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# **Perceived Negative Menstrual Cycle Symptoms, but not Changes in Estrogen or Progesterone, are Associated with Impaired Cycling Race Performance**

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## ABSTRACT

**Purpose:** To examine the relationship between menstrual cycle (MC) phase-dependent fluctuations of estrogen and progesterone and virtual cycling race performance, with a secondary aim of correlating perceived MC-related symptoms with performance. **Methods:** In a novel observational study design, thirty-seven female cyclists/triathletes not using any hormonal contraception completed one virtual cycling race [19.5 km time trial (TT)] per week across a one-month period (totaling four races). Participants completed MC characterization and tracking, including urinary ovulation kits, across two complete MCs. Venous blood samples were collected within 21 h of racing to determine serum 17- $\beta$ -estradiol and progesterone concentrations, as well as an assessment of self-reported, perceived race-day MC and gastrointestinal (GI) symptoms, which were all then correlated to race performance. **Results:** There was no relationship between race completion time and individual estradiol ( $r=-0.001$ ,  $p=0.992$ ) or progesterone ( $r=-0.023$ ,  $p=0.833$ ) concentrations. There was no difference between race time between MC phases (follicular/luteal,  $p=0.238$ ), whether MC bleeding or not bleeding ( $p=0.619$ ) and ovulating or not ovulating ( $p=0.423$ ). The total number of perceived MC symptoms recorded on race day was positively correlated to increased race time [ $r=0.268$  (95% CI 0.056 to 0.457),  $p=0.014$ ], as was the number of GI symptoms of at least “moderate” severity before the race ( $r=0.233$  [95% CI 0.021 to 0.425],  $p=0.031$ ), but not post-race ( $r=0.022$ ,  $p=0.841$ ). **Conclusions:** When implementing a novel, virtual cycling race, fluctuations in ovarian hormone concentrations across the MC do not appear to affect real-world cycling performance among trained cyclists, while perceived negative MC and GI symptoms may relate to impaired performance. Therefore, the management of negative MC and GI symptoms appears important for athletic performance enhancement or to mitigate performance decline. **Key Words:** WOMEN, FEMALE, MENSTRUAL CYCLE, OVARIAN HORMONES, CONTRACEPTION

## INTRODUCTION

Despite much discussion among the scientific community (1) and general population (2), consistent and high-quality evidence of changes in performance in response to fluctuations in the female sex-steroid hormones across the menstrual cycle (MC) is lacking. Alterations in estrogen and progesterone concentrations have the potential to influence multiple physiological systems associated with athletic performance, such as substrate utilization (3, 4) or force production (5, 6). Understanding if performance is systematically altered in response to changes in estrogen and/or progesterone concentrations is important for competitive athletes.

According to a recent meta-analysis, exercise performance might be trivially reduced during phase 1 (early follicular, low estrogen and progesterone concentration, begins at menstruation) of the MC comparative to all other MC phases, with the largest difference in performance between phase one (early follicular) and phase two (late follicular, highest estrogen and low progesterone concentrations) (1). These results suggest that the low concentrations of estrogen and progesterone observed during phase one may elicit a performance decrement, while elevated estrogen may be performance-enhancing. However, given that findings are highly inconsistent between studies, the magnitude of effect is trivial, and results are confounded by study quality variability (particularly regarding flaws in methodological control and classification of ovarian hormones), the conclusions from the available literature are considered weak (1). However, if trivial but consistent differences do exist, these may be important for elite athletes, for whom marginal gains are of consequence.

Other MC-related factors that may influence performance should also be considered. Indeed, symptoms (e.g., bloating, muscle aches, fatigue, gastrointestinal (GI) issues, headaches, poor sleep, and anxiety) commonly associated with the end of the luteal phase or beginning of

the follicular phase (during menses) may hinder performance. These negative symptoms are reportedly experienced by ~60-93% of naturally menstruating female athletes (7-12), with ~50-67% believing that such symptoms impair performance (9-11, 13). Disparities may further exist between trained [Tier 2 (14)] and recreational (Tier 1) athletes, with the possibility of detecting minor performance changes as a result of MC-related hormone fluctuations potentially greater in the former, given their superior performance consistency compared to recreational athletes (15). Therefore, it is important to examine performance indices across the MCs of trained athletes to account for these adaptations and for whom performance is more consistent; hence any small influence of the MC may more likely be detected.

Investigations of the MC in real-world performance settings (i.e., actual competition) are ideal given the high ecological validity but are simultaneously difficult to conduct and are therefore lacking. Indeed, most studies of performance across the MC are undertaken in a controlled laboratory environment (16, 17), in which participants are often blinded, fasted, and lack real-world motivation to perform. Furthermore, the few studies that have attempted to examine real-world performance have typically been hampered by self-reported MC phases and failure to verify ovulation and/or serum estrogen/progesterone concentrations (18, 19). Unsurprisingly, the outcomes from such studies are conflicting and highly variable (18-22).

Accordingly, the aim of this study was to examine the effect of estrogen and progesterone on sports performance among female athletes not using hormonal contraception, employing robust methodological control of menstrual status, the recruitment of trained athletes, and an ecologically valid measurement of performance using an online virtual (Zwift) competition. A secondary aim of the project was to examine the effect of perceived MC- and GI-related symptoms on performance.

## **METHODS**

### **Experimental overview**

In a novel observational study design (Figure 1), participants completed one virtual indoor cycling race per week across a one-month period (totaling four races) using the Zwift online cycling platform (2023 Zwift, Inc. v2.183.0). Venous blood samples were collected within 21 h of racing (pre- or post-race) to determine serum 17- $\beta$ -estradiol and progesterone concentration. The concentrations of these sex hormones were then matched with the respective race and correlated to each participants' race completion time. The incidence of MC and GI self-reported symptoms on race day were also correlated to race time as a secondary outcome measure. The study was approved by the Australian Catholic University Human Ethics Research Committee (2023-3192H) and conducted in accordance with the Declaration of Helsinki. All participants provided informed consent prior to participating.

### **Participants**

Thirty-seven Tier 2 (14) female cyclists/triathletes (mean age: 35 $\pm$ 6 y mean weight: 67.0 $\pm$ 10.3 kg, mean training volume: 8.0 $\pm$ 3.5 h/wk, mean age of menarche: 13 $\pm$ 3 y) were recruited. Inclusion criteria were: residing in Australia, pre-menopausal (confirmed via ovulation detection), absence of hormonal contraception for >three months prior to study commencement, not pregnant or breastfeeding (Figure 2). The only exclusion criteria based on MC function was current amenorrhea (absence of a MC for >three months) (23), given it results in the complete suppression of endogenous hormones and hence would prohibit the investigation of the primary outcome. Other menstrual irregularities that do not entirely suppress endogenous hormonal profiles were therefore included to increase study generalizability.

## **Menstrual cycle monitoring**

Prior to participation, athletes completed an initial questionnaire regarding their menstrual status, including MC length and frequency, prevalence of known MC dysfunction [e.g., polycystic ovary syndrome (PCOS), amenorrhea], and any current or previous hormonal contraceptive use. Participants' MCs were then tracked, according to best-practice protocols (23), across the four weeks of racing – with additional weeks before or after to capture two complete MCs per athlete. Participants completed daily online questionnaires (REDCap (24, 25)) pertaining to presence and heaviness of menstruation, symptom incidence, and medication use in the preceding 24 h (Supplemental Figure 1, Supplemental Digital Content 2). Athletes also used dual hormone urinary ovulation kits (Advanced Digital Ovulation Test, Clearblue, Geneva, Switzerland) from MC day 10 until ovulation occurrence (continuing until the next bleed if ovulation was not detected), recording the result on the online questionnaire. Venous blood samples were collected within 21 h of racing (pre- or post-race) to determine progesterone and serum  $17\beta$ -estradiol (the most potent form of estrogen among pre-menopausal women, henceforth referred to as “estradiol”) concentration.

## **Training monitoring**

Alongside MC tracking, participants logged all training via Strava or Garmin, and reported the type, duration, and intensity of sessions via the daily questionnaire, together with the presence of any injuries.

## **Zwift races**

Races were an individual time trial (TT) format: 19.5 km in length with 32 m elevation. The race was a private event open only to study participants, whereby participants could see all other competitors in the ride to replicate a real race environment. The Zwift software was programmed to a standardized bike setting, while drafting and powerups were disabled. The indoor trainer (n=33) or stationary bike (n=4) was consistent within each participant across all their races. Participants raced every Thursday evening across four consecutive weeks, commencing at 19:45 AEDT. Participants chose their own warm-up and replicated this each week. The race was completed indoors, with permission to use fans or air conditioning.

To enhance the ecological validity and motivation, prize money was available to the top performers. Participants were grouped into categories (A-D) based on ability ( $W \cdot kg^{-1}$ ) (26). At each race, participants provided a photo of themselves standing on a scale pre-race to verify body mass. Prize money was awarded separately across each category, such that riders were only directly competing against individuals of a similar ability. Participants voted on the prize money allocation system, and the number of prizes awarded was adjusted based on the total number of athletes, such that the top 30% of riders in each category were awarded a prize.

## **Pre-race standardization**

Dietary intake (all food, beverages and caffeine consumption) was standardized for 36 hours pre-race, with participants allowed to choose their own nutrition strategies but repeat them for each race. Dietary records were maintained to verify compliance with these instructions, via the use of meal photos posted on the MealLogger app (27). Alcohol was prohibited throughout both days. Training was permitted the day before the race but was kept consistent every week



and recorded on Strava/Garmin for verification. No training was permitted on the day of the race, with the exception of one athlete who completed the same 45 min run on the morning of each race.

### **Pre- and post- race questionnaires**

Before (within 15 mins of race commencement) and immediately after each race, participants completed an online questionnaire (REDCap (24, 25)) regarding GI symptoms (28), (a score  $\geq 5$  was deemed at least “moderate” severity, Supplemental Table 1, Supplemental Digital Content 1), thermal perception [thermal sensation (TS) and thermal comfort (TC), Supplemental Tables 2 and 3, Supplemental Digital Content 1 (29)]. Visual analogue scales (0-100) measured readiness to race (pre-race only): “how ready to race do you feel?”, with 0 representing “not at all ready” and 100 as “the most ready I have ever felt”, and race perception (post-race only): “how do you feel like you raced?”, with 0 representing “the worst I have ever raced” and 100 as “the best I have ever raced”.

### **Blood sampling**

Each week, using pre-organized pathology request slips, participants attended the same commercial pathology branch (Australian Clinical Labs) to have a rested blood sample drawn (total of four samples). An 8.5 mL venous blood sample was collected by a trained phlebotomist into a serum separator tube. Estradiol and progesterone were measured via a Siemens Atellica IM Analyzer using a direct chemiluminescent immunoassay. Four participants did not reside in the locale of an Australian Clinical Labs center and therefore attended an alternative pathology center (Healius Pathology). Participants were advised to complete their blood test the morning

prior to the race; however, athletes were not excluded from participation if this was not achievable. Hence, the blood samples were collected either the morning of (74% of participants) or after (26%) each race at the same time each week ( $\pm 1.3$  hours), at a mean time of within 11.5 h of the race start and all samples were collected within 21 h of the race.

### **Statistical analysis**

Statistical analyses were performed using R Studio (v3.5.2) with statistical significance accepted at an  $\alpha$  level of  $p \leq 0.05$ . Data are presented as mean  $\pm$  standard deviation (SD). Hormone concentrations  $>3$  SD from the group mean were removed as outliers [three elevated estradiol measures and one elevated progesterone measure, (30)]. Repeated measures correlations assessed associations between race time and estradiol/progesterone concentration and the progesterone:estradiol ratio (P:E. as  $\text{nmol} \cdot \text{L}^{-1}$ ) as our primary outcome measures, alongside our secondary outcome measures: total perceived MC symptoms, GI symptoms of at least moderate severity, and changes in TC/TS pre- to post-race. Because these secondary outcomes are ordinal measurements, they present some analysis limitations, however a non-parametric alternative to repeated measures correlation does not exist. A one-way ANOVA or paired t-test assessed differences in race completion time and participant weekly training volume across the four races, alongside participant mean weekly training volume during race weeks compared to volume across non-race weeks.

Sub-analyses using paired t-tests were conducted for athletes who experienced: menses during a race ( $n=24$ ) and/or ovulation within 24 hours of the race ( $n=9$ ) with race performance during these events compared to the mean performance across other races. Race completion time during follicular vs. luteal phases, as separated by ovulation, was also compared for athletes

completing at least one race in each phase (n=31). Finally, sensitivity analyses were performed (31), whereby results were analyzed separately excluding athletes with menstrual irregularities [(MI), n=8, 27 races] and races with minor protocol deviations (6 races).

## **RESULTS**

Thirty-seven participants competed in the race series, with n=19 cyclists completing all four races, n=15 completing three and n=3 completing two. Six participants had minor protocol deviations on one race occasion, including training prior to the blood test (n=4), lack of dietary replication (n=1) and lack of prior day training replication (n=1). However, sensitivity analyses removing these six races did not affect the results. In total, 127 individual races were completed, with five individual races excluded due to technical issues during the race while a single race was excluded due to a missed blood test. This totaled 121 individual races for final analysis. Weekly training time in the weeks before/after the races ( $497 \pm 211$  min $\cdot$ week $^{-1}$ ) did not differ from the training time completed during the four-week race period ( $484 \pm 273$  min $\cdot$ week $^{-1}$ ,  $p=0.714$ ); weekly training time also did not differ between the four race weeks ( $p=0.426$ ).

### **Menstrual characteristics**

A total of 2,493 questionnaires were completed across the study duration (four racing weeks, plus additional weeks pre- or post-racing weeks, to capture data for two complete MCs) with a compliance rate of 98%. Each participant recorded two complete MCs, with the exception of one athlete who, due to later study enrollment and long cycle length (41 days), only had complete data for a single MC. Our cohort had a MC length of  $28 \pm 4$  days, with  $5 \pm 1$  bleeding days and ovulation occurring on day  $15 \pm 3$ . Ovulation was detected in only one of two monitored

MCs for five athletes, and in both cycles for 30 athletes, while suspected anovulation was detected in two athletes (aged 28 and 29, with ovulation detected in all older athletes, confirming pre-menopausal inclusion criteria). Therefore, ovulation was detected in 90% of MCs observed across the 37 athletes. The two athletes with suspected anovulation were in the top 25% with regards to weekly training volume (>10 hours per week), however there was no difference in weekly training volume for athletes who displayed two ovulatory MCs ( $n=30$ ,  $468\pm 218$  min $\cdot$ week $^{-1}$ ) compared to those with disturbances to ovulation as outlined above ( $n=7$ ,  $577\pm 184$  min $\cdot$ week $^{-1}$ ,  $p=0.232$ ).

Participants' menstrual status was retrospectively classified through calendar counting, urinary ovulation, and serum hormone measurements (Supplemental Table 4, Supplemental Digital Content 1):  $n=18$ , eumenorrheic;  $n=11$ , naturally menstruating;  $n=8$ , with MI. Prior to MC monitoring, five athletes reported diagnoses of menstrual dysfunction: PCOS ( $n=3$ ) and endometriosis ( $n=2$ ). Following MC monitoring, we identified a further four MIs: suspected anovulation ( $n=2$ ), oligomenorrhea ( $n=2$ , one of whom also had PCOS) and polymenorrhea ( $n=1$ , who also was anovulatory). Moreover, based on initial data, two athletes had prior diagnosed primary amenorrhea, while a further eight reported onset of menses at  $\geq 15$  years of age. However, all participants were regularly menstruating for at least three years prior to and throughout the study. Sensitivity analyses, removing the eight athletes with MIs did not alter results, and hence they were included for analyses. Therefore, unless otherwise stated, results are presented for 37 athletes across 121 races.

## **Race performance**

There was no correlation between race completion time and estradiol or progesterone concentration (Figures 3A and 3B), nor the P:E ratio ( $r=-0.024$ ,  $p=0.834$ ). Mean race completion time was 31:13±03:04 (mm:ss) and not differ between the four races ( $p=0.458$ ). Performance variability between races was 58±51 s (3%)

There was no difference in race completion time on days when athletes were bleeding, comparative to non-bleeding days ( $n=24$ , Figure 4A), nor any difference between race performance on days when athletes were ovulating compared to other races ( $n=9$ , Figure 4B). Race performance was also not different in the follicular comparative to luteal phase, as separated by ovulation ( $n=31$ , Figure 4C).

## **Symptomology**

Most (92%,  $n=34$ ) athletes reported at least one perceived MC symptom on at least one race day. Of these,  $n=10$  had >3 symptoms,  $n=24$  had 1-3 symptoms, and 82% ( $n=30$ ) recorded symptoms across multiple races. Bloating was the most common self-reported MC symptom (17% of all symptoms reported), followed by fatigue (14%), abdominal cramps (9%) and appetite changes (9%).

Regarding GI symptoms specifically, 46% ( $n=17$ ) of athletes reported symptoms considered to be at least “moderate” in severity pre-race on at least one occasion; 27% ( $n=10$ ) experienced moderate symptoms before several races, and 62% ( $n=23$ ) experienced moderate symptoms post-race at least once. The most common GI symptom pre- and post-race was bloating, accounting for 47% of all moderate severity symptoms pre-race and 28% post-race; with nausea (16%) and urge to vomit (16%) also common post-race.

The number of GI symptoms of at least “moderate” severity pre- but not post-race positively correlated with race time (Figures 5A and 5B), as did the total number of perceived MC symptoms recorded on race day (Figure 5C). The total number of self-reported MC symptoms on race day negatively correlated with race perception [ $r=-0.307$  (95% CI -0.490 to -0.097),  $p=0.005$ ], but not readiness to race ( $r=-0.102$ ,  $p=0.363$ ). There was a negative relationship between estradiol concentration and the total number of perceived MC symptoms reported on race day [ $r=-0.267$  (95% CI -0.459 to -0.052),  $p=0.016$ ] but no relationship between total perceived MC symptoms and progesterone ( $r=-0.047$ ,  $p=0.672$ ) nor P:E ( $r=-0.068$ ,  $p=0.549$ ). There was also no correlation between estradiol and progesterone, nor their ratio, and moderate GI symptoms pre- or post-race (all  $p>0.050$ ).

Where follicular and luteal phase could be verified through ovulation, 14 instances of moderate GI symptoms pre-race occurred in the follicular phase, and 15 instances in the luteal. There were 35 instances of perceived MC-related symptoms on race day in the follicular phase, and 32 instances during luteal. For athletes reporting bloating as a GI symptom pre-race, there were 19 instances during the follicular phase, and 16 occasions during the luteal phase. There was no difference in body mass on occasions athletes reported “moderate” bloating compared to no bloating or that of less than “moderate” severity ( $p=0.476$ ). Body mass also did not differ between follicular and luteal phases ( $p=0.488$ ).

### **Thermal Perception**

There was a negative relationship between both the change in TS (Figure 6A) and TC (Figure 6B) pre- to post-race and race completion time.

## **DISCUSSION**

This study used a novel protocol to investigate whether real-world cycling competition performance was associated with fluctuations in MC phases and associated sex-steroid hormones estradiol and progesterone. Although we observed that estradiol and progesterone concentrations were not related to race completion time, small relationships were observed between race performance and the total number of negative symptoms the riders associated with their MC phase, as well as the number of pre-race GI symptoms of moderate severity. Our findings suggest that fluctuations in ovarian hormone concentrations across the MC are not associated with real-world cycling performance, but perception of negative self-reported MC or GI symptoms may have a greater effect. We also note our experiences with a research protocol that offers the potential to increase validity and flexibility with participant recruitment, to enable others to utilize and refine this type of research study design.

Our results agree with prior studies that have incorporated verified serum estradiol and progesterone concentrations and have failed to observe alterations in cycling TT (16-30 km) performance across MC phases in Tier 2 cyclists (32, 33). Here we note the considerably larger cohort in the present study ( $n=37$ ) compared to those investigations ( $n=5-9$ ) (32, 33). In addition, these studies either prohibited or failed to consider caffeine intake, and relied on TTs conducted in a laboratory environment, thus providing a less ecologically valid race scenario. Other studies of real-world performance have demonstrated a lack of group level/systematic alterations in football match metrics among Tier 3-4 athletes when serum estradiol and progesterone concentrations were confirmed (22). Moreover, our findings may also explain recent work suggesting that fluctuations in sex hormones across the MC do not contribute largely to performance changes when compared to the potential effects of individual or day-to-day

variation (22, 34), however these studies have examined team sports which entail different physiological demands to cycling.

By contrast, some studies verifying estradiol and progesterone concentrations have reported a decline in laboratory measures of endurance capacity during the luteal phase (when progesterone is elevated and estradiol is moderate) (35, 36). A rationale to explain the divergent results includes the recognition of a real-life competition as a dynamic environment in which potential subtle or trivial changes in performance due to ovarian hormone fluctuations may be outweighed by overriding factors such as day-to-day performance variability. This is of real-world significance given the dynamic nature of competitions in which athletes participate. The performance variability between the four races was 3% (58 s): 1% (20 s) for riders in Category A (n=7), 3% (51 and 48 s) for category B (n=6) and C (n=9), and 4% (84 s) for category D (n=15). This aligns with our knowledge that elite athletes demonstrate lesser variability in performance comparative to those less highly trained (15) . It is worth noting that even when a sub-analysis examining the relationship between ovarian hormones and race time was conducted among only the more highly trained athletes (categories A-C, n=22), no relationship was detected. This suggests that any variability due to hormonal influences is perhaps too small to be detected and potentially outweighed by intrinsic performance variability >1%. Another explanation for the divergent results may also be pre-race fueling in the present study, which may override any influence of estradiol or progesterone on performance (37). Indeed, many studies reporting alterations to endurance performance/capacity have been conducted in the fasted state (35, 37, 38), which is not reflective of real-world pre-competition practices.

While we did not detect an alteration in performance with physiological fluctuations in estradiol or progesterone concentration, negative symptomology (both MC and GI) was related



to a slower race performance (with bloating the most prevalent symptom). It is possible that athlete perception or subjective feelings are potentially more influential than physiological variations *per se*. It is common for women to perceive an impairment in training and competition performance during MC phases one and four, in association with negative symptoms (9, 10, 39-41). Indeed, some studies that report no performance alterations with hormonal fluctuations across the MC have observed performance changes related to psychological well-being (39) and negative MC symptoms (42). However, few studies have directly examined the influence of symptoms on performance, instead providing an indirect link by concentrating on the incidence of symptoms across the MC in conjunction with the athletes' perception of how symptoms influence performance. Future research should undertake a more direct investigation of this association, including the pre-tracking of perceived MC-related symptoms prior to examination under experimental conditions, as well as pre-trial assessment of athletes' personal beliefs around the impact that MC and/or GI will have on performance. We also note that the subjective collection of symptoms may be biased by the presentation of exclusively negative perceived MC symptoms. It is possible that recording, and therefore drawing attention to, positive symptoms (e.g., feeling energized) may counteract the reduced well-being that may be enhanced by focusing only on negative symptoms and feelings. Future research should investigate the potential performance benefits of positive MC symptomatology. Lastly, while we measured the severity of GI symptoms, other perceived MC-related symptoms were reported only in terms of incidence, and hence the influence of MC symptom severity on performance was not able to be examined. This therefore warrants future investigation with greater granularity.

It is important to consider that the correlation between symptoms (both MC and GI) and performance was weak in magnitude. Indeed, although the incidence of athletes self-reporting

numerous MC symptoms on a single day was relatively low, occurring at 24 of 121 race occasions (20%), others have reported that athletes who identify three or more symptoms are twice as likely to state they are affected by their MC (11). The presence of multiple GI symptoms at a single race was also relatively low. Of the 17 athletes reporting moderate symptoms before at least one race, just over half (59%, n=10) experienced symptoms at numerous races, while 20 athletes did not experience any GI symptoms of moderate severity prior to racing. It is possible that if more symptoms had been reported in our cohort, perhaps a stronger relationship to performance may have been observed. However, most (81%, n=30) athletes did report perceived MC symptoms on at least two separate races, while just four had symptoms at one race and three did not report any perceived MC symptoms across race days. Of course, symptoms typically associated with the MC (e.g., breast pain, bloating, abdominal cramps) have a range of other causes, and in the absence of exploring a differential diagnosis we cannot unequivocally attribute the reported symptoms to the MC. However, athletes who reported illness on race day were excluded from that race. Separately, medications were not restricted during this study and perhaps individuals experiencing negative perceived MC symptoms utilized analgesics or other relevant medications. However, of those experiencing symptoms, only 17% reported the use of paracetamol or ibuprofen on these days. Interestingly, of the occasions where paracetamol or ibuprofen was used, only 50% of these coincided with menstruation.

Of the 37 menstruating females in this study, only 48% (n=18) were classified as eumenorrheic, 30% were naturally menstruating, and 22% had MI, of which two athletes (5% of the 37) did not ovulate, despite menstruating. Further, despite a mean MC length of  $28 \pm 4$  days, only four out of 37 athletes (11%) had a “typical” 28-day MC, with considerable variability in cycle length within individual athletes (Supplemental Table 4, Supplemental Digital Content 1).

This further supports previous work demonstrating that methods to assess MC characteristics that do not include ovulation confirmation are inadequate to sufficiently characterize menstrual status (23, 43). Given the prevalence of anovulation in our Tier 2 athlete cohort, who average just eight hours of training per week, there is a need for future work to establish anovulation prevalence among elite athlete cohorts.

Finally, we implemented a novel protocol: remotely recruiting and managing participants and using a virtual cycling race for performance measurement. A live race protocol provided a competitive environment alongside an opportunity to engage a larger sample size than that typically employed in sports science research. On the contrary, it was not possible to replicate the dynamic tactical aspects, such as pacing strategies, of a live race between weeks. The controls present in a laboratory environment were also not possible, and we were unable to collect additional data such as heart rate or substrate oxidation which would have provided greater mechanistic detail. Further, the “live” nature of the races meant that rescheduling trials in the event of illness/injury was not possible. Nevertheless, standardization was implemented where possible, including racing on an identical Zwift course each week, using the same pathology lab for 33 out of 37 participants (as described above) and confirming participant compliance with study protocols. We encourage this novel “virtual” study design for future use/investigation given the opportunities presented by conducting a study in locations remote to the researchers.

Our findings must however consider potential limitations. Races were conducted in the evening, whereas morning exercise is typically used in most studies occurring in the fasted state. However, several competitions (including the Olympic Games) occur in the evening due to broadcasting requirements. Due to the real-world nature of this study, whereby participants competed in live races at specified times, we were unable to test specific MC phases and instead

correlated hormonal concentrations to performance, which prevents determining causality. The mean time between race and blood sample was 11.5 hours, and therefore measured hormonal profiles may not be fully reflective of the hormonal milieu exactly at race time, although this is unlikely. Moreover, while participants were instructed to complete their blood sample pre-race, post-race was permitted, if necessary (with between seven and ten athletes completing the blood test post-race each week), and hence we cannot exclude the possibility of an altered sex-hormonal profiles associated with post-race stress in these instances. Finally, although we tracked participants' MCs according to best practice protocols, and hence were able to identify eumenorrhea, some methodological considerations (23) required to achieve a "gold" (44) standard were not achieved. A minimum of two MCs were tracked per athlete; however, guidelines stipulate tracking for at least two months prior to testing. Moreover, races were conducted across a four-week period, and hence were not repeated across a second MC. These decisions were taken to reduce participant burden and increase adherence. Lastly, as explained above, we included individuals with MI (except amenorrhea) to increase generalizability and maximize data retention. Sensitivity analyses indicate that individuals with MIs did not affect the results.

## **CONCLUSIONS AND FUTURE RESEARCH**

Cycling race performance in a virtual competition setting appears not to be systemically altered with fluctuations in estradiol or progesterone across the MC in trained cyclists, but performance may be influenced by negative MC/GI symptoms. Therefore, an individualized approach, including monitoring and managing any negative symptoms, may be better for uncovering any links to individual athlete performance or mitigating performance decline. Future

research should seek further understanding of the relationship between symptoms and performance, both examining if specific symptoms are driving an association, and if this relationship persists into other activities beyond cycling.

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## FIGURE LEGENDS

**Figure 1.** Study protocol. Participants completed a monitoring period (to capture both their menstrual cycle and training) of between four and six weeks prior to, or after if necessary, the commencement of the four-week race series. \*The blood sample was collected either the morning of (Thursday) or after (Friday) each race at the same time each week.

**Figure 2.** Participant flow chart from pre-screening to the final sample size include for analysis. \*Exclusion criteria: living outside of Australia, exercising <150 min per week, no access to Zwift cycling app, use of hormonal contraceptives within three months of study commencement, currently pregnant or breastfeeding, current amenorrhea.

**Figure 3.** Repeated measures correlation between race competition time and (A) estradiol concentration, and (B) progesterone concentration. Different colors represent individual participants.

**Figure 4.** Race time, separated by (A) races when athletes were bleeding compared to the mean time across non-bleeding race days (n=24), (B) days when athletes ovulated on race day compared to the mean time across other races (n=9), and (C) between follicular and luteal phases, as separated by ovulation for ovulatory athletes who completed at least one race in each phase (n=31). The different color lines and symbols represent individual participants: triangle denotes participants present on all three graphs, a circle for two graphs, and a square for one graph.

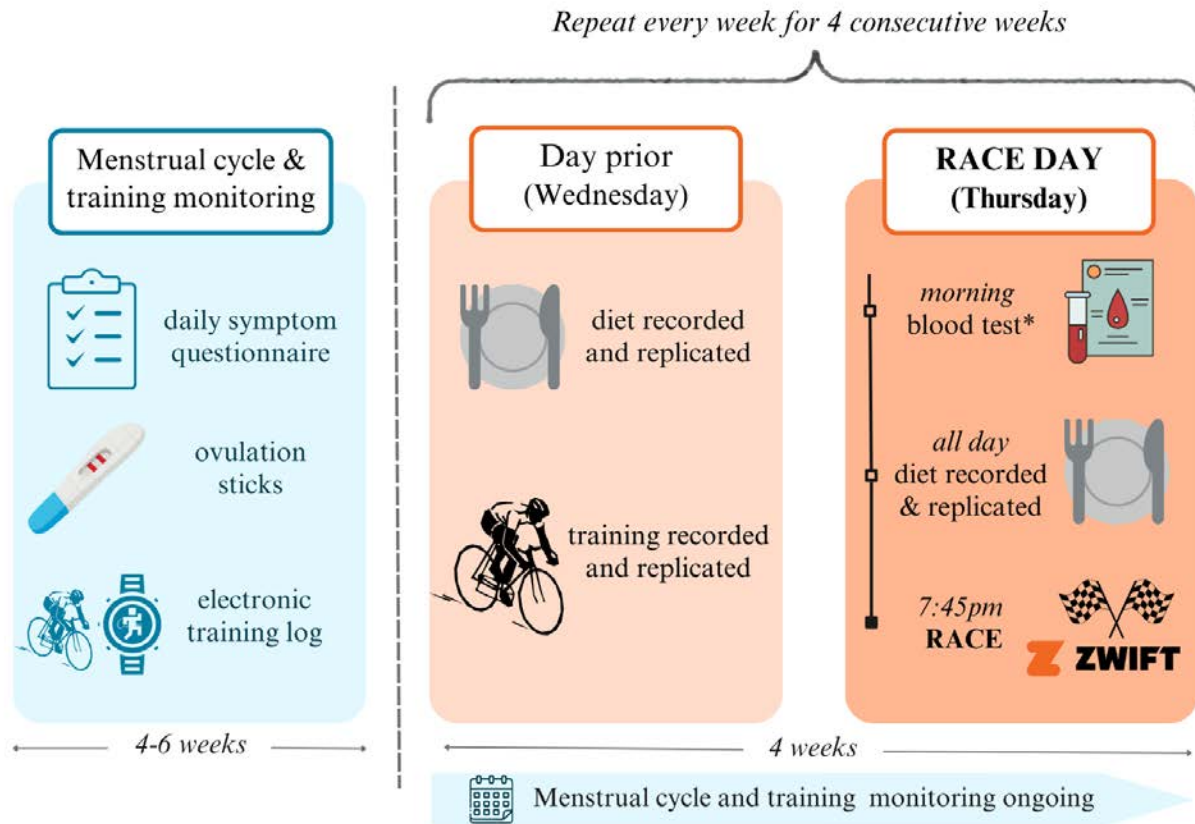
**Figure 5.** Repeated measures correlation between race competition time and (A) the number of “moderate” severity GI symptoms reported pre-race, (B) the number of “moderate” severity GI symptoms reported post-race, and (C) the total number of MC symptoms recorded on the day of the race. Different colors represent individual participants. *GI*; gastrointestinal, *MC*; menstrual cycle.

**Figure 6.** Repeated measures correlation between race competition time and (A) the change in participant ratings of thermal sensation pre-to-post race, and (B) the change in participant ratings of thermal comfort pre-to-post race. Different colors represent individual participants. *TS*; thermal sensation, *TC*; thermal comfort.

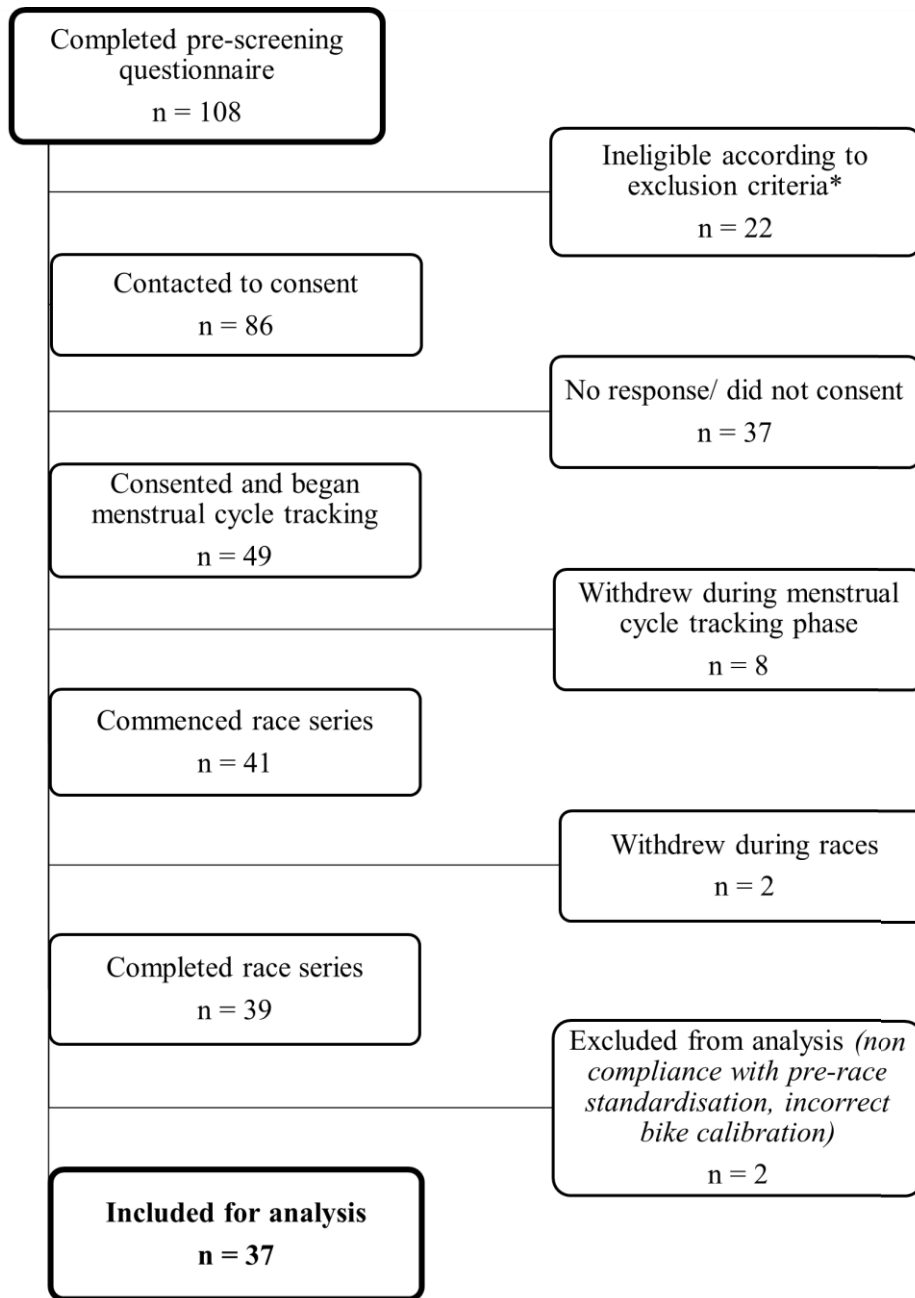
## **SUPPLEMENTAL DIGITAL CONTENT**

**SDC 1:** Supplemental Digital Content 1.docx

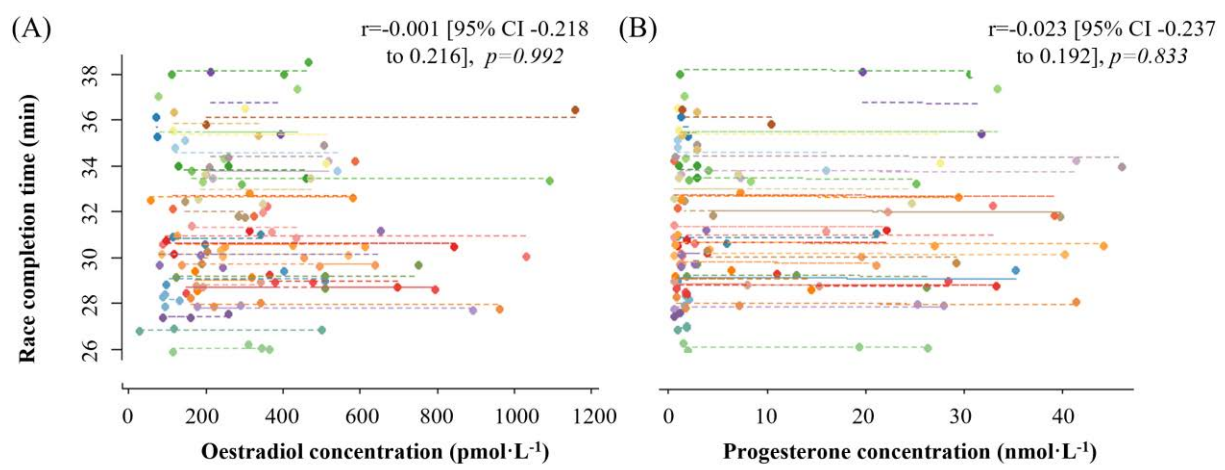
**SDC 2:** Supplemental Digital Content 2.pdf



**Figure 1**

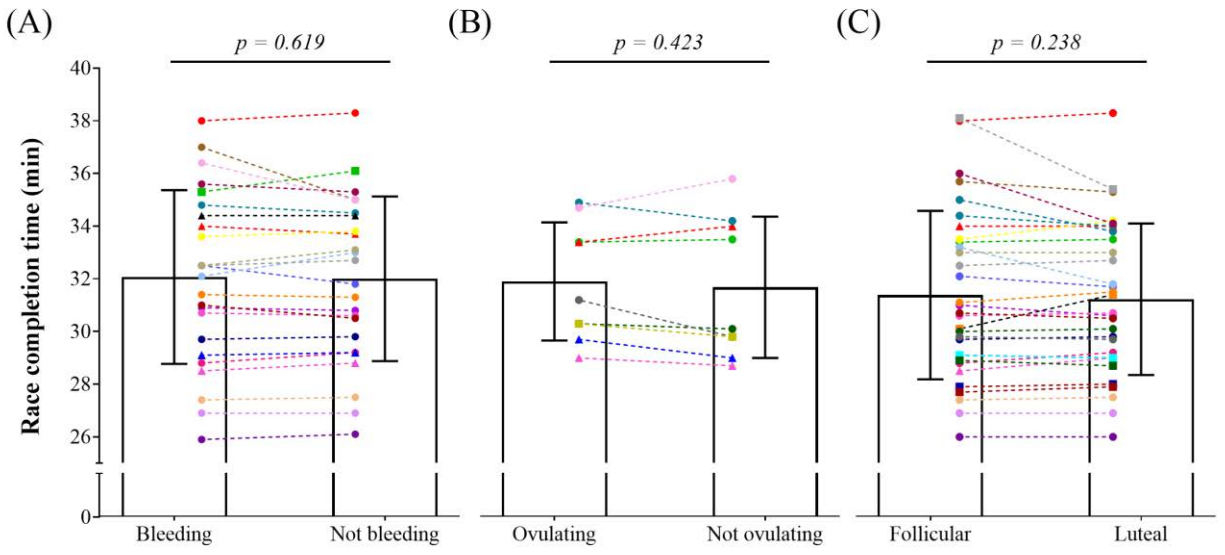


**Figure 2**

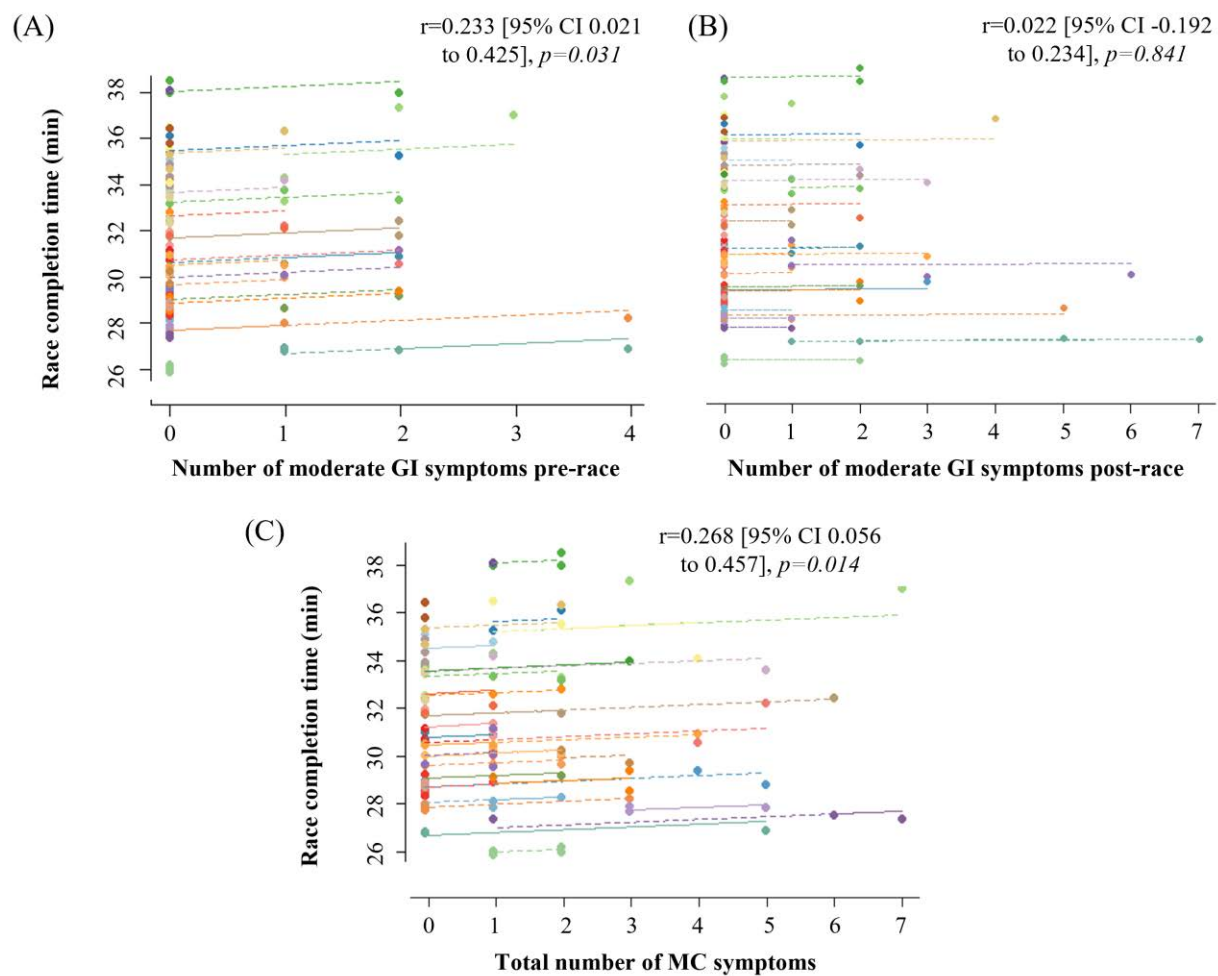


**Figure 3**





**Figure 4**



**Figure 5**

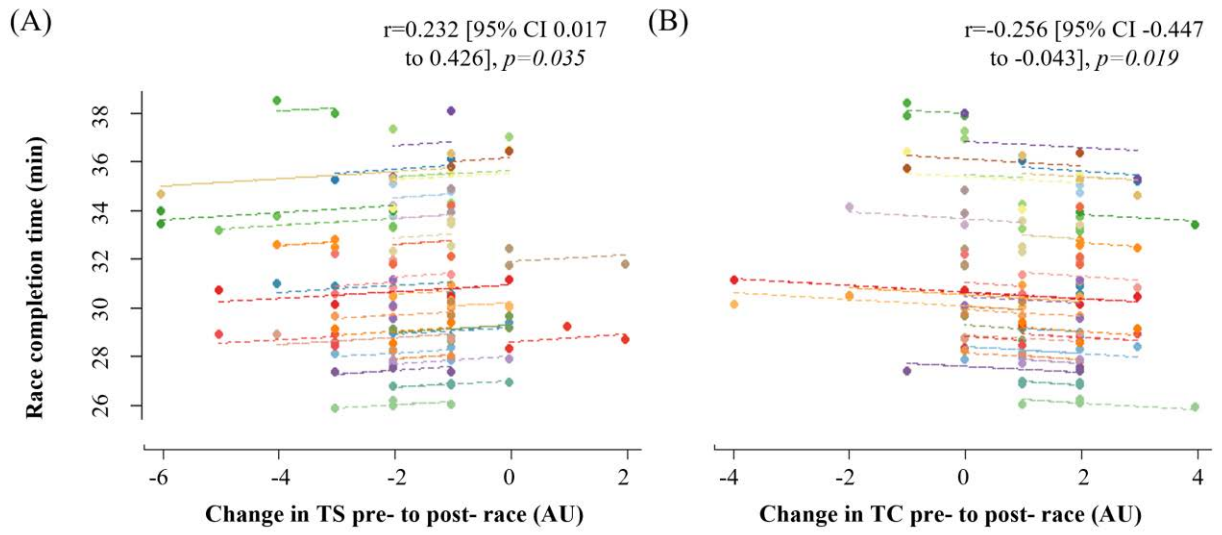


Figure 6





**Table S2.** Likert scale for thermal sensation.

*Please rate your current thermal sensation*

|   |               |
|---|---------------|
| 0 | very hot      |
| 1 | hot           |
| 2 | warm          |
| 3 | slightly warm |
| 4 | neutral       |
| 5 | slightly cool |
| 6 | cool          |
| 7 | cold          |
| 8 | very cold     |

**Table S3.** Likert scale for thermal comfort.

*Please rate your current thermal comfort*

- |   |                    |
|---|--------------------|
| 0 | very comfortable   |
| 1 | comfortable        |
| 2 | just comfortable   |
| 3 | just uncomfortable |
| 4 | uncomfortable      |
| 5 | very uncomfortable |

**Table S4.** Individual participant menstrual cycle information, as confirmed through retrospective classification following two months of menstrual cycle monitoring. Classifications as defined by Elliott-Sale (22): eumenorrhea (menstrual cycle length 21-35 days, confirmed urinary luteinizing hormone surge, serum progesterone concentration  $>16 \text{ nmol}\cdot\text{L}^{-1}$ ), naturally menstruating (cycle length 21-35 days without confirmed ovulation or hormonal profiles), oligomenorrhea (cycle length  $>35$  days), polymenorrhea (cycle length  $<21$  days), anovulatory (negative urinary luteinizing hormone surge testing for two consecutive cycles). “Unknown” is reported if the participant did not record the characteristic.

| Athlete | Cycle 1             |               |               | Cycle 2             |               |               | Progesterone $>16$<br>$\text{nmol}\cdot\text{L}^{-1}$ detected? | Classification (and any diagnoses)        |
|---------|---------------------|---------------|---------------|---------------------|---------------|---------------|---|---|
|         | Cycle length (days) | Bleeding days | Ovulation day | Cycle length (days) | Bleeding days | Ovulation day |   |   |
| 1       | 28                  | 9             | 17            | 27                  | 9             | 16            | Yes   | Eumenorrhea                               |
| 2       | 14                  | 2             | not detected  | 25                  | 6             | not detected  | No  | Polymenorrhea* with suspected anovulation |
| 3       | 37                  | 5             | 17            | 29                  | 7             | 12            | Yes   | Eumenorrhea*                              |
| 4       | 36                  | 8             | 20            | 35                  | 6             | 20            | No  | Oligomenorrhea                            |
| 5       | 26                  | 5             | 15            | 26                  | 3             | 13            | Yes   | Eumenorrhea                               |
| 6       | 40                  | 6             | 12            | 31                  | 6             | 10            | Yes   | Oligomenorrhea* (Endometriosis)           |
| 7       | 25                  | 6             | not detected  | 24                  | 6             | 11            | Yes   | Naturally menstruating                    |
| 8       | 28                  | 7             | 16            | 28                  | 7             | 15            | Yes   | Eumenorrhea                               |
| 9       | 29                  | 5             | 15            | 28                  | 5             | 14            | Yes   | Eumenorrhea                               |
| 10      | 37                  | 6             | 21            | 33                  | 5             | 16            | Yes   | Eumenorrhea*                              |
| 11      | 27                  | 6             | 12            | 28                  | 6             | 13            | No  | Naturally menstruating with ovulation     |
| 12      | 27                  | 6             | not detected  | 27                  | 4             | 12            | Yes   | Naturally menstruating                    |



|    |    |   |              |    |         |              |     |  |
|----|----|---|--------------|----|---------|--------------|-----|--|
| 13 | 29 | 3 | 14           | 28 | 6       | 20           | Yes | Eumenorrhea  |
| 14 | 24 | 4 | 11           | 26 | 5       | 11           | Yes | Eumenorrhea (PCOS)                                   |
| 15 | 26 | 5 | 11           | 27 | 5       | 11           | Yes | Eumenorrhea  |
| 16 | 27 | 4 | 15           | 27 | 4       | 14           | Yes | Eumenorrhea (PCOS)                                   |
| 17 | 26 | 4 | 13           | 24 | 5       | 13           | Yes | Eumenorrhea  |
| 18 | 29 | 5 | not detected | 32 | 5       | not detected | Yes | Naturally menstruating with suspected<br>anovulation |
| 19 | 31 | 5 | 13           | 30 | 7       | 15           | Yes | Eumenorrhea  |
| 20 | 31 | 6 | 17           | 28 | 5       | 15           | Yes | Eumenorrhea  |
| 21 | 28 | 5 | 13           | 29 | 6       | 14           | Yes | Eumenorrhea  |
| 22 | 27 | 5 | 11           | 29 | 6       | 12           | Yes | Eumenorrhea  |
| 23 | 32 | 5 | 17           | 30 | 6       | 16           | Yes | Eumenorrhea  |
| 24 | 26 | 5 | 14           | 24 | 5       | 16           | No  | Naturally menstruating with ovulation                |
| 25 | 27 | 5 | 13           | 22 | 4       | 19           | Yes | Eumenorrhea  |
| 26 | 32 | 7 | 20           | 27 | 6       | not detected | No  | Naturally menstruating                               |
| 27 | 28 | 5 | 24           | 28 | 5       | 18           | Yes | Eumenorrhea  |
| 28 | 28 | 6 | 13           | 28 | 5       | not detected | Yes | Naturally menstruating                               |
| 29 | 28 | 6 | 15           | 34 | 5       | 21           | No  | Naturally menstruating with ovulation                |
| 30 | 26 | 4 | 12           | 24 | 5       | 18           | No  | Naturally menstruating with ovulation                |
| 31 | 24 | 5 | 13           | 41 | unknown | unknown      | No  | Naturally menstruating with ovulation* (PCOS)        |
| 32 | 24 | 6 | 13           | 25 | 6       | 13           | Yes | Eumenorrhea  |
| 33 | 30 | 5 | 17           | 31 | 6       | not detected | Yes | Naturally menstruating                               |
| 34 | 25 | 4 | 12           | 27 | 4       | 13           | Yes | Eumenorrhea  |
| 35 | 23 | 4 | 12           | 26 | 4       | 13           | No  | Naturally menstruating with ovulation (Endo)         |
| 36 | 23 | 7 | 12           | 27 | 8       | 16           | No  | Naturally menstruating with ovulation                |

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|    |    |   |    |    |   |    |    |  |
|----|----|---|----|----|---|----|----|--|
| 37 | 17 | 7 | 16 | 24 | 8 | 14 | No | Naturally menstruating with ovulation* |
|----|----|---|----|----|---|----|----|--|

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*Blood hormone concentrations were measured during one menstrual cycle per athlete. \*denotes some irregularity in menstrual cycle length. Endo, endometriosis; PCOS, polycystic ovary syndrome*