-leg hop test. This was tested in moderately active premenopausal females with testing in the EFP, OV and MLP of the MC (confirmed by hormone sex analyses in serum and LH surge). No significant variation in muscle strength and muscle endurance during the MC was detected (Fridén et al., 2003).

Mhenni et al. (2017) investigated sports-specific physical performances in team handball female athletes during one MC in the luteal phase, between day 22-25 (monitored by menstrual calendar, basal body temperature charts and serum progesterone measurements), and examined the two different time-of-day effect (morning: 07:00-0830 am, evening: 05:00-0630) had on the performance. They concluded that among young female team handball players, handgrip, ball throwing velocity and modified agility T-test performances of the players were better in the evening than the morning (Mhenni et al., 2017). Dasa et al. (2021) examined the MC's effect on handgrip strength, 20-m sprint, counter movement jump and leg press in both eumenorrheic and HC-users (n = 29) who were highly trained female team sport-athletes (soccer, handball, and volleyball). When comparing the results from tests conducted in the follicular phase and luteal phase (confirmed by serum hormonal levels through venous blood samples, no statistically significant changes were observed).

2.2 The menstrual cycle

The menstrual cycle (MC) has been recognized as a vital sign that gives information about the overall health of an adolescent or young adult female. The first occurrence of menstruation (menarche) typically occurs at the age of 12-13 years (Hillard, 2014). It is common to have irregular periods during the first few years after menarche (Hillard, 2014). Women between the ages of approximately 13 and 50 years' experience a circamensal rhythm referred to as the menstrual cycle, during which the ovarian hormones fluctuate predictably. The MC usually comprises 28 days, but it can vary within the typical range of 21–35 days and is defined as eumenorrheic (Keizer & Rogol, 1990); Diaz et al., 2006). The MC is divided broadly into two phases, the follicular phase (FP), and the luteal phase (LP), separated by OV. The first day of menstrual bleeding is referred to as cycle day 1; the first day of the FP, which lasts approximately 14-15 days, until OV. The LP, which also lasts about 14 days, until the next onset of menstrual bleeding, follows OV. These phases are regulated by two female sex hormones, estrogen, and progesterone, as their levels rise and fall throughout the cycle (Brukner & Khan, 2010; Oosthuyse & Bosch, 2010).

2.2.1 The phases of the menstrual cycle

The MC is commonly divided into three phases, (1) the early follicular phase (EFP), characterized by low estrogen and progesterone, (2) the ovulatory phase (OVP), characterized by high estrogen and low progesterone, and (3) the mid-luteal phase (MLP), characterized by high estrogen and progesterone (De Jonge et al., 2019). The EFP begins with menstruation, which usually takes 4 to 6 days to complete; during this time, female sex hormone concentrations are all relatively low and stable. The EFP continues until ovulation occurs (late follicular phase) where there is a significant increase in the luteinizing hormone (LH) and estrogen to trigger OV. The increase in LH occurs 34–36 hours before OV and is a very reliable predictor of OV. After OV, the level of estrogen decreases but moderately rise later in the MLP, when progesterone levels reach its peak (Barriga-Pooley & Brantes-Glavic, 2019). The approximate timing of OV can variate, and therefore the whole MC can be highly variable (Soumpasis et al., 2020). If the oocyte is not fertilized, the corpus luteum dies off, estrogen and progesterone levels decrease, and is causing the uterine lining to be expelled as a menstrual bleeding, and marks a “stop” for the MC, and a new cycle begins (Marieb & Hoehn, 2007).

2.2.2 The female sex hormones

From adolescence until menopause, almost all women will experience monthly fluctuations in the female sex hormones estrogen and progesterone throughout the menstrual cycle (Davis & Hackney, 2017; Landgren et al., 1980). Other than reproductive function, these hormones influence many other physiological systems, and their action during exercise may have implications for exercise performance, including cardiovascular, respiratory, metabolic, and neuromuscular parameters (Oosthuyse & Bosch, 2010; Davis & Hackney, 2017; Chrousos et al, 1998).

2.2.3 Estrogen and progesterone

Estrogen and progesterone are released in fluctuating concentrations throughout the MC, resulting in the follicular (low estrogen) phase and the luteal (high estrogen) phase (Davis & Hackney, 2016). The sex hormone estrogen has been proposed to induce anabolic and muscle building processes in females (Lowe, Baltgalvis & Greising., 2010; Hansen et al., 2012). It has also been shown to attenuate muscle damage during phases of the MC with elevated circulating estrogen concentrations (Carter, Dobridge, & Hackney, 2001). Estrogen can influence the cardiovascular system, substrate metabolism, and the brain (Constantini et al., 2005).

Progesterone, another female sex hormone that plays an important role in the MC, as it's preparing the female body for conception and pregnancy (Dante et al., 2013). Progesterone has been associated with protein catabolism, conceivably attenuating muscle strength (Oosthuyse & Bosch, 2010). Progesterone and other progestins appear to mainly affect thermoregulation, ventilation, and the choice and use of fuel for energy needs (Constantini et al., 2005).

2.2.4 Hormonal contraceptives

In the general Nordic population 40% of women (15-49 years) are reported as users of hormonal contraceptives (HC), where combined oral contraceptives (OC) are the most common choice of HCs (Lindh et al., 2017). HCs can be divided into two main categories, progestin-only or combined, based on their concentration of synthetic estrogen and progestin (Burke, 2011; Shulman, 2011). The oral contraceptive pill (OCP) is usually prescribed in the form of a combined estrogen and progesterone tablet. The most obvious benefit of the OCP is its action as a reliable and reversible form of contraception. Other benefits include decreasing the risk of iron deficiency anemia by decreasing menstrual blood loss and allowing manipulation of the menstrual cycle for travel, training, and competition commitments. This allows one to take control of eventual premenstrual symptoms (Bennell et al., 1999).

Among endurance and team sports athletes in Norway, Denmark, Ireland, and United Kingdom, approximately half of the females choose to suppress the production of endogenous sex hormones using HC (Brynhildsen et al., 1997; Solli et al., 2020; Martin et al., 2018). Athletes in technical sports (e.g., golf, table tennis etc.) are showing a higher use of HC (80%) compared to athletes competing in endurance sports (50%) (Oxfeldt et al., 2020). A meta-analysis by Martin et al. (2018) showed that of 430 elite female athletes, 213 of them were HC users, meaning that almost 50% of the population surveyed did not have a eumenorrheic MC. Of these, 145 (68%) athletes reported using OCPs, making them the most common type of HC used and the second most common hormonal profile, after non-hormonal contraceptive users (Martin et al, 2018).

Although the use of hormonal contraceptives is widespread among female athletes, descriptive information about female athletes' experiences of using HC in the context of menstrual symptoms and exercise training and sports performance is today limited in both endurance and team-sports athletes (De Jonge et al., 2019).

2.3 Handball

Team-handball is an Olympic sport ball game that is characterized by fast-paced defensive and offensive action during the game with the objective of the game to score goals. The seven playing positions are: goalkeeper, left wing, left back, middle back, line player, right back and right wing. To score goals, the offensive players (6 players and one goalkeeper) attempt to establish an optimal position for the throwing player by fast movements over short distances performing powerful changes in direction (with and without the ball), one-on-one action against defensive players and passing the ball using different offensive tactics (Wagner et al., 2014). The intensities during 60 minutes of play vary between standing and walking, jogging and moderate running, stopping, jumping, sprinting, tackling and fast forward, sideward, and backward movements (Michalsik et al., 2012; Povoas et al., 2012; Karcher & Bucheitt, 2014). Despite the short duration of a game, these are actions that require a well-developed speed- and muscle strength (Massuca et al., 2014; Karcher & Bucheitt, 2014). Because of this, a sufficient level of endurance is important to keep up a high intensity in different actions throughout an entire game (2×30min) (Wagner et al., 2014).

2.4 Performance factors

2.4.1 Maximal muscle strength

Maximal strength refers to the maximum force or torque that can be exerted by the muscles (Knuttegen et al., 2003). Maximal dynamic strength is frequently assessed via a one-repetition maximum (1RM) protocol, which means that the maximum load that can be lifted one time is identified. There is a great importance of muscle strength to perform several high intensity speed-strength tasks during a handball match such as sprints, sudden changes of direction, jumps, blocks, pushes and throws and shots (Gorostiaga et al., 1999, 2005, 2006; Thorlund et al., 2008). This is supported by Grostiaga et al. (2005) who suggested that higher values of maximal strength and muscle power would result in a clear advantage in sustaining the forceful muscle contractions during team-handball specific movements (Wagner et al., 2014).

2.4.2 Handgrip strength

The maximum handgrip strength can be defined as a forceful flexion of all finger joints with the maximum voluntary force that the subject can exert under normal biokinetic conditions (Richards et al., 1996; Bohannon, 1997), which involves several muscles in the hand and the forearm (Bassey and Harries, 1993) The handgrip of a skilled arm in handball is important for the holding and throwing of the ball (Zapartidis et al., 2009).

2.4.3 Jumping ability

Maximal jumps aim to cover the highest vertical and/or horizontal distance from a large acceleration of the body during one quick legs extension (Samozino et al., 2010). The maximal vertical jumping height is influenced by several biomechanical and physiological factors. Ultimately, the jumping height is determined by the vertical velocity of the center of gravity at take-off (Oddson, 2008). A great force and power are produced in jumping movements. During jumping, the strengths of the ankle, knee and hip joint is central for a good, or sufficient, jumping performance (Dieserud et al., 2012, p.95).

Vertical resilience has been shown to have a high correlation with running speed and sprint acceleration (Cronin & Hansen, 2005; Requena et al., 2011; Wisloff et al., 2004), which indicates that many of the same factors are important for resilience and speed. A handball players jump height is important for the jump-throw action in team-handball, this to reach a high vertical position to throw over the block of the rival defensive players when throwing from a backcourt position, or to have more time for throwing (an increase in flight time) to mimic or to react to the movements of the goalkeeper. When in defense, jump height is important to block the rival offensive player when throwing (Wagner et al., 2014).

2.4.4 Agility

Team game players need to be exceptional movers in forward, lateral, back, and multidirectional movements in a very reduced area (Bloomfield et al, 2007). Handball has many high-intensity actions such as accelerations, decelerations, rapid changes of direction, sprints, and jumps (Michalsik et al., 2012). Most research (Bloomfield et al., 1994; Draper and Landcaster, 1985; Moreno, 1985) has applied the term ‘‘agility’’ to describe any dynamic sporting action that involves a change in body position or change of direction speed. Wagner et al. (2014) insist that tests including changes in direction might be more suitable to measure team-handball performance. The modified agility T-test (MAT) is recommended for sports such as volleyball, basketball, and tennis because they are based on short, repeated displacements (Sassi et al., 2009).

2.4.5 Maximal sprinting

Running speed is somewhat an important prerequisite factor in competitive handball (Fleck et al., 1992). The importance of sprint capacity may vary between the playing positions, and it is observed for the ving players it may be of a greater importance (Waldron et al., 2011). During matches, players usually do not cover large distances, they almost only perform short sprints (less than 20 m) (Alexander & Boreskie, 1989). According to Wagner et al. (2014) sprints are often used in practice to increase the performance in team ball sport games.

3 Method

3.1 Participants

Twenty-two subjects were recruited to this study (age = 20 ± 3.6) yrs; body mass = 69 ± 6,1 kg; body height = 167,8 ± 6,8 cm; body fat percentage = 27.7 ± 3.3). Subjects were both users and non – users of hormonal contraception, such as oral combined birth control pills (n = 4), mini-pills (n = 3), arm implant (n = 3), hormonal IUD (n = 2), non-hormonal IUD, copper IUD, (n = *1*) and non – users of hormonal contraception (n = 9), who were considered as eumenorrheic. Subjects were active female handball players playing in 2nd (national) and 3rd (regional) division as well as age-specific series like 16yrs-series, in Northern-Norway. Twenty-two completed the baseline testing, and four subjects dropped out due to injury or motivational reasons, leaving the participant number at n = 18. In total, 10 subjects completed the MC-phase based testing, and were divided into three groups based on hormonal contraceptive status: normal menstruation group (NMG) (n = 3), oral contraceptive group (OCG) (n = 2) and hormonal implant group (HIG) (n = 5). The HIG included users of arm implant (n = 3), mini-pills (n = 1) and hormonal IUD (n = 1).

3.2 Overall study design

This study aimed to investigate the fluctuations of physiological sports-specific capabilities during three different phases of the menstrual cycle (MC) of female handball players. The three phases of the MC were EFP, OV and MLP, because they represent the main events occurring during the menstrual cycle (i.e., menses, pre-ovulation, and peak progesterone concentration, respectively (De Jonge, 2003).

To investigate this topic 22 female handball players in Tromsø (Norway) were recruited to perform a testing of physiological sports-specific capabilities such as the maximal lifting weight in a 1 repetition maximum (1RM) back squat using a Smith machine, maximal handgrip strength, maximal vertical jumping ability, modified agility T-test and sprint ability, all tested in three critical phases (EFP, OV, MLP) of the MC. The subjects were divided into a) naturally menstruating group (NMG), b) oral contraceptive group (OCG) and c) hormonal implants group (HIG). In total, 10 subjects completed the MC-phase based testing. The MC phases were confirmed through serum hormonal levels determined from blood samples and saliva tests taken each of three test days during one MC. The testing took place from April 2022 – May 2022, and due to Covid-reasons this was during the end of the season for most players. As the study was considered by the regional ethical committee to be outside their mandate, the study protocol was approved by the Norwegian center for research data (NSD).

3.3 Equipment and procedure

3.3.1 Baseline testing

At baseline, all subjects completed the LEAF-Q questionnaire: a screening tool for the identification of female athletes at risk for the female athlete triad (Melin et al., 2014). The questionnaire comprises questions about age, height, weight, and BMI, as well as more comprehensive information (sleep, nutrition, digestion, and metabolism) for purposes beyond the scope of this study.

All tests were performed indoors at the university's gym and laboratory located at Alfheim Stadion. Subjects went through a first session, independent of the MC with all the physical tests as a familiarization of the phase-specific MC related tests. The first session also included treadmill running to determine running economy, maximum oxygen uptake (VO_{2max}) and anaerobic capacity. In addition, a DXA scan (GE Healthcare, Madison, WI, USA) to measure body composition was completed on a separate day within approximately 2 weeks of the first session. Upon arrival to the laboratory, height and weight were measured. Before testing subjects performed a 10-minute warmup including 5 min cycling on a cycle ergometer and 5 minutes easy stretching through a standard protocol (see Figure 1). In the following order subjects preformed a 1RM back squat test in the Smith Machine, CMJ and SJ jumps on a force platform, a maximal handgrip strength test, a modified agility T-test, 15 m sprint, and a treadmill running test to determine running economy, VO_{2max} and anaerobic capacity (see Table 2 and Table 3 for results). The running test was excluded for the phase specific MC-testing. Although maximum oxygen uptake was not a MC- phase specific test, this physiology-based outcome was included as it can be used as an indicator of the subject's cardiovascular fitness. The performance time for the modified agility T-test and the 15-m sprint was measured via the use of electronic timing systems that were placed at the start and the finish lines. For the 15-m sprint, split-times at 5 and 10 m were also determined. Subjects were instructed to use preferable and appropriate clothing and shoes for the physical testing. There were 3-4 min rest between all the tests. Subjects were encouraged to avoid hard resistance strength training two days prior to the testing day, and to have a normal food and liquid intake. Specific details related to the testing procedures for each specific test are described below.

Afterward, subjects performed three experimental trials within their MC and repeated the same tests, i.e., apart from the LEAF-Q, DXA and the treadmill running test (see Figure 1 for an overview of the testing day). The MC-phase based testing took place at the same time of the day for each subject, this to avoid variation in muscle function indicators (Mhenni et al., 2016).

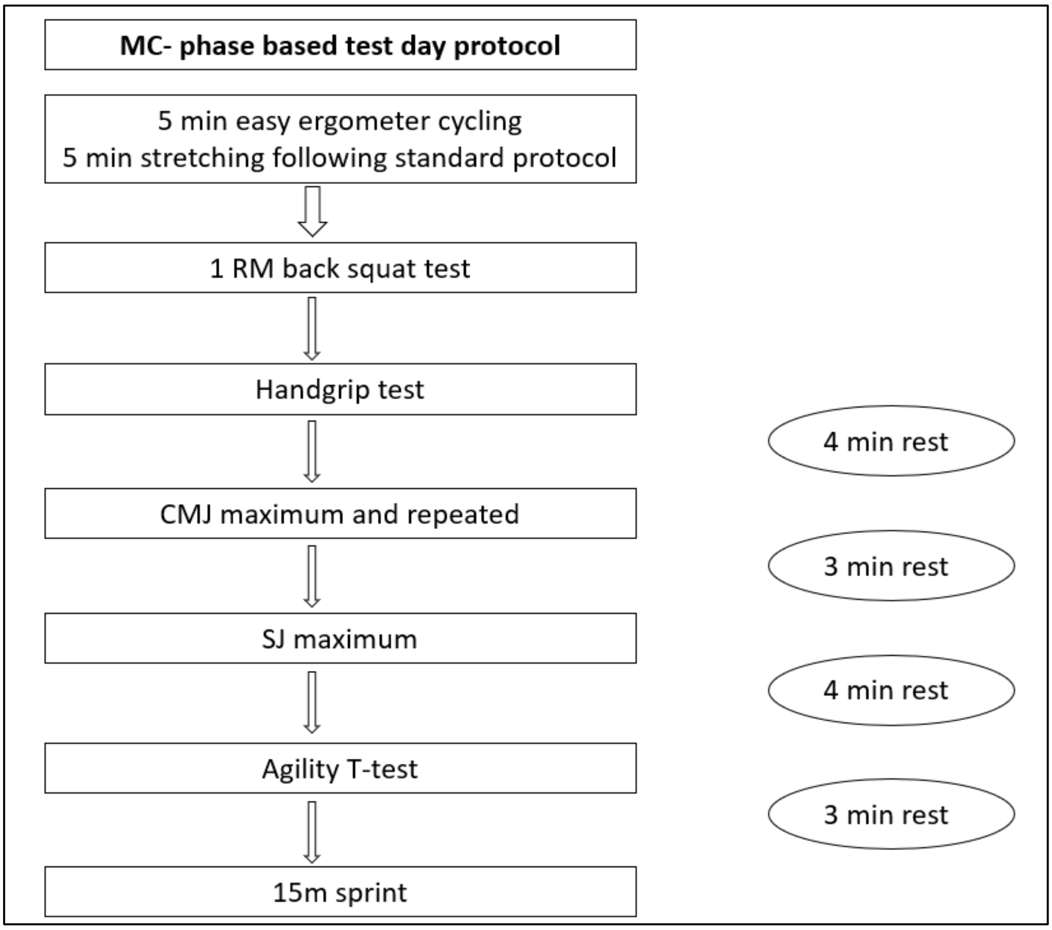

Figure 1. *Flow chart display of the protocol followed in the MC-phase based testing for the physical testing in 1 repetition maximum back squat, maximal handgrip strength, countermovement jump (CMJ), squat jump (SJ), agility T-test and 15 m sprint. Abbreviations: MC, menstrual cycle; min, minutes.*

3.3.2 Determination of the menstrual cycle

The duration of the MC and the onset of each phase were determined using: 1) saliva hormone test, 2) venous blood sample tests, 3) an OV-test and 4) a menstrual cycle tracker application. To provide hormonal confirmation of MC phase, subjects provided fasted saliva tests and venous blood samples at the hospital in the morning, the same day as the physical testing. This was to avoid the effect of physical strain and nutritional intake on hormonal levels. Measurement of serum estrogen and progesterone concentration is recommended as the gold standard for research purposes (De Jonge, 2019). The eumenorrheic subjects (NMG) used a calendar app (Flo Fem®), to predict their menstruation and OV. All subjects were given a digital OV- test kit (Clearblue®) to determinate the day of OV. For this purpose, subjects were instructed to use the test kit from “day 9” after their first menstruation day. This method is a less invasive and less expensive method to get a more direct indication of hormone fluctuations over the MC by measurement of the luteinizing hormone (LH) surge in urine (De Jonge, 2019). It was important that they did not urinate 4 hours before testing and avoided any intake of fluid or food before testing for OV. When a positive result (a smiley) appeared, subjects informed the test leader, and the time and date were noted. The subjects that were on hormonal contraceptives were told it was expected and normal if they did not get any positive result. The NMG came in for testing the day after a positive OV-test. The subjects were given one bag with an OV-test kit and tubes for the blood and saliva tests. This bag included smaller marked bags with tubes for the tests in EFP, OV, and MLP throughout the MC. When the menstrual cycle was detected, there was booked an appointment for the subjects to go to the hospital to take the blood- and saliva tests. Before eating breakfast or brushing their teeth they did the first saliva test at home and brought it for their hospital appointment. The appointments were between 0730-0930 am. Subjects were informed that after their hospital visit, they could go home and eat etc., and later in the afternoon they would complete the physical testing. The physical testing took place at the same time for every testing.

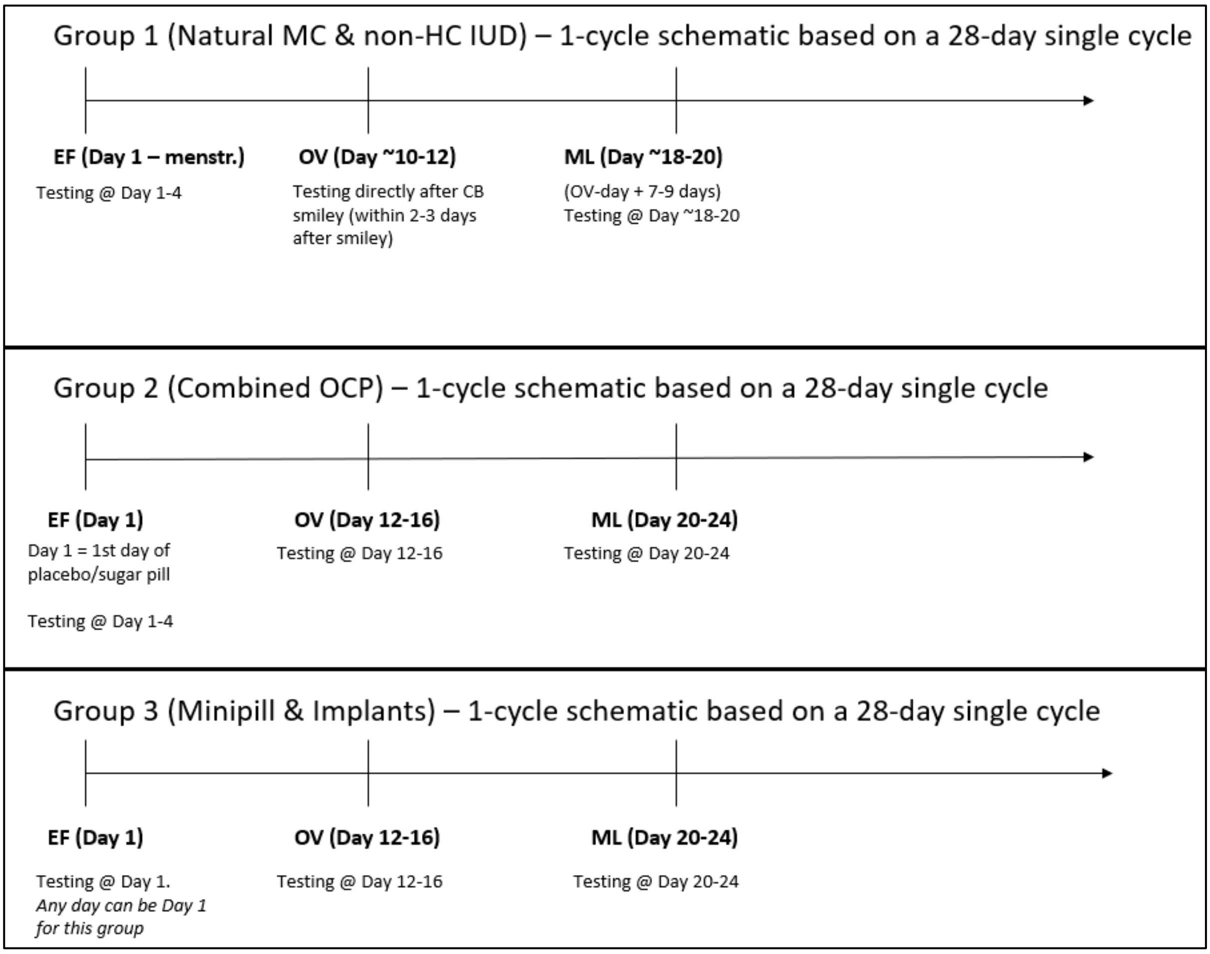

Figure 2. *Flow chart of the testing procedure for the naturally menstruating group (group 1), oral contraceptive group (group 2) and hormonal IUD group (group 3) during the three different phases of the MC i.e., the early follicular phase (EFP), ovulation (OV) and mid luteal phase (MLP). Abbreviations: MC, menstrual cycle; non-HC IUD, non-hormonal contraceptive intrauterine contraceptive device.*

3.3.3 DXA

Knowledge of the physical and anthropometric characteristics of athletes has been considered valuable in talent identification and training planning (Abdelkrim et al., 2010). The dual-energy absorptiometry (DXA) analyses body composition at the molecular level that is basically translated into a clinical model made up of fat mass, non-bone lean mass, and bone mineral content (Bazzochi et al., 2016). DXA (GE Healthcare, Madison, WI, USA) is increasingly used to measure body composition in terms of fat and fat-free mass (Laskey, 1995). The subjects came in for the DXA scan in the morning between 0730-0900 am before having breakfast. This was done as a single test in connection with the baseline testing (within ~1-2 weeks). Subjects were told to use sports clothes without any metal and to remove eventual body piercings.

3.3.4 1RM back squat in Smith Machine

To examine the subjects' maximal dynamic leg strength, a 1RM test using a Smith-machine was performed. Two-three weeks before the onset of the experiment, participants performed a familiarization session to minimize any learning effects during the experiment, and to standardize the loads in the subsequent experimental sessions. Subjects were instructed to perform a squat to 90º of knee flexion until the barbell was touching or almost touching the safety stoppers on the side, and then ascend back to an upright position. The protocol for the familiarization test were ~40% predicted 1RM (6 REP), ~55% predicted 1RM (5 REP), ~70% (4 REP) predicted 1RM, ~80% predicted 1RM (3 REP), ~90% predicted 1RM (2 REP), ~95% (1 REP). This protocol was used because most of the subjects did not know their 1RM and for the subjects to get used to the Smith machine.

In the MC specific testing the subjects commenced the 1RM assessment with sets of increasing loads estimated to be ~20% 1RM (3-REPs), ~40% 1RM (3-REPs), ~60% 1RM (3-REPs), ~80% 1RM (1-REP), and ~90% 1RM (1-REP; Banyard, Nosaka & Haff, 2017). This was then followed by the first 1RM attempt with a maximum of five 1RM attempts permitted. After a successful 1RM attempt, the barbell load was increased, in consultation with the subject, between 1.25 and 2.5 kg. The last successful lift with a correct technique was classified as the 1RM load. The rest periods between all warm-up sets were ~2 minutes with ~3 minutes of rest between the 1RM attempts. One subject did not complete the 1RM-test throughout the MC because of injury, leaving the participant number for this test to n = 9.

3.3.5 Handgrip strength

The maximal handgrip strength of both hands was measured with a calibrated hydraulic hand dynamometer. Grip strength in pounds was measured using a standardized protocol; subjects were standing with their arm straight down leaning towards the side of the body, the handle was adjusted according to hand size and subjects were squeezing the dynamometer for ~5 s max as hard as they could. The palm did not flex on the wrist joint (Visnapuu & Jürïäe, 2007). The left hand was measured first then the right hand and the dominant hand was noted. Time was taken by the test leader. Two trials were performed with 2 min rest in between both trials (Mhenni et al, 2017). The highest readings for the left and right hands were used for further analyses.

3.3.6 Countermovement jump (CMJ)

The subjects preformed the vertical jumping tests on a force platform. Maximum vertical jumping ability was assessed using countermovement jump (CMJ) and squat jump (SJ). Subjects were instructed to keep their hands on the hip throughout the whole movement. CMJ was initialized with a squat down to preferred bottom, then jumping upward with maximum effort without letting go of the hands on the hip during the whole movement. Both of their feet should naturally land on the force plate. The protocol for the CMJ's was three jumps with ~30 s passive rest in-between, then followed by five repeated jumps with ~7 s passive rest in-between. Maximum jump height (cm) was assessed, based on force impulse, using an automated software (MuscleLab software; MuscleLab Technology, Langesund, Norway). All trials were recorded, and the highest of the three first jumps were noted as well as the average jump height of the five repeated jumps.

3.3.7 Squat jump

There was a ~2 min rest between CMJ and squat jump (SJ). The subject was standing on a force platform with both feet at shoulder width squatting down to 90° with both hands on the hip and holding the position for ~2 s, then jumping upwards with maximal effort without making a "drop" from the bottom position. Three jumps were performed with ~30 s passive rest in-between. Maximum jump height (cm) was assessed, based on force impulse using the same automated software as was used for the CMJ tests (MuscleLab software; MuscleLab Technology, Langesund, Norway). All trials were recorded, and the highest and jump were noted.

3.3.8 Modified agility T-test

The modified agility T-test (MAT) was used as a relevant test for handball players to determine speed with directional changes such as forward sprinting, left and right shuffling, and backpedaling (Mhenni et al., 2016). Based on the protocol outlined by Pauole et al. (2000), subjects began with both feet 30 cm behind cone A. Preferred forward foot was placed at the line 30 cm in front of cone A. A pair of infrared Brower Timing System Photocells, model TRD- T175 (Draper, UT, USA) were placed by cone A facing each other. At her own discretion, each subject sprinted forward to cone B and touched the base of it. Facing forward and without crossing the feet, the subject shuffled to the left to cone C and touched its base, then shuffled to the right to cone D and touched its base and shuffled back to the left to cone B and touch its base. When touching cones, the preferred hand was used. Finally, subjects ran backward as quickly as possible and returned to line A. Any subject who failed to touch the base of the cone, and/or failed to face forward throughout had to repeat the test. The subjects preformed three test trials, with ~3 min of rest between trials. The recorded score for this test was the best of the three trials.

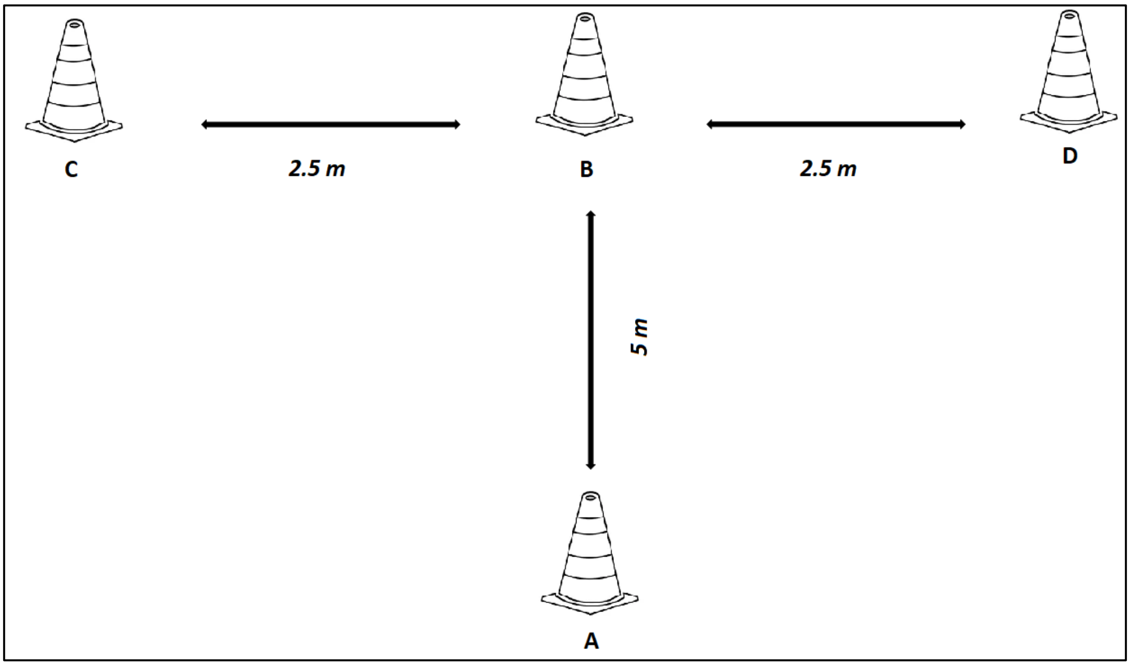

Figure 3. *Illustration of the modified agility T-test.*

3.3.9 15-m sprint

There was a ~ 4 min rest between the agility test and the 15-m sprint. The maximal 15-m sprint effort was measured by using a linear sprint test, i.e., forward sprinting over 15 m as fast as possible from the start position. Subjects were instructed to be in a preferred start position with the preferred forward foot in front of the line that was 30 cm from the first laser. They started on a "ready – set – go"- command from the test leader. The subjects preformed three test trials with a ~3 min rest between each trial. Time for 5 m, 10 m and 15 m meters were determined by single-beam electronic photocells (ATU-X, IC control AB, Stockholm, Sweden) mounted to the floor and walls. The recorded time for this trial was the best of the three trials.

3.4 Statistical analyses

The differences in the performance data between the three phases of the menstrual cycle (EFP, OV, MLP) were analyzed using one-way repeated-measures analysis of variance (ANOVA). The statistical threshold was set at $p < 0.05$. The results are presented as mean ± standard deviation (SD). The one-way repeated measures ANOVA was used to analyze the results for the three MC-phases (EFP, OV and MLP). The Statistical Package for the Social Sciences (SPSS, Version 26, IBM, Armonk, NY, United States) was used for the statistical analyses.

4 Results

A total of 19 participants were included in the study, and 10 were included for the MC-phase based testing and were divided into three groups: a normally menstruating group (NMG) (n = 3), an oral contraceptive group (OCG) (n = 2) and a hormonal implant group (HIG) (n = 5). The descriptive data showing mean values ± standard deviation (±) of subject characteristics in the hormonal contraceptive groups and the non-hormonal contraceptive group as well as for all ten subjects combined are presented in Table 1. Nine out of ten subjects completed the 1RM-back squat test, with the results being presented in Figure 3A, and there was a statistically significant difference between the three phases of the MC phases with EFP showing significantly lower values than the OV and the MLP (both $P < 0.001$).
For the maximal handgrip strength there were no statistically significant differences between the three phases of the MC for the left hand and the same goes for the right hand (see Figures 3B-C).

There no statistically significant differences between the three phases of the menstrual cycle phases in the maximal CMJ, for the EFP, OV and MLP, and the same goes for the results for EFP, OV and MLP for the repeated CMJ-s (see Figures 3D-E). There were no statistical differences between the three phases of the MC in maximal SJ jump height (see Figure 3F). For the 15-m linear sprinting, there were no statistical differences between the three phases of the MC (see Figure 3G), and the same goes for the results for EFP, OV and MLP for the modified agility T-test.

Table 1: *Descriptive data presented as mean values ± standard deviation of subjects (n = 10) characteristics in the non-hormonal contraceptive group (NMG), oral hormonal contraceptive group (OCG), hormonal implants contraceptive group (HIG) and in total.*

	Total	**NMG**	**OCG**	**HIG**
(n)	10	3	2	5
Age (yrs)	19.9 ± 2.9	20.3 ± 3.0	18.0 ± 2.0	20.4 ± 2.8
Height (cm)	166.7 ± 6.0	169.6 ± 0.4	166.3 ± 7.1	165.0 ± 5.4
Body mass (kg)	67.7 ± 4.1	68.1 ± 2.9	63.2 ± 1.0	69.3 ± 3.4
VO2max (ml·kg·min)	46.4 ± 4.15	49.0 ± 3.8	42.2 ± 0.6	46.5 ± 2.0
Whole body fat (% of body mass)	27.6 ± 3.1	27.9 ± 3.4	24.7 ± 1.0	28.6 ± 3.2
Fat mass (kg)	18.7 ± 3.7	19.2 ± 3.3	15.3 ± 0.7	28.6 ± 3.2
Lean mass (kg)	46.3 ± 2.3	47.4 ± 0.8	44.0 ± 0.4	46.5 ± 2.8
Bone mineral content (g)	2623 ± 228	2619 ± 301	2527 ± 129	2663 ± 247

Table 2: *Descriptive data presented as mean values ± standard deviation of subjects (n =* 22) *anthropometric characteristics.*

Age (yrs)	**Bodymass (kg)**	**Height (cm)**	**Whole body fat (%) of body mass**	**Fat mass (g)**	**Lean mass (kg)**	**Bone mineral content (g)**
20	69	167.8	27,7	19176,1	46989	2683,6
± 3.6	± 6.1	± 6.8	± 3	± 3563,1	± 3357,7	± 282,7

Table 3: *Descriptive summary of test results in 1RM back squat, handgrip left hand (L), handgrip right hand (R), maximal countermovement jump (CMJ max), countermovement jumps repeated average (CMJ repeated AVG), maximal squat jump (SJ max), 15 m sprint, agility T-test and VO_{2max} from the baseline session presented as mean ± standard deviation. Subjects (n=* 22*) with age* 20 ± 3.6 *and with height and weight of* 167.8 ± 6.8 *cm and* 69.0 ± 6.1 kg *constituted this sample.*

Strength			Jump			Sprint		VO2max
Squat (kg)	**Handgrip L** (lf)	**Handgrip R** (lf)	**CMJ max** (cm)	**CMJ** **repeated AVG** (cm)	**SJ max** (cm)	**15-m** (s)	**Agility** (s)	**(mL/kg/min)**
89.4 ± 16.1	104.9 ± 10.8	113.2 ± 11.4	27.1 ± 4.0	23.1 ± 3.2	25.3 ± 3.6	2.72 ± 0.14	6.86 ± 0.49	45.14 ± 4.15

Table 4*: Descriptive summary of test results in 1RM back squat, handgrip left hand (L), handgrip right hand (R), maximal countermovement jump (CMJ max), countermovement jumps repeated average (CMJ repeated AVG), maximal squat jump (SJ max), 15 m sprint, agility T-test and VO_{2max} from the baseline session presented as mean ± standard deviation. Subjects (n=* 10) *with age* 19.9 ± 2.9 *who completed the MC-specific testing with height and weight of* 166.7 ± 6.0 cm *and* 67.7 ± 4.1 kg *constituted this sample.*

Strength			Jump			Sprint		VO2max
Squat (kg)	**Handgrip L** (lf)	**Handgrip R** (lf)	**CMJ max** (cm)	**CMJ** **repeated AVG** (cm)	**SJ max** (cm)	**15-m** (s)	**Agility** (s)	**(mL/kg/min)**
87.2 ± 15.1	105.6 ± 13.7	114.7 ± 12.8	25.9 ± 4.2	22.7 ± 3.4	24.4 ± 2.3	2.7 ± 0.1 s	7.0 ± 0.5	46.4 ± 3.7

5 Discussion

The aim of this study was to examine potential changes in performance in 1RM back squat, maximal hand grip strength, maximal vertical jumping ability, agility, and 15-m maximal linear sprinting during three critical phases of one MC in a group of female handball players. One-way ANOVA did not reveal any statistically significant changes for the three different phases of the MC (i.e., EFP, OV and MLP), in terms of physical performance in maximal handgrip strength, maximal vertical jumping height, agility, or maximal sprinting for the whole group. To the authors knowledge this is the first study to investigate the effects of the menstrual cycle phase on different standardized sport-specific physical parameters in female handball players following the methodological recommendations in such research (De Jonge, 2019).

The findings in this study are consistent with the finding by Romero-Moraleda et al. (2019) who investigated the effect of the menstrual cycle on strength and power performance in resistance-trained eumenorrheic females (*n* = 13), together with Lebrun et al. (1995), Grü (1997), Fridén et al. (2003), Montgomery & Schultz (2010), Ansdell et al. (2019) and Romero-Parra et al. (2020) who all did not find any differences in physical performance in muscle strength and power. The mentioned studies included isokinetic muscle strength exercises in their experiments, which may not be a very sports-specific choice of exercises, nor is it very transferable to everyday physical actions. In this study we choose to include more handball-specific physical exercises that athletes are familiar with and used standardized protocols for all tests. Mhenni et al. (2016) investigated jumping ability with CMJ-test during repeated shuttle sprints. Compared to isolated jump protocols this test is even more transferable and specific for handball. Wagner et al. (2014) developed and validated a game-based performance test that is suitable to measure team-handball specific agility and sprinting performance, VO_{2max} and HR_{max} under conditions similar to team-handball competition. To sum this up, it is difficult to directly compare this study with not only the mentioned studies, but also the majority of studies done in this research field of female athletes and the MC because of variating methodological methods detecting the MC, participant characteristics including age, hormone status, exercise level, and very different types of exercises.

Although, the one-way ANOVA did reveal statistically significant changes in the 1RM back squat throughout the MC (EFP: 99.9 ± 14.6, OV: 110.9 ± 13.7 and MLP: 113.9 ± 14.8), the author believes that this was due mainly to a learning effect. This is because magnitude of the change was surprisingly high and there were no statistically significant changes in the other respective physical performances. The baseline 1RM strength did not provide a strong predicative measure of strength adaptation for most subjects for the MC-phase testing. The protocol for 1RM back squat in the baseline testing had more repetitions and sets than the one used in the MC-phase specific testing. The subjects did complete one experimental attempt for every test, to minimize potential bias through a learning effect. A few more familiarization trials, especially for the 1RM back squat test using the MC-specific protocol, should have been included.

The subjects were all active handball players, but the training volume in both team practice and physiological exercising between the players, teams, and the levels they were playing at (16yrs series, regional 3rd, and national 2nd division) were variating. Some subjects stated that they were not so familiar with heavy-loaded back squats. We chose the 1RM back squat testing to be performed with the Smith machine because the decreased need for balancing the bar and weight plates may increase the safety through the movement as it requires less balance of an external load, compared to a maximal lift with free weights (Cotterman et al., 2005).

Future research in team handball should include participants with similar training backgrounds and choose sports-specific exercises that can be used effectively in women's handball programs. Information on training-related issues of female handball-athletes such as anthropometric measurements, physiological attributes together with on-court performances can be used effectively for optimizing strength and condition programs (Manchado et al., 2013). The MC of female athletes should be taken into consideration by the players and coaches for optimizing training. Finally, because of the small participant number ($n = 10$) no analysis between-groups were made, and this might be a line of investigation interesting to follow in future studies.

5.1 Limitations

As with any scientific study, the present findings include some limitations. The handball season for the respective subjects was strongly affected by COVID-19, with a long break from the middle of December 2021 to the end of February 2022, where no team training or games were allowed, which lead to an extended season and therefore delayed the start of this study considerably, also the easter break made the testing more problematic. This is mentioned because it is likely that it would have been easier with the recruitment of more players if the study were performed after the season, which could have resulted in a larger participant number. Because of deadlines it was impossible to get all subjects recruited through the MC-phase based testing. The subjects were divided into three groups based on their HC status and they all completed the same procedures, and because of the small participant number no analysis between-groups were made. With a higher total sample of participants completing the MC-specific testing, it would be interesting to compare the sex hormone concentrations between all the three groups. Unfortunately, the total final sample were quite low (n = 10), and due to the submission deadline, we were not able to get the results from the blood analyzes, so we could not confirm that the subjects were in the different stages of their respective cycles, i.e., changes in hormone values could not be observed.

A limitation of this study is that the order of the MC phases was not randomized, whereby all participants first completed testing in the EFP followed by the OV and then the MLP, this was due to the current method of cycle phase determination. Except for the NMG who first completed testing either between day 1 to day 4, or when a positive result appeared on the digital OV- kit. By tracking serum hormone concentrations to capture the hormone environments during the same day of testing we would be able to create individual hormone profiles for each participant (Montgomery & Schultz, 2010). Such information would be interesting as the fluctuation in sex hormones is highly individual during the MC (Dasa et al., 2021).

A strength of this study is the determination of the MC by following recommendations from De Jonge (2019) by using calendar-based counting, salivary testing, serum venous blood sample measuring the concentrations of estrogen and progesterone, as well as measuring the LH surge in urine to get a direct indication of the OV (De Jonge, 2019). In contrast to this study where only measurements were conducted over one MC, measurements over more than one MC would increase the validity and might have resulted in significant changes in the physical performance variables due to a potentially lowered test-to test variation due to reduced “learning/training effects”. However, such an approach would then have been even harder to fulfill as it would have been even more problematic with participant recruitment and increased the number of dropouts during testing.

6 Conclusion

This study detected no statistically significant changes for the three different phases of the MC, in terms of physical performance in maximal handgrip, CMJ, SJ, agility and 15-m sprint. However, a statistically significant change was found in the 1RM back squat test during one MC (EFP: 99.9 ± 14.6, OV: 110.9 ± 13.7, MLP: 113.9 ± 14.8) which was probably caused by a learning effect and not affected by the phases of the MC.

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