Methodological Recommendations for Menstrual Cycle Research in Sports and Exercise

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ABSTRACT

JANSE DE JONGE, X., B. THOMPSON, and A. HAN. Methodological Recommendations for Menstrual Cycle Research in Sports and Exercise. *Med. Sci. Sports Exerc.*, Vol. 51, No. 12, pp. 2610–2617, 2019. **Introduction:** The aim of this review is to provide methodological recommendations for menstrual cycle research in exercise science and sports medicine based on a review of recent literature. Research in this area is growing but often reports conflicting results, and it is proposed that some of this may be explained by methodological issues. **Methods:** This review examined the menstrual cycle verification methods used in recent literature on exercise performance over the menstrual cycle identified through a literature search of PubMed and SportDiscus from 2008 until 2018. **Results:** Potential changes over the menstrual cycle are likely related to hormone fluctuations; however, only 44% of the selected studies measured the actual concentrations of the female steroid hormones estrogen and progesterone. It was shown that the likely inclusion of participants with anovulatory or luteal phase–deficient cycles in combination with small participant numbers has affected results in recent menstrual cycle research and, consequently, our understanding of this area. **Conclusion:** To improve the quality of future menstrual cycle research, it is recommended that a combination of three methods is used to verify menstrual cycle phase: the calendar-based counting method combined with urinary luteinizing hormone surge testing and the measurement of serum estrogen and progesterone concentrations at the time of testing. A strict luteal phase verification limit of >16 $nmol \cdot L^{-1}$ for progesterone should be set. It is also recommended that future research should focus on the inclusion of the late follicular estrogen peak. It is envisaged that these methodological recommendations will assist in clarifying some of the disagreement around the effects of the menstrual cycle on exercise performance and other aspects of exercise science and sports medicine. **Key Words:** ESTROGEN, PROGESTERONE, HORMONAL VERIFICATION, REVIEW, PERFORMANCE

A recent review of sports and exercise medicine research demonstrated a clear underrepresentation of female participants with just 4%–13% of articles including females only (1). Even when female participants are included in exercise science research, often no allowance is made for female hormone fluctuations, or testing is set to occur when hormone levels are low to reduce potential effects (2). The female steroid hormones, estrogen and progesterone (and their interactions), have numerous physiological effects, which are likely to have implications for outcomes in exercise science and sports medicine research. Therefore, it is important to not only include more female participants in research but also stop regarding the hormone fluctuations throughout the menstrual cycle as a confounding factor and instead focus specific research on the important physiological implications of these female hormone fluctuations.

Regular fluctuations in female steroid hormones occur during ovulatory menstrual cycles. Despite individual variations, the menstrual cycle is conventionally allocated 28 days (see Fig. 1). The onset of menses is day 1 of the cycle and marks the start of the follicular phase. In the early follicular phase, gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. FSH and LH stimulate follicle maturation and estrogen production. Via positive feedback, the increased secretion

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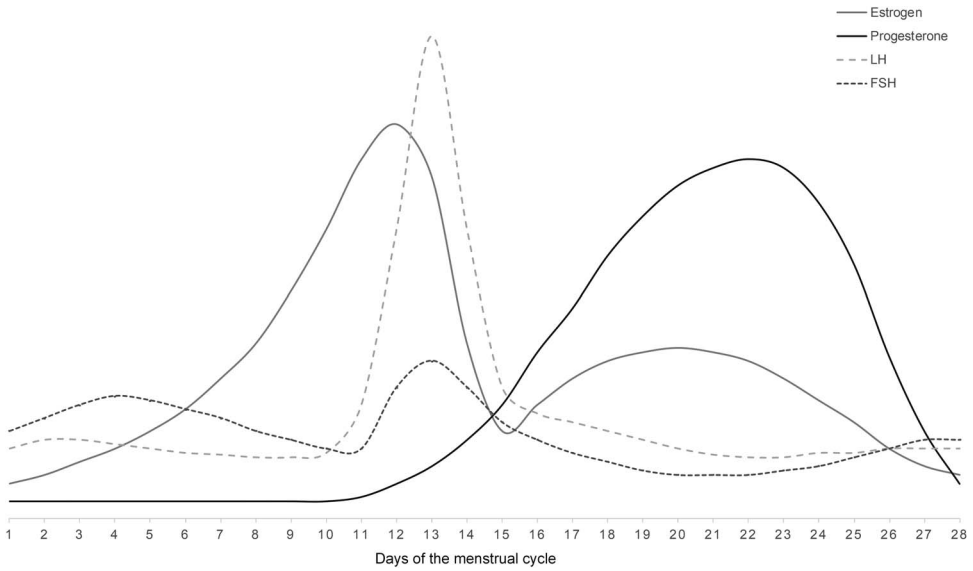

FIGURE 1—Overview of the fluctuations of estrogen, progesterone, FSH, and LH throughout a regular ovulatory menstrual cycle lasting 28 days (day 1 is onset of menses and day 14 is ovulation).

of estrogen causes a surge of LH, which triggers ovulation. At ovulation (day 14 in Fig. 1), the mature egg (oocyte) is released from the follicle and the corpus luteum is formed. This marks the start of the luteal phase. During the luteal phase, both progesterone and estrogen are secreted in large quantities by the corpus luteum. Rising levels of estrogen and progesterone act via negative feedback to reduce the secretion of FSH and LH. If the oocyte is not fertilized, the corpus luteum dies off, estrogen and progesterone levels decrease, and a new cycle begins (3). On the basis of these fluctuations, three distinctly different hormonal environments can be identified during the early follicular phase (low estrogen and progesterone), the late follicular phase (high estrogen and low progesterone), and the mid–luteal phase (high estrogen and progesterone).

Many reproductive-age females with regular menstrual cycles, however, do not experience these monthly hormone fluctuations. Luteal phase–deficient (LPD) and anovulatory cycles are characterized by an inadequate LH surge due to disrupted GnRH pulsatility, resulting in reduced estrogen and progesterone production (4). In particular, in physically active females, there is a high prevalence of anovulation and LPD (30%) (5). This percentage can be as high as 50% in heavily exercising females (>450 min·wk^{-1}) (6). These ovulatory disturbances in active females are thought to be an adaptation to negative energy balance. In this state, it has been suggested that changes in ghrelin, peptide YY, and leptin mediate reproductive dysfunction (for further details see a comprehensive review by Scheid and De Souza (4)). In an energy-deficient state, vital physiological processes are prioritized over reproductive function (6). As ovulatory disturbances often occur in females with apparently normal cycles without any clinical presentation or symptoms of abnormality (5), the presence of a regular menstrual cycle with monthly bleeding does not confirm an ovulatory menstrual cycle with its associated hormone fluctuations. To be able to draw conclusions about potential differences in exercise performance and other parameters over the menstrual cycle, it is therefore paramount to accurately verify if participants are experiencing a regular ovulatory menstrual cycle at the time of testing.

The limited amount of sports medicine and exercise science research that has considered the female hormone fluctuations throughout the menstrual cycle has provided conflicting results, with many studies reporting no changes over the menstrual cycle. It is hypothesized that much of the disagreement in recent research on exercise performance over the menstrual cycle may be explained by methodological differences. The purpose of this review is to firstly focus on the menstrual cycle verification methods used in recent research (past 10 years) on exercise performance over the menstrual cycle. A brief review of the exercise performance outcomes in conjunction with each of the methods used will then assist in the formulation of recommendations for menstrual cycle verification and further methodological considerations to assist in the growth of high quality menstrual cycle research.

LITERATURE SEARCH

The focus of this review is on the methodological aspects of current menstrual cycle research. The aim is not to provide a comprehensive overview of effects of the menstrual cycle on elements of exercise performance but instead to highlight some of the methodological issues that may affect our understanding of this topic. The general topic "effects of the menstrual cycle on exercise performance" was selected to include a wide range of research in exercise science and sports medicine. An electronic literature search of PubMed and SportDiscus databases was conducted from July 1, 2008, to June 30, 2018. Search terms used were "menstrual cycle" in the title and "performance" or "exercise" in any field. The search was restricted to English language only. Only published full-text original studies that investigated a measure of exercise performance

TABLE 1. Overview of methods of included studies on exercise performance over the menstrual cycle.

Reference	*n*	Method of MC Verification	Ovulation Criteria	Test Day of MC
Bandyopadhyay and Dalui (18)	45	Counting days BBT	Rise in BBT of 0.4°F to 0.6°F	Days 3, 10, and 20–24
Cook et al. (15)	22	Counting days	None reported	Days 1, 14, and 21
Dos Santos Andrade et al. (16)	26	Counting days	None reported	FP, LP, days not specified
Fazil et al. (13)	80	Counting days	None reported	Days 1–6, 7–12, and 26–28
Forsyth and Reilly (19)	10	Counting days BBT Urinary LH Serum E and P	No E and P limits set, mentions normal range per Landgren (20)	Days 6–10 and 6–10 d after LH surge
Girija and Veeraiah (8)	40	Serial follicular scanning Counting days	Phase confirmed by follicular scanning	Days 1–5, 9–12, and 19–21
Gordon et al. (21)	10	Counting days Salivary E and P	None reported, mentions salivary E and P suggest regular MC	Days 1–3, 9–11, 19–20, and 27–28
Hashimoto et al. (22)	6	Counting days BBT Serum E and P	Serum P limit >16.3 nmol·L^{-1} in LP	FP, LP, days not specified
Janse de Jonge et al. (23)	8 (12–4)	Counting days BBT Serum E and P	Serum P limit >16 nmol·L^{-1} in LP	Days 3–6 and 19–25
Julian et al. (24)	9 (10–1)	Counting days MC diary Serum E and P	Mentions serum E and P used to verify timing of test, but no limits set	Days 5–7 and 21–22
Kumar et al. (14)	30	Counting days	None reported	Second day of each phase
Pallavi et al. (17)	100	No method reported	None reported	M, FP, LP, days not specified
Shakhlina et al. (25)	13	BBT Ferning test	Rise in BBT Positive ferning test	Days 1–5, 6–12, 13–15, 16–24, and 25–27
Stefanovsky et al. (12)	8	Counting days	None reported	Days 6–10 and 20–24
Tenan et al. (26)	9	Counting days BBT	Clear biphasic response in BBT	Five times evenly spread over cycle
Tsampoukos et al. (27)	8 (14–6)	Counting days MC diary Urinary LH Serum E and P	Serum P limit > 9.54 nmol·L^{-1} in LP LP length of 11–17 d	FP, LP, days not specified and midcycle, day of LH surge
Vaiksaar et al. (28)	15	Counting days MC diary Serum E and P	Serum P limit > 16 nmol·L^{-1} in LP	Days 8 ± 3 and 20 ± 2
Wiecek et al. (7)	16	Counting days BBT Serum E and P	Rise in BBT of 0.5°C No E and P limits set	Days 6–9 and 5–8 d after rise in BBT

E, estrogen; FP, follicular phase; LP, luteal phase; M, menstrual phase; MC, menstrual cycle; P, progesterone.

over the phases of the menstrual cycle were included. Studies with an oral contraceptive group were included if there was also a menstrual cycle group. For these studies, only the results from the menstrual cycle group and not those from the oral contraceptive group were included in this review. The electronic searches were performed by one reviewer (BT). Titles and abstracts were assessed independently by two reviewers (BT and AH). Any disagreement about the inclusion of studies was resolved by consensus or a third reviewer (XJ) where necessary. The initial database search produced a total of 250 articles. After removing the duplicates (7) and completing selection criteria, 18 articles were included in this review (see Table 1; and Supplemental Digital Content 1, which provides an overview of the characteristics and main findings of the included studies on exercise performance over the menstrual cycle, http://links.lww.com/MSS/B662).

METHODS FOR VERIFICATION OF MENSTRUAL CYCLE PHASE

Serial follicular scanning. One of the 18 included studies used serial follicular scanning via transvaginal ultrasonography for menstrual cycle phase verification (8). This is the most direct method for monitoring ovulation; however, it has several limitations. First, numerous daily scans are required in each cycle, which poses a significant time commitment for the participants. Furthermore, this is an invasive assessment, which is likely to result in a decrease in participant numbers. Finally, this method requires expensive ultrasound equipment and technical expertise (9). Therefore, in menstrual cycle research in exercise science, the use of indirect methods to verify menstrual cycle phase is more common.

Calendar-based counting. Seventeen of the 18 selected studies used the calendar-based counting method to identify phases of the menstrual cycle. This indirect method sets the self-reported onset of menses as day 1, and the phases are then established by counting days from this point. The length of the follicular phase is more prone to variation than the luteal phase (10). Therefore, unless days are counted backwards retrospectively, it is very difficult to estimate the day that ovulation occurred. The main limitation of this method, however, is that it does not distinguish between ovulatory and anovulatory or LPD cycles. This method assumes that all participants with regular menstruation experience ovulatory cycles with normal hormonal fluctuations. As LPD and anovulation often occur in active women with regular bleeding (5), the use of calendar-based counting methods in isolation is not recommended when accurate identification of

menstrual cycle phase is required (11). Five of the selected studies in this review (12–16), however, used the counting method as their sole method to verify menstrual cycle phase. One of the 18 selected studies only indicated that testing was conducted in the menstrual, follicular, and luteal phases without providing any further information on how these phases were established (17). Therefore, a total of 33% of studies relied on very basic methods only to determine menstrual cycle phase.

Basal body temperature. To estimate the day of ovulation, eight studies included daily recording of basal body temperature (BBT) (see Table 1). BBT charting is another widely used method for menstrual cycle phase determination, which requires the participant to measure their body temperature with a sensitive thermometer (scale of 0.05°C) before rising in the morning. These daily BBT measurements are then plotted over the menstrual cycle. After ovulation, many women experience an increase in BBT of approximately 0.3°C throughout the luteal phase (29,30). Although BBT may be useful to identify the approximate day of ovulation and the relative length of the follicular and luteal phases, it does not provide information about the actual hormone levels. Although it is often assumed that an increase in BBT reflects an increase in progesterone levels, a poor correlation between the two has been found (31,32). Furthermore, some females with ovulatory cycles show no increase in BBT during the luteal phase (31,33). Temperature readings may also be influenced by factors such as stress, illness, alcohol consumption, sleep patterns, and medications (34). Hence, BBT charting in insolation is not considered reliable for verification of menstrual cycle phase. However, two of the selected studies in this review only used BBT (combined with the calendar-based counting method) to confirm menstrual cycle phase (18,26), whereas one study combined BBT with salivary mucus fern testing (25).

Salivary ferning. The fern leaf crystallization of saliva can be seen under a microscope and has been shown to correlate well with the BBT method (35). However, when compared with ovulation confirmed by ultrasound, the ferning method only showed 53% sensitivity suggesting this test is unreliable (36). Thus, in addition to the six studies that only counted days, a further three studies added BBT and one of these combined BBT with salivary ferning. This results in 9 (50%) of 18 recently published studies relying only on indirect estimation of the menstrual cycle phases.

Urinary LH measurement. The least invasive and least expensive method to get a more direct indication of hormone fluctuations over the menstrual cycle is the measurement of the LH surge in urine. However, this method was only used by two of the 18 studies (19,27). This method uses an ovulation predictor kit, which identifies the surge of LH in urine. For this method, the participant collects her urine at the same time of day (midmorning is generally recommended) from day 8 of the menstrual cycle until a positive test result occurs. The test strip is inserted into the urine according to the manufacturer's instructions and will display either a positive or a negative result for urinary LH. In the majority of cases, ovulation has been shown to occur within 14–26 h of the urinary LH peak (37). A limitation of this method is that it is not uncommon for at home interpretation by the participant to result in false-positive results (38). Furthermore, apparently normal follicular and periovulatory events may be followed by a deficient luteal phase (39). LPD has been shown to be as high as 30% in participants who experienced a positive urinary ovulation test (5). Thus, although the use of an LH surge ovulation prediction kit may increase the likelihood of accurately estimating the point of ovulation and the timing of testing in the mid–luteal phase, this method does not exclude LPD cycles. Therefore, additional menstrual cycle phase verification testing is recommended (38).

Salivary hormone analysis. One of the studies measured progesterone and estrogen in saliva (21). Salivary hormone analysis is noninvasive and convenient; however, salivary estrogen in individual women appears in a pulsatile pattern of 60- to 90-min cycles (40), whereas salivary progesterone concentrations have been shown to have a greater variation over a 24-h period than serum progesterone (41). Therefore, samples may be required to be taken at multiple time points to obtain reliable information. Furthermore, the actual concentration of estrogen and progesterone in saliva is much lower than the concentration in serum. The total concentration of estrogen in saliva equals approximately 1%–2% of the total concentration of estrogen in serum, and the concentration of progesterone in saliva is approximately 2% of total serum concentration (42). Because of these low concentrations, tests with a much greater sensitivity must be used to obtain accurate measurements in saliva. Despite these limitations, salivary estrogen and progesterone have been used to differentiate between normal and abnormal cycles (43).

Serum hormone analysis. Seven of the 18 selected studies (39%) measured concentrations of estrogen and progesterone in serum. As potential effects of the menstrual cycle on exercise performance are expected to be related to the secondary effects of female steroid hormones, the measurement of serum estrogen and progesterone concentration is recommended as the gold standard for research purposes. This method requires the collection of a venous blood sample (approximately 8 mL) before testing. The blood sample is then left to clot before centrifugation. The serum is separated and stored frozen at −80°C until analysis of estrogen and progesterone using either ELISA kits or a pathology laboratory. A rise in progesterone from the follicular phase to the luteal phase is used to verify that ovulation has occurred. However, there does not appear to be a consensus on the minimum luteal phase progesterone level required as a reliable indicator of an ovulatory non-LPD cycle. Four of the studies in this review set a clear limit for progesterone during the luteal phase. Three of these selected a minimum progesterone limit of 16 $nmol \cdot L^{-1}$ (22,23,28), whereas one set the limit at 9.54 $nmol \cdot L^{-1}$ (27). Daily hormone monitoring in 68 females demonstrated a serum progesterone concentration greater than 16 $nmol \cdot L^{-1}$ for a minimum of 5 days in 95% of normal ovulatory cycles (20). Therefore, where a single luteal phase progesterone measurement is taken, a conservative limit of at least 16 $nmol \cdot L^{-1}$ should reduce the risk of including nonovulatory or LPD cycles. The remaining three studies that performed serum hormone analysis did not set a minimum for progesterone. One of these mentioned that the measured hormone levels were within the normal range

without providing further information (19), one reported a progesterone range of 3.5 nmol·L^{-1} to 89 nmol·L^{-1} (7), and one reported a mean progesterone concentration of 6.5 ± 2.0 nmol·L^{-1} (24). The large range and the low mean of these progesterone concentrations suggest that some anovulatory and/or LDP participants were included in these studies.

PARTICIPANT EXCLUSIONS BASED ON SERUM HORMONE LEVELS

Once hormone concentrations have been measured, the next step is to exclude those participants who do not appear to have a regular ovulatory cycle. Only two studies reported exclusions for not meeting menstrual cycle verification requirements (23,27). Janse de Jonge et al. (23) excluded three of the 12 participants for both of their testing cycles, whereas another two participants were excluded for one testing cycle each because of not meeting the 16 nmol·L^{-1} limit for progesterone in that cycle. Tsampoukos et al. (27) excluded 6 of 14 participants for not meeting their hormonal inclusion criteria (urinary LH surge, luteal phase progesterone greater than 9.54 nmol·L^{-1}). One additional study reported exclusions (1 of 10 participants) because the luteal phase test timing was incorrect based on the menstruation diary; however, they did not report on a clear limit for progesterone (24). Therefore, of all the 18 included studies, only three (17%) reported participant exclusions and only two of these were based on hormone verification. The total percentage of exclusions ranged from 10% to 43%, whereas the hormone-based exclusions ranged from 25% to 43%. A further two studies did not report exclusions; however, they provided the range of hormone concentrations for their participants. From this information, it was determined that all included participants had a luteal phase progesterone level of 16 nmol·L^{-1} or greater (19,22). In conclusion, this review has shown that only 4 of 18 recent menstrual cycle studies thoroughly verified the luteal phase based on serum hormone measurements. Therefore, given the high prevalence of LDP and anovulation in active females reported in the literature (5) and the large percentages of excluded participants in the studies mentioned above, 14 (78%) of 18 recent menstrual cycle studies are likely to have included anovulatory or LPD participants in their results.

TESTING IN THE LATE FOLLICULAR PHASE

As outlined earlier, the late follicular phase presents another distinctly different hormonal environment with low progesterone and high estrogen levels. The actual measurement of estrogen concentration is important to confirm the late follicular estrogen peak. Of the seven studies with hormone measurement, only one tested during the late follicular phase (27). This study clearly explained their late follicular verification methodology and only included the late follicular testing if the estrogen concentration was higher than during the luteal phase and the progesterone concentration was higher than during the early follicular phase but lower than 6.36 nmol·L^{-1}. The other six studies with hormone measurements only compared the follicular phase with the luteal phase. Two of these studies specified testing during the early follicular phase (23,24), three tested during the mid–follicular phase (7,19,28), and one did not provide information on timing within the follicular phase (22). Testing in the mid–follicular phase poses the risk of including participants with a large range of estrogen concentrations depending on how close to the late follicular estrogen peak testing takes place. As the follicular phase is more variable in length than the luteal phase, it is difficult to estimate when the late follicular estrogen peak will occur. Testing of the LH surge in urine can assist in determining the best late follicular testing day. Tsampoukos et al. (27) managed to conduct their testing on the actual day of the LH surge in urine. The logistical problems of participant, researcher, and equipment availability for testing on such short notice are likely to contribute to the limited amount of studies including the late follicular phase in their investigations.

FINDINGS ON EXERCISE PERFORMANCE OVER THE MENSTRUAL CYCLE

Of the 18 studies on exercise performance over the menstrual cycle, one study performed the valid, but invasive, method of transvaginal follicular scanning and they found changes in both exercise performance and physiological variables (8). Nine studies did not actually measure estrogen and progesterone concentrations, and two of these found no significant changes in exercise performance and physiological responses over the menstrual cycle (12,13). One study found no change over the menstrual cycle in the dominant leg, whereas the nondominant leg showed change (16). One study found no change in performance, but some changes in physiological responses over the menstrual cycle (26). Five studies did find changes in exercise performance (14,15,17,18,25), and two of these also found physiological changes (18,25). It is very likely, however, that these nine studies without hormone measurements included participants who did not experience regular fluctuations in female sex hormones throughout the testing cycle. In particular, in studies with small participant numbers, the inclusion of anovulatory or LPD participants may have masked significant outcomes. For example, two of the studies that found no significant changes included less than 15 participants (12,26), whereas four of the five studies that did find significant changes in exercise performance included from 22 up to 100 participants (14,15,17,18). The methodological limitation of small participant numbers combined with limited menstrual cycle verification may partly explain the conflicting findings of these studies without hormone verification.

Only 7 of 18 studies on exercise performance over the menstrual cycle published in the last 10 years actually measured serum concentrations of the hormones likely to be responsible for potential changes. Of the four studies with participants that clearly had a progesterone concentration greater than 16 nmol·L^{-1} in the luteal phase, three found significant changes in either exercise performance or physiological responses or both over the menstrual cycle (19,22,23), whereas one found no differences (28). The study with the lower progesterone limit of

9.54 nmol·L^{-1} found no changes over the menstrual cycle (27). The two studies that are likely to have included some participants who were anovulatory or LPD (suggested by a range of 3.5 to 89 nmol·L^{-1} (7) and a mean of 6.5 ± 2.0 nmol·L^{-1} (24) for progesterone in the luteal phase) also found no significant changes over the menstrual cycle. In summary, setting the more conservative progesterone limit of 16 nmol·L^{-1} for luteal phase verification reduces the risk of inclusion of anovulatory or LPD cycles and appears more likely to reveal potential changes in exercise performance and physiological responses over the menstrual cycle.

The one study with hormone measurement that included testing during the late follicular estrogen peak did not show any changes in performance over the menstrual cycle (27). This study followed strict hormonal criteria resulting in exclusion of 6 of 14 participants. The resulting small participant number (n = 8) may have masked potential differences over the menstrual cycle. This highlights another methodological problem in menstrual cycle research, which is the low participant number in many studies and especially in those with strict exclusion criteria (see Table 1).

DISCUSSION

This review of studies from the past 10 years on exercise performance over the menstrual cycle found conflicting results. More detailed investigation of the methodology showed that 78% of these studies are likely to have included participants with anovulatory or LPD cycles in their results. The inconsistent findings in menstrual cycle research on exercise performance may therefore be explained by methodological problems masking potential changes over the cycle. To improve the quality of menstrual cycle research, clear methodological standards should be set and adhered. The most important methodological consideration is the verification of the menstrual cycle phase at the time of testing.

Verification methods generally rely on the identification of ovulation to divide the cycle into the follicular and luteal phase. This ensures that the intended phase is being examined, and it aims to avoid the inclusion of LPD and anovulatory cycles. This review showed that, besides serial follicular transvaginal ultrasound scanning, there are six main methods used for menstrual cycle phase verification. The calendar-based counting method, the BBT method, and the salivary fern methods on their own or combined do not provide enough information to confirm a regular ovulatory cycle. The urinary ovulation detection kits provide information on the LH surge and can assist in determining the best days for testing. Again, this method, however, cannot confirm that the luteal phase will not be deficient. Greater sensitivity in the measurement of progesterone in saliva is a promising development. However, the main method found in this review that could verify that a regular ovulatory cycle had taken place was the measurement of progesterone concentration in serum during the luteal phase.

Several studies used a combination of these six methods to verify menstrual cycle phase at the time of testing (see Table 1). Wideman et al. (11) showed that the use of calendar-based counting methods with the addition of urinary ovulation tests and strategic serial blood sampling for 3 to 5 days to measure levels of postovulatory progesterone enhanced the proper identification of menstrual cycle events. Schaumberg et al. (5) also recommended a three-step method, including

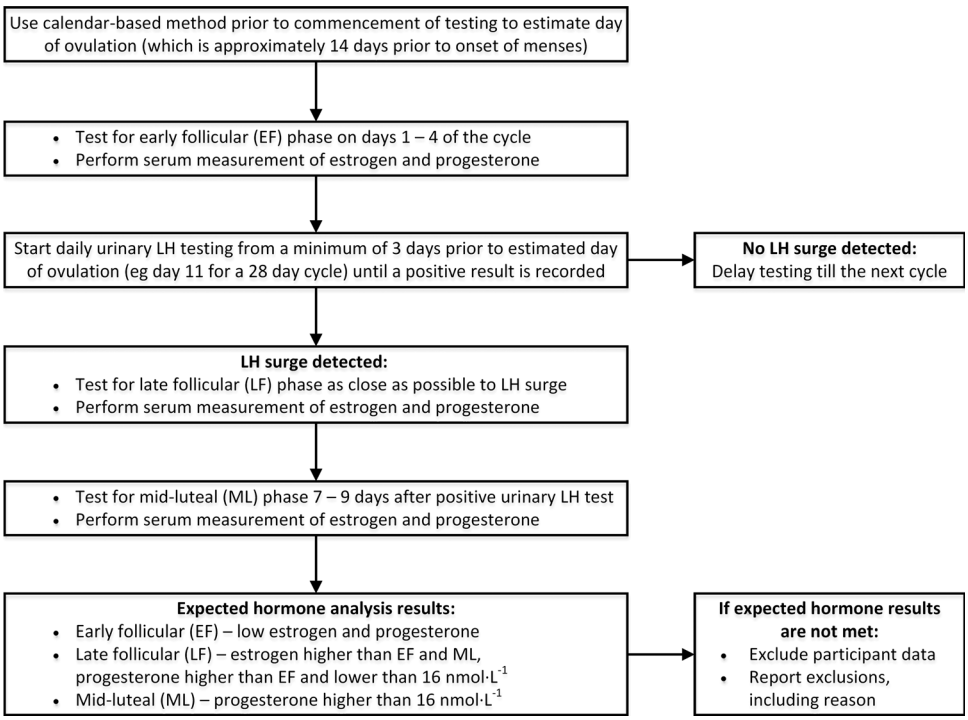

FIGURE 2—Flow chart of the methodological steps recommended to verify regular ovulatory menstrual cycle phases.

menstrual cycle mapping, urinary ovulation tests, and serum hormone measurement, to increase the success of verifying menstrual cycle phase. To minimize the need for serial blood sampling, they investigated how many days after the positive urinary ovulation test blood sampling was most likely to confirm the mid–luteal phase. The authors showed that taking a single blood sample between 7 and 9 days after a positive urinary ovulation prediction test was better than either 6 or 11 to 12 days after the positive ovulation test (5). These studies highlight the importance of combining the calendar-based counting method with urinary LH surge testing and serum progesterone measurement.

RECOMMENDATIONS

The presence of regular menstrual bleeding does not confirm a consistent menstrual cycle with regular hormone fluctuations. To be able to draw conclusions about potential differences in exercise performance and other parameters between menstrual cycle phases, it is paramount to accurately verify if participants are experiencing a regular ovulatory menstrual cycle at the time of testing. Figure 2 shows a flow chart of the methodological steps recommended to verify regular ovulatory menstrual cycle phases, as described in further detail below. Given the high prevalence of ovulatory disturbances commonly reported among females, and especially physically active females, it is recommended that menstrual cycle research includes serum measurement of both estrogen and progesterone. To assist in setting the timing of testing throughout the menstrual cycle, the calendar-based counting method in combination with urinary LH surge ovulation detection kits is recommended. Setting luteal phase testing to occur 7 to 9 days after the positive urinary ovulation test increases the likelihood of testing at high progesterone levels (5). At this time point, it is recommended that a conservative minimum serum progesterone concentration of 16 $nmol \cdot L^{-1}$ should be met. Participants not meeting this limit should be excluded from further analysis, and it is recommended that the number of excluded participants is clearly reported. To assist with the interpretation of research findings, it is also recommended that the actual range of progesterone concentrations of the included participants is reported and not just the mean and SD. The accurate verification of a regular ovulatory menstrual cycle in research participants will assist in identifying true menstrual cycle–related changes.

This review also showed that most menstrual cycle research only compares the follicular with the luteal phase and does not take into account the late follicular estrogen peak. As estrogen and progesterone have many antagonistic effects on numerous physiological functions (2), it is important to test not only when both concentrations are low (early follicular phase) or high (mid–luteal phase) but also during the late follicular estrogen peak. It is therefore recommended that menstrual cycle research aims to test during these three different hormonal environments to gain a better understanding of the potential effects of the female hormone fluctuations. To assist with the timing of the late follicular phase, testing should take place as soon as possible after a positive urinary LH surge. Serum measurement of estrogen should confirm that the concentration is higher than during the early follicular and luteal phases, whereas progesterone concentration should be higher than in the early follicular phase and lower than in the luteal phase. Again, participants not meeting these criteria should be excluded from analysis. Inclusion of the late follicular phase in future research will assist in developing more specific hormone concentration limits for confirmation of this phase.

The final methodological recommendation for menstrual cycle research is to take into account in the planning stage that it is likely that a very large percentage (up to 40%) of participants may need to be excluded for not having an ovulatory menstrual cycle. Most researchers do not have the facilities to measure hormone concentrations directly after each testing session and, therefore, will not know the number of exclusions until hormone verification is conducted upon completion of the study. If at that point up to 40% of participants needs to be excluded, it may be too late to conduct further testing. The lower number of participants than expected is therefore likely to result in reduced power to identify potential changes over the menstrual cycle. Considering the potential large percentage of exclusions in the planning stage of menstrual cycle research should assist in improving research quality.

Of course, it is acknowledged that for financial reasons or lack of laboratory facilities, the measurement of serum progesterone may not be an option for every menstrual cycle study. This review does not intend to discourage investigators from conducting research in this area and, in contrary, strongly encourages further research. However, this review aims to highlight that if hormone measurements are not feasible, then at least studies should aim for high participant numbers and measurement of urinary LH. Furthermore, the likelihood of inclusion of anovulatory and/or LPD participants should be acknowledged and conclusions should be drawn with this limitation in mind.

CONCLUSION

This review has demonstrated that methodological limitations often result in the conclusion that the menstrual cycle does not affect exercise performance, whereas in fact the distinctly different hormonal environments of the menstrual cycle may not have been properly compared. Methodological recommendations have therefore been made to avoid the likely inclusion of participants with anovulatory and LPD cycles and to include the late follicular estrogen peak. It is envisaged that these recommendations will assist in the growth in both quality and quantity of research in this important area and will reduce further confusion around the potential effects of the menstrual cycle on exercise performance.

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