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Bachelor's thesis in Human Movement Science
Supervisor: Dionne Noordhof
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Abstract

Purpose: The menstrual cycle (MC) is a fundamental part of the female body. It is unclear whether the MC could influence sport-related factors like training, recovery, and performance. One indirect marker of muscle damage is creatine kinase (CK). The limited research on this subject is conflicting. The purpose of this literature review was therefore to further investigate the influence of the MC phases on CK concentration in physically active women after exercise. **Methods:** Two literature searches were conducted on databases PubMed and BASE. Keywords used were “menstrual phase” and “creatine kinase post-exercise” for the first search and “influence of menstrual cycle” and “blood” and “muscle damage” for the second search. Inclusion criteria: 1) testing in both FP and LP but not necessarily the same participants in both groups; 2) participants had to be physically active women; 3) keywords had to be present in title, abstract or keywords; 4) the text had to be published in English. Exclusion criteria: 1) no full text available; 2) no information about participants’ physical activity; 3) missing results section. **Results:** Five studies were found to be relevant for this review. Three studies found significantly higher CK concentrations in FP compared to LP 24 hours or more post-exercise, one study found significantly higher CK concentration in LP compared to FP 24 hours or more post-exercise, and one study found no significant differences. **Conclusions:** The limited and conflicting results makes it difficult to conclude. Further research using standardized protocols is needed for a conclusion.

Bakgrunn: Menstruasjonssyklusen (MC) er en stor del av den kvinnelige kroppen. Det er usikkert om MC kan påvirke idrettsrelaterte faktorer som trening, restitusjon og prestasjon. En indirekte markør for muskelskade er creatine kinase (CK). Den begrensede forskningen på dette temaet er motsigende.. Hensikten med dette litteratursøket var dermed å videre undersøke MC-fasene sin påvirkning på CK-konsentrasjonen i fysisk aktive kvinner etter trening. **Metode:** To litteratursøk ble gjort i databasene PubMed og BASE. Nøkkelordene som ble brukt var “menstrual phase” og “creatine kinase post-exercise” for det første søket, og “influence of menstrual cycle” og “blood” and “muscle damage” for det andre søket. Inklusjonskriterier: 1) testing i både FP og LP, ikke nødvendigvis samme grupper på begge, 2) fysisk aktive kvinner, 3) nøkkelord måtte være i tittel, abstrakt eller nøkkelord, 4) teksten måtte være på engelsk. Eksklusjonskriterier: 1) full tekst ikke tilgjengelig, 2) ingen informasjon om fysisk aktivitet i deltakerne, 3) manglende resultatdel. **Resultat:** Fem studier ble inkludert. Tre studier fant signifikant høyere verdier av CK i FP sammenlignet med LP 24 timer eller mer etter trening, en studie fant signifikant høyere verdier av CK i LP sammenlignet med FP 24 timer eller mer etter trening, og en studie fant ingen signifikante forskjeller. **Konklusjon:** De begrensede og motsigende resultatene gjør det vanskelig å konkludere. Fremtidig forskning bør bruke standardiserte protokoller for testing for å kunne konkludere.

1. Introduction

With an increasing amount of women participating in sports, the need for research on women's health is clear. According to Cowley et al. (2021), females are significantly underrepresented in exercise and sport research. Cowley et al. (2021) investigated how underrepresented women are in Sport and Exercise research including six different journals (2014 – 2020) and found that out of all the participants included, 66 % were male, with only 34 % females. Which makes females significantly underrepresented in Sport and Exercise research (Cowley et al., 2021). Furthermore, only 0.6 % of the studies included were male-specific (n = 9 prostate cancer, n = 1 semen quality) (Cowley et al., 2021)**.** Cowley et al. (2021) states "This findings suggests that most of the research conducted exclusively on males are topics or questions that potentially have equal application to females" (Cowley et al., 2021). Further on, Elliot-Sale et al. (2021) states that until now there has been inconsistencies research design and terminology, in addition to differences in defining the reproductive status, which potentially could make it harder to include women as participants or to include on the right terms.

With the lack of research on women's health combined with the MC being a fundamental part of the female body, one could easily question whether the MC could influence factors related to sports, such as training, recovery, and performance. A study published by Kissow et al. (2022) investigated the potential effects of cycle-based resistance training. It was uncovered that follicular phase (FP)-based resistance training enhanced muscle strength and mass in a greater way than luteal phase (LP)-based resistance training (Kissow et al., 2022). It was noted, however, that while FP-based resistance training may be superior to LP-based resistance training for the general woman, more research is needed to determine whether the same difference can be seen in athletes. Studies have shown that there might also be some differences related to endurance training. Benito et al. (2023) investigated the recovery processes after a high-intensity interval session and found that recovery was slightly more delayed in MLP compared to EFP and LFP (Benito et al., 2023). Self-perceived fitness and performance during the MC was studied by Solli et al. (2020). The participants in this study had the worst perceived fitness and performance during the bleeding phase (EFP) (Solli et al., 2020). The following phase post-bleeding (LFP) was found to be the best phase regarding perceived fitness and performance.

Female endurance athletes report that they lack enough knowledge about the effect of the menstrual cycles (MC) on training and performance, and also about communication with coaches about this specific topic (Solli et al., 2020). Solli et al. (2020) also reports that only 27 % of female athletes communicate with their coach about their MC, especially if it is a male coach. This is a strong indicator of taboos around this topic. These findings indicate that it is not common yet to talk about the MC, its side effects, irregularities, and its effect on training and recovery in different phases of the MC.

An eumenorrheic MC is, according to Elliot-Sale et al. (2021), a cycle of 21 to 35 days, where there has not been used any hormonal contraceptives (HC) for the past 3 months. There should be a luteinizing hormone (LH)-surge in addition to the correct hormonal profile. Throughout a MC, there is variation in the ovarian hormones (Elliott-Sale et al., 2021). Elliott-Sale et al. (2021) states that based on the hormonal fluctuations, four distinct hormonal environments can be identified.

Menstrual cycle phases

Table 1: The four hormonal phases, according to Elliot-Sale et al. (2021).

Phase	**When**	**Hormonal profile**	**Abbreviation**
Phase 1	Onset of bleeding until day 5	Progesterone: low Estrogen: low	EFP
Phase 2	14-26 h prior to ovulation	LH-surge Progesterone: lower than 6.36 $nmol \cdot L^{-1}$ but higher than phase 1 Estrogen: higher than in phase 1, 3 and 4	LFP
Phase 3	Ovulation, lasts for 24-36 h	Indicated by a urinary ovulation test. Progesterone: lower than 6.4 $nmol \cdot L^{-1}$ but higher than phase Estrogen: lower than in phase 2 and 4, but higher than in phase 1	OP
Phase 4	+ 7 days post-ovulation confirmed	Progesterone: > 16 $nmol \cdot L^{-1}$. Estrogen: higher than phase 1 and 3, but lower than phase 2	MLP

EFP, early follicular phase; LFP, late follicular phase; OP, ovulary phase; MLP, mid-luteal phase

A MC begins with a menses during EFP, and LP is post-ovulation (Elliott-Sale et al., 2021). During FP, serum estradiol rises simultaneously as the follicle size grows and there is an increasing number of granulosa cells (Reed & Carr, 2018). At the end of FP a mature ovarian follicle is released during the ovulation, which happens 10-12 hours post LH peak (Reed & Carr, 2018). Following the ovulation, is LP. When looking at hormones, the estrogen level rises and falls twice during the MC; once during the mid-follicular phase (MFP) following a drop post-ovulation, and a rise during the mid-luteal phase (MLP) and then proceed to decrease at the end of the MC (Reed & Carr, 2018). The hormonal fluctuations throughout the MC might influence recovery after exercise, and some studies indicates that estrogen might have a protective effect on muscle damage (Carter et al., 2001; Enns & Tiidus, 2010; Williams et al., 2015).

One of the indirect markers of muscle damage, and thus recovery, is the level of Creatine Kinase (CK) in the blood. CK is an enzyme that all humans have in their muscle cells. When the muscles get damaged, CK leaks into the blood stream. The CK enzyme reaches its peak value 3-5 days after exercise (Funaki et al., 2022). Due to hormonal differences throughout the MC, it is possible that the MC could affect the CK values post-exercise differently depending on the MC phase.

Even though there is limited research on the MC phase and its influence on blood variables post-exercise, some has been conducted. Markofski and Braun (2014) found greater CK concentration 96 hours after exercise in LP, compared to FP. This study compared groups, which is a limitation as there is large individual variability regarding CK concentration (Koch et al., 2014). Hackney et al. (2019) found significantly higher CK concentrations 24 and 72 hours after exercise in FP compared to the LP.

As the studies published on this topic showed conflicting results, the purpose of this review was to further investigate the effect of MC phase on post-exercise CK levels in physically active women.

2. Methods

PubMed and BASE were the databases used to conduct the literature search for. Two different searches were performed. The key words used were "menstrual phase" and "creatine kinase post-exercise" for the first search and "influence of menstrual cycle" and "blood and "muscle damage" for the second search. This resulted in 23 unique publications. All studies that were included had to: 1) perform testing in both FP and LP but not necessarily the same participants in both groups; 2) participants had to be physically active women; 3) keywords had to be present in title, abstract or keywords and 4) the text had to be published in English. Exclusion criteria were: 1) no full text available; 2) no information about physical activity in the participants; 3) missing results section. After the papers were checked for these inclusion and exclusion criteria, five studies were found to be relevant for this review.

3. Results

The study characteristics and result from the five included studies are found in the Table 2 below. The studies included a total of 67 physically active, eumenorrheic women. Three studies used a running test to measure CK level. Two of the studies used a regular running test and one study used a downhill running test. The two remaining studies used a strength session to induce muscle damage, as measured by CK levels after exercise. One study used a maximal strength test, and the final study used a higher repetition-based session.

Results from the included studies

Table 2: Study characteristics and results of the studies included in this review.

Study	Participants	Protocol	MC phase verification	Recovery measures	CK (U/L)	
Hackney et al. (2019)	n=8 trained female runners	Treadmill running at 70% VO_{2max} for 90 min in MFP and MLP	Calendar-based counting, ovulation test, blood test	Rest, IP, 24 h post-exercise and 72 h post-exercise	Rest: MFP: 89.7 ± 16.7 MLP: 92.6 ± 9.6 24 h post-exercise: MFP: 510.8* ± 344.9 MLP: 275.1 ± 55.1	IP: MFP: 109.7 ± 10.7 MLP: 112.7 ± 16.8 72 h post-exercise: MFP: 425.7* ± 249.7 MLP: 211.2 ± 23.4
White (2019)	n=12 physically active women	Downhill running for 30 min at 60% VO_{2max} in EFP, LFP and LP	Questionnaire, tracking 2 months with an ovulation test	Rest, 5 min post-exercise, 24 h post-exercise, 48 h post-exercise and 72 h post-exercise	This study only provides five min post-exercise CK level. 24 h post-exercise CK level was found to be significantly higher during the LFP compared to the EFP and the LP (p=0.014)	
Williams et al. (2015)	n=10 healthy, highly trained females	60 min of treadmill running at 65% VO_{2max} in MFP and MLP	Blood samples during MFP and MLP, determined by forward counting	Rest, IP, 30 min post-exercise and 24 h post-exercise	Rest: MFP: 106.8 ± 34.5 MLP: 104.2 ± 3.1 30 min post-exercise: MFP: 161.2 ± 60.9 MLP: 153.4 ± 28.9	IP: MFP: 129.9 ± 38.0 MLP: 118.6 ± 18.7 24 h post-exercise: MFP: 378.7* ± 176.5 MLP: 279.6 ± 100.8

Markofski and Braun (2014)	n=18 resistance-trained women	1RM biceps curl in a preacher curl bench using the non-dominant arm in FP and LP	Verbal discussion, estradiol measure using serum blood sampling	Rest, IP, 24 h post-exercise, 48 h post-exercise, 72 h post-exercise, 96 h post-exercise and 168 hs post-exercise	This study does not provide the specific numbers from their testing, but CK in LP was significantly higher than in the FP 96 h post-exercise (p=0.016)	
Romero-Parra et al. (2020)	n=19 well-trained females	10 sets of 10 reps of plate-loaded barbell back squats at 60 % of 1RM in EFP, LFP and MLP	Self –reported cycle lengths previous past 6 m, phase lengths and cycle phases determined by a gynecologist, urine-sample to determine LH-surge, blood-sample to determine hormones	Rest, 2 h post-exercise, 24 h post-exercise and 48 h post-exercise	Rest: EFP: 108.6 ± 48.0 LFP: 105.7 ± 33.1 MLP: 100.7 ± 29.9 24 h post-exercise: EFP: 154.1 ± 69.3 LFP: 195.5 ± 95.3 MLP: 172.1 ± 85.8	2 h post-exercise: EFP: 151.6 ± 70.0 LFP: 155.1 ± 44.9 MLP: 150.6 ± 43.8 48 h post-exercise: EFP: 117.3 ± 40.1 LFP: 130.6 ± 47.7 MLP: 128.8 ± 49.5

CK = creatine-kinase, VO_{2max} = maximal oxygen uptake, FP = follicular phase, EFP = early follicular phase, MFP = mid-follicular phase, LFP = late follicular phase, LP = luteal phase, MLP = mid-luteal phase, IP = immediately post-exercise, 1RM = one-repetition maximum, *Significantly higher than other menstrual cycle phases at the same time point (p<0.05).

Three out of five included studies found significantly higher CK levels 24 hours and 72 hours after exercise in FP compared to LP (Hackney et al., 2019; White, 2019; Williams et al., 2015). Romero-Parra et al. (2020) found no significant differences between MC phases at any post-exercise time point. The study of Markofski and Braun (2014) was the only study that found significantly higher CK levels in LP compared to FP. Hackney et al. (2019), Williams et al. (2015) and Romero-Parra et al. (2020) did not provide p-values, but rather stated it was below 0.05.

4. Discussion

The purpose of this review was to examine the effect of MC phase on post-exercise CK levels in physically active women. Five studies were included after a comprehensive search. Two of the included studies found significantly higher CK levels in MFP compared to MLP, one study found significantly higher CK levels in LFP compared to EFP and MLP, one study found significantly higher CK levels in LP compared to FP, and one study found no significant differences between MC phases.

Three studies looked at CK levels after an endurance workout, and the two other studies looked at CK levels after a strength exercise. According to Funaki et al. (2022) CK levels reach peak values about three to five days post-exercise. When looking at 72 hours post-exercise, only two studies provided results about this: Hackney et al. (2019) and Markofski and Braun (2014). Hackney et al. (2019) found that the CK level was significantly higher in MFP compared to MLP both 24 and 72 hours after exercise, but the levels decreased from 24 hours post-exercise until 72 hours post-exercise. Markofski and Braun (2014), at 72 hours post-exercise, showed the results from FP and LP as relatively similar compared to each other, but no statistical significance was found. Markofski and Braun (2014) did not provide any testing 24 hours after exercise, but the testing done after 72 hours shows slightly higher values than the testing done 48 hours after exercise. When looking at testing done 96 hours after exercise, Markofski and Braun (2014) was the only study who measured CK concentration at this point. CK concentrations in LP peaked at this point, which strengthens the statement by Funaki et al. (2022). Markofski and Braun (2014) was also the only study that tested 168 hours post-exercise. CK values in FP peaked at this point, but it is unknown if concentrations would have continued rising had they performed another test later. With the statement from Funaki et al. (2022) in mind, results could have been different if more than two studies had tested 96 hours or more post-exercise. Williams et al. (2015) is the best example of this. In this study, testing was only done until 24 hours post-exercise, and CK concentration was still rising both in MFP and MLP.

4.1 Sample Size and Study Design

The sample size of the five included studies was mostly low, ranging from 8 (Hackney et al., 2019) to 19 (Romero-Parra et al., 2020) participants. All studies, except one, used a within-subject design. Only Markofski and Braun (2014) randomized the participants into either FP-group or LP-group. This leads to those four studies having a greater statistical power, than the one study that used a between-group design.

4.2 Estrogen and Recovery

Estrogen rises twice during the MC, once during MFP and once during MLP. As Carter et al. (2001), Enns & Tiidus (2010) and Williams et al. (2015) stated, estrogen might have a protective effect on muscle damage (i. e. CK concentration). When looking at the results from Hackney et al. (2019) and Williams et al. (2015), the CK levels in MFP (low estrogen) are higher than i LP (high estrogen), which could confirm the protective role of estrogen. Williams et al. (2015) hypothesized that higher estrogen level would lead to lower CK levels, which is what their results showed. However, when looking at the results from Markofski and Braun (2014), the CK levels are higher in LP where the estrogen levels are raised, leading to the conclusion that estrogen might not have a protective role regarding muscle damage. On the other hand, Romero-Parra et al. (2020) indicates that progesterone interferes with estrogen-protective responses, and thus lead to increase in inflammation (i. e. higher CK values). Markofski and Braun (2014) hypothesized the opposite of what was found and were surprised by their results, stating that “the effect of estrogen on signs and symptoms of muscle damage is a complex puzzle” (Markofski & Braun, 2014). Markofski and Braun (2014) also state that with these findings in mind, estrogen level might not be the only factor affecting muscle damage, but also the “interaction between estrogen and estrogen receptors” (Markofski & Braun, 2014). In Markofski and Braun (2014), the luteal group had a significantly higher estrogen level than the follicular group. Further on, Markofski and Braun (2014) randomized subjects into one of two groups, and thus only tested each subject in one phase. This could lead to subjects whose CK level naturally increased more after exercise being put in one group. This is a potential source of error, and the results could have been different had they tested all subjects in both phases.

4.3 Methods

In three of the studies the subjects performed a running test to induce muscle damages, of which two studies used level running (Hackney et al., 2019; Williams et al., 2015) and one study used downhill running (White, 2019). In the two other studies the participants performed a strength exercise bout (Markofski & Braun, 2014; Romero-Parra et al., 2020). This could potentially have an impact on the results, as CK levels are not a direct marker of muscle damage, but an indirect marker. A study looking at the CK response to resistance exercise stated that the exercise protocol could affect CK levels, with some exercises not being able to result in peak CK levels (Koch et al., 2014). Koch et al. (2014) also stated that several recent studies found that upper body exercises consistently led to higher CK concentrations than lower-body exercises (Koch et al., 2014). The only study of the five included in this paper that used an upper-body exercise bout to test CK response post-exercise, was the study of Markofski and Braun (2014). The study of Romero-Parra et al. (2020) was the only study that found no significant differences between MC phases. This is also the only study that used a lower-body resistance exercise. Even though Hackney (2019), White (2019) and Williams et al. (2015) all found significant differences using a lower-body endurance session, different results could have been seen had all studies used the same exercise protocol.

One other consideration for the methods part of the included studies, were if the participants were told to refrain from training in the days with testing. Only two of the studies provided information about this. Markofski and Braun (2014) instructed their participants to refrain from "performing any upper-body or high-intensity lower-body weightlifting or downhill running; and icing, heating, stretching, or massaging the arm for the duration of the study" (Markofski & Braun, 2014), and Williams et al. (2015) asked their participants to refrain from intense physical activity during the period of testing. The three other studies did not write about providing any clear guidelines regarding exercising during the period of testing. Hackney et al. (2019) told their participants to refrain from exercising for 24 hours but explained that by fear of dehydration. If all the studies provided clear guideline regarding not to work out during the period of testing, the results might have been different.

4.4 Menstrual Cycle Phase Verification

All studies provided information on how they determined MC phases. All studies used some sort of self-report data (questionnaire, verbal discussion, self-tracking), but in addition Hackney et al. (2019) and Williams et al. (2015) and Markofski and Braun (2014) provided a blood sample test to further confirm an eumenorrheic MC. By also doing an ovulatory test, such as White (2019) and Hackney et al. (2019) did, it easy to divide LFP from LP. Romero-Parra et al. (2020) used urine-samples to confirm MC phases of their participants to further know if they were to assign them to the FP-group or the LP-group.

4.5 Limitations

A clear weakness of this selection of studies is the different methods utilized. Four of the studies include the same participants in both phases, whilst one included a random selection of women in the different phases. As stated in the results, all studies, except Romero-Parra et al. (2020), indicated that there is a significant effect on CK levels after exercise in the different phases of the MC. It is worth noting that testing within the FP (early, mid, and late) vary between the different studies. Due to hormonal differences, the results may vary within the different phases. One other consideration is that not all studies provided test results from the exact same post-exercise time points. This is a possible weakness since it is then not possible to compare the exact same time points to each other. All studies tested resting CK levels, but the next testing stage ranged from immediately post-exercise to two hours post-exercise. The final tests were carried out at vastly different times as well. Williams et al. (2015) only tested until 24 hours post-exercise, while Markofski and Braun (2014) tested until 196 hours post-exercise. The study of Markofski and Braun (2014) was the only study that tested 96 hours or more post-exercise, and the only study that found significantly higher CK level in LP compared to the FP. One should not exclude the possibility that the results in the four other studies could have been different had they tested for 96 hours or more post-exercise.

4.6 Limitations of this review

With limited research available on the topic of the MC's effect on post-exercise recovery, this review has multiple weaknesses. CK has been shown to have a certain variability, and thus may not be the best variable to use when investigating this topic. A better option would be to look at multiple variables, such as Romero-Parra et al. (2020), who used 8 different variables (CK, lactate dehydrogenase, interleukin-6, tumor necrosis factor, C reactive protein, aspartate aminotransferase and alanine aminotransferate). With the limited scope of this paper, including all these blood markers would be difficult to achieve, though including some would be possible. Another limitation of this review is the number of studies and participants included. With just five studies included and a total of 67 participants it is difficult to conclude one way or the other, especially considering the different methods utilized and the fact that the studies provide three different results. This makes the need for further research clear, considering women make up half of the world population.

4.7 Future Directions

For future studies we would recommend testing the same subjects in all phases in order to reduce the possibility of individual differences affecting the results. In addition, studies with women as participants should follow Janse De Jonge et al.'s (2019) "flow chart of the methodological steps recommended to verify regular ovulatory menstrual cycle phases" (Janse De Jonge et al., 2019) as shown in figure 1.

Flow chart

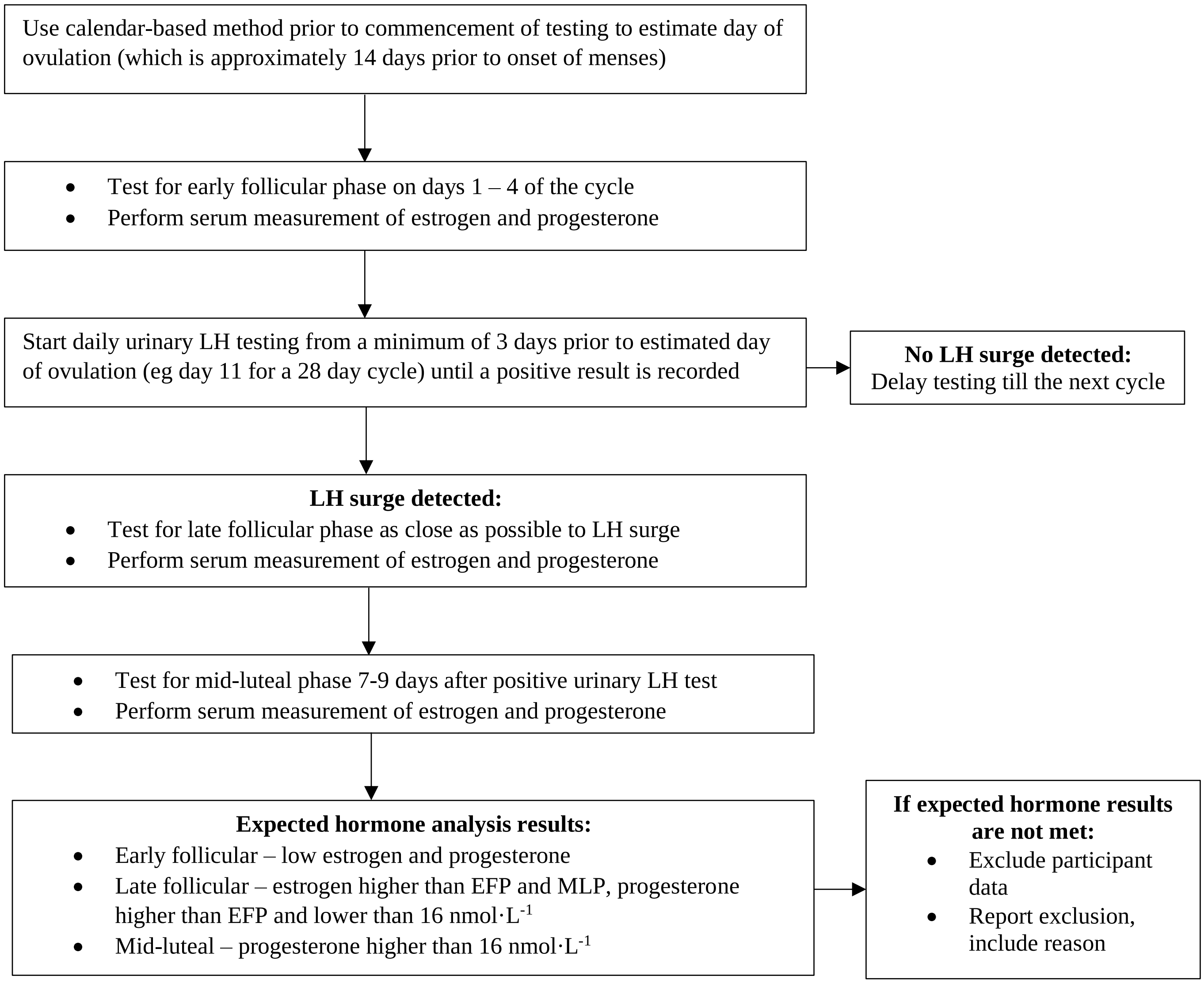

Figure 1: Recommended methodological steps, retrieved from Janse De Jonge et al. (2019).

Testing all women in all phases may give a more precise answer to whether MC phases have an impact on post-exercise CK levels. In order to fully confirm which MC phase the participants have at a given time, it is important to follow the steps of Janse De Jonge et al. (2019). Bleeding regularly does not confirm an eumenorrheic MC, only testing for the correct hormonal concentration does (Janse De Jonge et al., 2019). Furthermore, with research showing a peak CK level three to five days post-exercise, future studies should test subjects for a longer time frame than 24-48 hours post-exercise. Lastly, with studies showing upper-body exercises yielding higher

CK levels than lower-body exercises, there is a clear need for more standardized studies that follow a similar protocol (Koch et al., 2014).

5. Conclusions

The limited number of studies included in this review, and the low quality of some of these studies, prevent us from concluding whether MC phases affect CK levels or not. The included studies merely give a small indication that CK levels post-exercise could vary between the different MC phases, but further research is needed for a conclusion.

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