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Three-step method for menstrual and oral contraceptive cycle verification

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Abstract

Objectives: Fluctuating endogenous and exogenous ovarian hormones may influence exercise parameters; yet control and verification of ovarian hormone status is rarely reported and limits current exercise science and sports medicine research. The purpose of this study was to determine the effectiveness of an individualised three-step method in identifying the mid-luteal or high hormone phase in endogenous and exogenous hormone cycles in recreationally-active women and determine hormone and demographic characteristics associated with unsuccessful classification.

Design: Cross-sectional study design.

Methods: Fifty-four recreationally-active women who were either long-term oral contraceptive users (n=28) or experiencing regular natural menstrual cycles (n=26) completed step-wise menstrual mapping, urinary ovulation prediction testing and venous blood sampling for serum/plasma hormone analysis on two days, six to 12 days after positive ovulation prediction to verify ovarian hormone concentrations.

Results: Mid-luteal phase was successfully verified in 100% of oral contraceptive users, and 70% of naturally-menstruating women. Thirty percent of participants were classified as luteal phase deficient; when excluded, the success of the method was 89%. Lower age, body fat and longer menstrual cycles were significantly associated with luteal phase deficiency.

Conclusions: A step-wise method including menstrual cycle mapping, urinary ovulation prediction and serum/plasma hormone measurement was effective at verifying ovarian hormone status. Additional consideration of age, body fat and cycle length enhanced identification of luteal phase deficiency in physically-active women. These findings enable the development of stricter exclusion criteria for

female participants in research studies and minimise the influence of ovarian hormone variations within sports and exercise science and medicine research.

Key words

ovarian hormones; menstruation; luteal phase; anovulation; contraceptive agents; menstrual disturbances

1. Introduction

Fluctuations in endogenous oestrogen and progesterone throughout the menstrual cycle, and in exogenous hormones such as those present in oral contraceptives (OC), may influence exercise performance[1]. Furthermore, hormone status in physically-active women is highly individual and commonly influenced by OC use, anovulation, luteal phase deficiency, or menstrual disturbances such as amenorrhoea. Indeed, research investigating the influence of hormone status on exercise performance in women has yielded inconsistent data; this, at least in part, can be attributed to poor control and/or verification of menstrual cycle phase[2].

Early-follicular menstrual phase is simply identified by the onset of menstruation, and does not provide insight into luteal function and high ovarian hormone conditions, therefore the present method focuses on mid-luteal menstrual phase. Direct methods for identifying mid-luteal menstrual phase, such as ultrasound of follicular development and endometrial biopsies[3,4], combined with frequent measures of serum/plasma ovarian hormone concentrations are time-consuming, costly and invasive. In applied exercise science and sports medicine research, indirect methods of menstrual cycle verification and/or control have been employed, including calendar cycle tracking[5], basal body temperature fluctuations[6], and use of ovulation prediction tests[7]. In isolation, these methods have low success and significant limitations, especially within physically-active women, who are at higher risk of experiencing anovulation or luteal phase deficiency[8,9]. Furthermore, without specifically measuring circulating oestradiol and progesterone levels, accurate identification of cycle phase is unlikely[8,10]. There is a clear need to develop more accurate methods for cycle verification given this is a significant limitation in female specific research.

The primary aim of this study was to investigate the effectiveness of an individualised three-step method of hormonal cycle verification for determination of mid-luteal or high ovarian hormone phase in both endogenous and exogenous ovarian cycles in physically-active women. The secondary aim was to explore hormonal and demographic characteristics associated with successful or unsuccessful

classification of mid-luteal or high hormone phase in both endogenous and exogenous ovarian hormone cycles from the three-step method of menstrual cycle verification.

2. Methods

Fifty-four recreationally-active women (≥150 min.week^{-1} of physical activity) who were either long-term (minimum six months) oral contraceptive users (OC-group; n=28) or experiencing regular natural menstrual cycles 25-40 days in length, with no OC use for a minimum of six months prior to study inclusion (MC-group; n=26) participated in the study. All experimental procedures were approved by the Institutional Medical Human Research Ethics Committee (ethical clearance #2012001438) and participants provided written informed consent.

Participants completed a menstrual cycle diary (adapted from Prior et al.[5]) for three consecutive cycles to determine average cycle length; calculated as the number of days between the onset of consecutive menses. The menstrual diary determined approximate follicular and luteal phases and estimated point of ovulation[11]. All participants in the OC-group were taking a monophasic, combined OC, with ethinyl oestradiol (20-30 mcg, i.e. low dose) and a second or third generation progestin. The OC-group mapped their cycle based on their pill packaging, with day one coinciding with the first withdrawal day (inactive pill); if participants reported missing two or more consecutive pills in one cycle, testing was delayed until adherence was confirmed.

Urinary ovulation prediction testing was performed during the experimental cycle to verify cycle phase and ovulation in the MC-group and confirm exogenous hormone control in the OC-group. Participants were provided a home urine ovulation prediction test for luteinising hormone surge detection (Discover® 7-Day Pregnancy Planning kit, Church and Dwight Australia Pty Ltd.; 95% specificity; 99% accuracy) and instructed to follow the manufacturer's directions to perform ovulation prediction testing for seven consecutive days during one cycle. Results were confirmed by visual inspection of the test strip (participant) and photographic record confirmation (project staff). Two days following the urinary luteinising hormone surge, ovulation was assumed to occur, with the mid-luteal phase beginning six to eight days following ovulation. An absence of the luteinising hormone surge during the menstrual cycle

(MC-group only) indicated absence of ovulation. In this case, testing was delayed by a further cycle until a positive urinary ovulation test was experienced. If three consecutive non-ovulatory cycles were experienced, participants were excluded from the study.

Six to 12 days following positive ovulation prediction, participants attended the laboratory on two occasions, separated by at least two days. Both sessions occurred at the same time of day to minimise diurnal hormone fluctuations[9]. MC participants attended during the estimated mid-luteal phase, six to 12 days following ovulation[12]; OC participants attended in the final two weeks of the consumption phase (days 15-28). At each visit, venous blood (12 mL) was sampled from an antecubital vein for later serum/plasma hormone analysis. Height and body mass were measured using a stadiometer (Seca, Birmingham, UK) and electronic scales (A&D Mercury, Pty Ltd., Thebarton, AUS), respectively. Body composition (body mass, lean body mass, fat mass and body fat percentage) was assessed by dual-energy x-ray absorptiometry (Hologic Discovery W, QDR 4500A, Waltham, Mass., USA). Scans were analysed using software (APEX version 3.3) provided by the manufacturer (Hologic, Bedford, Va., USA) and according to manufacturer instructions. The coefficient of variation (CV) in our laboratory for whole body mass, lean body mass, fat mass and body fat percentage are 0.1%, 0.4%, 1.2% and 1.2%, respectively.

Successful verification of mid-luteal phase in the MC-group was defined as a progesterone concentration >6 $ng.mL^{-1}$ [13,14]. Higher sex-hormone binding globulin concentrations are observed in women taking OC compared to women not taking an OC[15-17] and was used as a physiological indicator of OC compliance in the OC-group[16]. Free androgen index was calculated by the method of Vermeulen et al.[14]. Total testosterone and the free androgen index are higher in women who exhibit menstrual disturbances[17]; therefore, these measures were explored in relation to unsuccessful classification of mid-luteal phase.

Venous blood was collected into prepared vacuum tubes containing K3EDTA or micronised silica until centrifugation. Serum tubes (micronised silica) were allowed to clot at room temperature, and plasma tubes (K3EDTA) were stored on ice. After 30 min, samples were centrifuged at 1100 x G for 10 min at

4° C. Serum and plasma was removed, placed into separate 0.4 mL aliquots and stored at -80° C until analysis. Plasma samples were analysed for oestradiol, progesterone and testosterone, whilst serum samples were analysed for sex-hormone binding globulin using a Cobas e411 electrochemilumescence immunoassay autoanalyser (Roche Diagnostics, Germany) and manufacturer-recommended Elecsys assays. Manufacturer-supplied reagents were used, and instruments were calibrated according to the manufacturer's instructions. The CV's in our laboratory for oestradiol-II, progesterone, testosterone and sex-hormone binding globulin are 3.1%, 5.1%, 4.8% and 3.1%, respectively.

Data were analysed using Microsoft Excel 2007 and SPSS (version 22.0, SPSS, Inc., Chicago, IL, USA). Where data were not normally distributed (assessed by the Shapiro-Wilk test; $p<0.05$), data were log-transformed and re-checked for normality of distribution. Analyses included standard descriptive statistics, frequency counts, Pearson's (r_p) and Spearman's (r_s) correlation coefficients and independent samples t-tests. Variables that were significantly different between successful and unsuccessful classification were placed into unadjusted (univariate) and significant predictors were placed into adjusted (multivariate) binary logistic regression models to ascertain the effects of variables on the likelihood that participants had luteal phase deficiency. All models were tested for goodness of fit using the Hosmer and Lemshow test with significance for fit set at $p<0.05$. All tests were two-tailed and statistical significance was set at $p<0.05$. Results are given as mean±SD, unless stated otherwise.

3. Results

There were no differences ($p>0.05$) between OC- and MC-groups for age, body mass index or body composition (Table 1). Following serum/plasma hormone analysis, the MC-group was sub-divided into 'normal' (MC_{NORM}; n=18; 70%) and luteal phase deficient (i.e. not meeting minimum progesterone concentration criteria for mid-luteal phase; MC_{LPD}; n=8; 30%) groups. MC_{LPD} participants were younger ($p=0.024$), had lower body mass ($p=0.048$) than MC_{NORM} participants, were shorter than OC participants ($p=0.042$), and had lower fat mass ($p=0.002$ vs. MC_{NORM}; $p=0.022$ vs. OC) and body fat % than both MC_{NORM} ($p=0.005$) and OC ($p=0.034$) participants. The MC-group had longer cycles than the OC-group

($p<0.001$); MC_{LPD} participants had longer cycles than both MC_{NORM} ($p=0.003$) and OC ($p<0.001$) participants.

Two (7.7%) MC participants reported anovulatory cycles, and completed a second cycle of ovulation prediction testing prior to serum/plasma hormone sampling. Both participants reported a positive test in the second cycle, and did not cluster in either normal or luteal phase deficient groups. Four (14.3%) OC participants reported a positive ovulation prediction test (no illness/compliance issues were reported) and completed a second cycle of testing; none of these participants reported ovulation in the second cycle. Following inclusion in the study and completion of the test cycle, two (7.7%) MC participants reported abnormal (late or absent) menstruation in the following cycle. Positive ovulation prediction tests in the MC-group occurred 15±2 days after the onset of menstruation (range 10-19 days).

All OC participants recorded suppressed progestogen concentrations with no differences ($p=0.847$) between Day 1 and Day 2 (Table 2). Plasma progesterone analysis indicated that 18 (70%) MC participants met the >6 $ng.mL^{-1}$ progesterone criterion for mid-luteal phase on one (n=18; 70%) or both (n=14; 54%) days and were classified as 'normal' (MC_{NORM}). Despite confirming a positive ovulation prediction test, eight (30%) participants exhibited progesterone concentrations <6.0 $ng.mL^{-1}$ on both testing days, indicating probable luteal phase deficiency (MC_{LPD})[9,13]. When MC_{LPD} participants were excluded from analysis, the three-step method was 89% (n=32) successful at determining mid-luteal phase and 100% (n=24) successful when testing occurred between days 21-24 of the menstrual cycle or seven to ten days following positive testing, indicating higher success than early (days six; n=3/4; 75%) or late (days 11-12; n=5/8; 62.5%) in the testing period.

Logistic regression was performed to ascertain the individual effects of age, cycle length, body mass, body fat percentage, and fat mass on the likelihood that participants had luteal phase deficiency (Table 3). Lower age ($p=0.041$), longer cycle length ($p=0.016$), lower body fat percentage ($p=0.035$), and less fat mass ($p=0.028$) were associated with higher likelihood of luteal phase deficiency, and correctly classified 80.8%, 84.6%, 84.6% and 88.5% of cases, respectively. When combined into one prediction model, age, cycle length and body fat percentage (fat mass was excluded due to its similarity with body

fat percentage), the model was statistically significant (X^2=27.127; p<0.001) and correctly classified 96.2% of cases; however, due to the low sample size, no predictors were considered significantly predictive within the model (all p>0.05).

4. Discussion

The present three-step method for hormone cycle verification was successful in identifying mid-luteal menstrual phase in 70% of participants experiencing natural menstrual cycles. In the remaining 30% of cases, even though participants reported positive urinary ovulation prediction testing, serum/plasma hormone concentrations did not satisfy the criterion for mid-luteal phase. The findings suggest there are significant hormonal, body composition and menstrual cycle characteristic differences between regularly-menstruating women who exhibit normal luteal phase characteristics and those who exhibit luteal phase deficiency. When luteal phase deficient participants were excluded, the method was 90% successful, demonstrating the importance of identifying abnormal luteal phase characteristics in menstrual cycle verification.

The three-step method successfully identified low endogenous oestradiol and progesterone hormone concentrations in 100% of participants using an OC. However, four (14%) OC participants reported ovulation in the first testing cycle, highlighting the importance of utilising ovulation testing in research studies involving OC users to confirm anovulation. Previous research has not verified OC use with a biomarker, likely due to the assumption that the active hormone pill has been correctly consumed for a sufficient number of consecutive days to ensure exogenous hormone control of the phases[18,19]. In the present study, sex-hormone binding globulin concentrations were three-fold higher in the OC-group compared to the MC-group. All OC participants exhibited sex-hormone binding globulin concentrations above physiologically normal concentrations for naturally-menstruating women (approximately three times higher; p<0.001), verifying 100% OC adherence. This novel biomarker of OC adherence has potential application in future research where OC use should be biochemically confirmed.

An important finding of the present study are the significant hormonal, body composition and demographic differences between participants who were successfully classified in mid-luteal phase

compared to those who displayed apparent luteal phase deficiency. Luteal phase deficiency is defined as either reduced progesterone concentrations or shortening of the luteal phase (<10 days), characterised by a minimal progesterone surge[9, 20,21]. Previously, Wideman et al.[9] demonstrated that anovulatory and luteal phase deficient women had lower body mass index than ovulatory women; reflective of the present findings where MC_{LPD} participants had lower body fat indices. Additionally, previous research suggests women with higher physical activity levels and/or lower caloric intake are likely to show more variable sex hormone concentrations across menstrual cycles[22]. Physically-active women also have increased likelihood of anovulation and luteal phase deficiency, which have no perceptible symptoms[23,24], are not reflected in bleeding patterns[20,21], and are not identified through questionnaires[25]. In physically-active women, the prevalence of luteal phase deficiency has been reported as high as 79%[20,21]. Therefore, as participants in the present study were all recreationally physically-active, the 30% incidence of luteal phase deficiency is not surprising and may indeed be lower than previously reported. It is also important to note that self-reported physical activity was not different between MC_{NORM} and MC_{LPD} participants, therefore the difference between groups may be more related to energy balance. Biochemically, participants exhibiting characteristics of luteal phase deficiency had a higher free androgen index, but similar total testosterone concentrations compared to regularly-menstruating women. This could potentially allow identification of luteal phase deficiency through free androgen measurement, which does not require stringent cycle phase verification and could be completed at any time point, regardless of menstrual cycle phase[26].

When combined in a multivariate binary logistic regression age, cycle length and body fat percentage correctly classified 96.2% of cases, despite none of the predictors statistically significantly contributing to the outcome. This is likely due to the low numbers involved in the study; a larger sample size may have increased the ability to determine significant contributions of each of the variables. Despite this, our finding demonstrates that simply-measured characteristics such as age, cycle length and body composition may have the potential to identify luteal phase deficient phases cases. Luteal phase deficiency has no perceptible symptoms[23,24] and as such, may have been implicated in the incorrect classification of mid-luteal phase in previous research. Assessment of these measures prior to study

inclusion may assist in predicting participants who may not exhibit normal luteal phase characteristics, despite exhibiting normal ovulatory characteristics. The ability to differentiate individuals with luteal phase deficiency would enable development of stricter exclusion criteria for research studies that require female participants with normal luteal phase characteristics, within sports and exercise science and medicine, fertility, and intervention studies.

We recognise several limitations of the present study. Firstly, while urinary ovulation prediction has been shown to be superior to other methods of ovulation prediction[7,27,28], it remains a predictive test and no direct measure of ovulation was employed. Urinary ovulation prediction is limited by subjective interpretation of the test result and accuracy is reduced in populations where ovulatory disturbances are prevalent[30]. The predictive methods employed were minimally invasive and cost-effective, and therefore more applicable in practical research environments. Secondly, menstrual dysfunction is less likely to occur when women have adequate caloric intake[23] and when there is positive energy balance[20,29]. Measurement of caloric intake and energy expenditure within this study may have enhanced identification of luteal phase deficiency, especially considering self-report physical activity was not different among groups. Thirdly, we acknowledge the potential limitations of clinical diagnostic auto-immunoassay techniques compared to traditional mass spectrometry techniques for steroid hormone analysis. Detection sensitivity limits were too high for OC-group oestradiol measures, and further investigation should consider mass spectrometry as a potentially more sensitive technique. Finally, the testing window was possibly too wide, with the majority of incorrect classifications occurring early and late (Day six and 12 following the positive ovulation prediction test) coinciding with Days 19 and 27 following the onset of menstruation, respectively. With insights from the present findings, we recommended testing between days seven and nine following the positive ovulation prediction test (days 20-22 following the onset of menstruation), where 100% of trials in participants with normal luteal function were successfully classified.

5. Conclusion

Our findings suggest the three-step method comprising menstrual cycle mapping, home urinary ovulation prediction testing and serum/plasma hormone measurement is effective at verifying hormone status in women using OC 100% of the time, and 70% of the time in naturally-menstruating women. Once luteal phase deficient participants were excluded the method was successful 90% of the time in normally-menstruating women. If tight control of ovarian hormones is required within a study design, women taking an OC may be more suitable participants than naturally-menstruating women; but ovulation testing should still be included. Given the impracticalities surrounding daily serum/plasma hormone measures or direct measures of ovulation, the use of the three-step method for hormone cycle verification is a more cost-effective and less invasive method for verifying ovarian hormone status in the majority of women. Consideration of age, body composition (specifically indices of fat mass) and additional hormone characteristics, including free androgen index and sex-hormone binding globulin could inform more accurate and cost-effective methods for successfully categorising menstrual phase in physically-active women.

6. Practical implications

- Step-wise menstrual cycle mapping, urinary ovulation prediction testing, and serum/plasma hormone verification are an effective combination for accurate verification of mid-luteal menstrual phase. Identification of mid-luteal phase is essential to determine the influence of ovarian hormone concentrations on both acute and chronic physiological and performance adaptations in sport and exercise science and medicine.
- We recommend testing seven to nine days following positive ovulation prediction testing, or 20-22 days following onset of menstruation, where we determined the highest likelihood of correct mid-luteal phase classification.
- Screening of potential luteal phase deficient women should consider age, cycle length and body fat indices.
- Identification of biomarkers with minimal cyclic fluctuations, including sex-hormone binding globulin (for OC use), and free androgen index (for potential luteal phase deficiency) will enhance identification of menstrual characteristics/disturbances.

- The ability to identify individuals with luteal phase deficiency enables development of stricter exclusion criteria for research studies that require female participants with normal menstrual characteristics within sports and exercise science and medicine.

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References

1. Lebrun, CM, Effect of the different phases of the menstrual cycle and oral contraceptives on athletic performance. *Sports Med.,* 1993. **16**(6): p. 400-430.
2. Rickenlund, A, Carlstrom, K, Ekblom, B, et al., Effects of oral contraceptives on body composition and physical performance in female athletes. *J. Clin. Endocrinol. Metab.,* 2004. **89**(9): p. 4364-4370.
3. Ecochard, R, Boehringer, H, Rabilloud, M, et al., Chronological aspects of ultrasonic, hormonal, and other indirect indices of ovulation. *BJOG,* 2001. **108**(8): p. 822-829.
4. Grunfeld, L, Sandler, B, Fox, J, et al., Luteal phase deficiency after completely normal follicular and periovulatory phases. *Fertil. Steril.,* 1989. **52**(6): p. 919-923.
5. Prior, JC, Vigna, Y, and Alojada, N, Conditioning exercise decreases premenstrual symptoms. A prospective controlled three month trial. *Eur. J. Appl. Physiol. Occup. Physiol.,* 1986. **55**(4): p. 349-355.
6. Constantini, NW, Dubnov, G, and Lebrun, CM, The menstrual cycle and sport performance. *Clin. Sports Med.,* 2005. **24**(2): p. e51-82.
7. Miller, PB and Soules, MR, The usefulness of a urinary lh kit for ovulation prediction during menstrual cycles of normal women. *Obstet. Gynecol.,* 1996. **87**(1): p. 13-17.
8. Janse de Jonge, XAK, Effects of the menstrual cycle on exercise performance. *Sports Med.,* 2003. **33**(11): p. 833 - 851.
9. Wideman, L, Montgomery, MM, Levine, BJ, et al., Accuracy of calendar-based methods for assigning menstrual cycle phase in women. *Sports Health,* 2013. **5**(2): p. 143-149.
10. De Souza, MJ, Maguire, MS, Rubin, KR, et al., Effects of menstrual phase and amenorhea on exercise performance in runners. *Med. Sci. Sports Exerc.,* 1990. **22**(5): p. 575-580.
11. Oosthuyse, T and Bosch, AN, The effect of the menstrual cycle on exercise metabolism - implications for exercise performance in eumenorrheic women. *Sports Med.,* 2010. **40**(3): p. 207-227.
12. Prior, JC and Vigna, Y, Gonadal steroids in athletic women contraception, complications and performance. *Sports Med.,* 1985. **2**(4): p. 287-295.
13. Liu, Y, Gold, EB, Lasley, BL, et al., Factors affecting menstrual cycle characteristics. *Am. J. Epidemiol.,* 2004. **160**(2): p. 131-140.
14. Vermeulen, A, Verdonck, L, and Kaufman, JM, A critical evaluation of simple methods for the estimation of free testosterone in serum. *J. Clin. Endocrinol. Metab.,* 1999. **84**(10): p. 3666-3672.
15. Burger, HG, Androgen production in women. *Fertil. Steril.,* 2002. **77 Suppl 4**: p. S3-5.
16. Westhoff, CL, Petrie, KA, and Cremers, S, Using changes in binding globulins to assess oral contraceptive compliance. *Contraception,* 2013. **87**(2): p. 176-181.
17. Bermon, S, Garnier, PY, Hirschberg, AL, et al., Serum androgen levels in elite female athletes. *J. Clin. Endocrinol. Metab.,* 2014. **99**(11): p. 4328-4335.
18. Casazza, GA, Suh, SH, Miller, BF, et al., Effects of oral contraceptives on peak exercise capacity. *J. Appl. Physiol.,* 2002. **93**(5): p. 1698-1702.

19. Rechichi, C and Dawson, B, Oral contracteptive cycle phase does not affect 200-m swim time trial performance. *J. Strength Cond. Res.,* 2012. **26**(4): p. 961-967.
20. De Souza, MJ, Menstrual distrubances in athletes: A focus on luteal phase defects. *Med. Sci. Sports Exerc.,* 2003. **35**(9): p. 1553-1563.
21. De Souza, MJ, Miller, BE, Loucks, AB, et al., High frequency of luteal phase deficiency and anovulation in recreational women runners: Blunted elevation in follicle-stimulating hormone observed during luteal-follicular transition. *J. Clin. Endocrinol. Metab.,* 1998. **83**(12): p. 4220-4232.
22. Shultz, SJ, Wideman, L, Montgomery, MM, et al., Some sex hormone profiles are consistent over time in normal menstruating women: Implications for sports injury epidemiology. *Br. J. Sports Med.,* 2009. **45**: p. 735-742.
23. Nattiv, A, Loucks, AB, Manore, MM, et al., American college of sports medicine position stand. The female athlete triad. *Med. Sci. Sports Exerc.,* 2007. **39**(10): p. 1867-1882.
24. Chen, EC and Brzyski, RG, Exercise and reproductive dysfunction. *Fertil. Steril.,* 1999. **71**(1): p. 1-6.
25. Vescovi, JD, The menstrual cycle and anterior cruciate ligament injury risk: Implications of menstrual cycle variability. *Sports Med.,* 2011. **41**(2): p. 91-101.
26. De Cree, C, Sex steroid metabolism and menstrual irregularities in the exercising female. *Sports Med.,* 1998. **25**(6): p. 369-406.
27. Gudgeon, K, Leader, L, and Howard, B, Evaluation of the accuracy of the home ovulation detection kit, clearplan, at predicting ovulation. *Med. J. Aust.,* 1990. **152**(7): p. 344, 346, 349.
28. Corson, SL, Self-prediction of ovulation using a urinary luteinizing hormone test. *J. Reprod. Med.,* 1986. **31**(8 Suppl): p. 760-763.
29. Redman, LM and Loucks, AB, Menstrual disorders in athletes. *Sports Med.,* 2005. **35**(9): p. 747-755.
30. Guermandi, E, Vegetti, W, Bianchi, MM, et al., Reliability of ovulation tests in infertile women. *Obstet. Gynecol.,* 2001. **97**(1): p. 92-96.

Table 1: Participant characteristics

	Oral contraceptive group (OC; n=28)	Menstrual cycle group (MC; n=26)		
		MC_{ALL} (n=26)	**MC_{NORM} n=18**	**MC_{LPD} n=8**
Age (years)	25±5 (23-27)	26±5 (24-28)	27±5 (25-30)	23±3 (20-25)*
Body mass (kg)	65±7 (63-68)	65±9 (61-68)	67±9 (63-72)	60±7 (54-66)*
Stature (cm)	169±5 (167-172)	168±7 (165-171)	170±7 (166-173)	165±6 (160-170)$^{\#}$
Body mass index ($kg.m^{-2}$)	22.6±1.9 (22.0-23.5)	22.9±2.1 (22.0-23.7)	23.3±2.1 (22.3-24.4)	21.2±2.1 (20.2-23.7)
Bone mineral content (kg)	1.7±0.3 (1.6-1.8)	1.8±0.3 (1.6-1.9)	1.8±0.3 (1.6-1.9)	1.8±0.3 (1.5-2.0)
Fat mass (kg)	19.7±4.2 (18.5-21.8)	19.8±5.0 (17.6-21.7)	21.7±4.2 (19.6-23.8)	15.7±4.0 (12.4-19.0)*$^{\#}$
Lean body mass (kg)	38.2±3.9 (36.6-39.8)	38.1±5.6 (35.5-40.3)	38.5±6.1 (35.5-41.6)	37.1±4.3 (33.5-40.6)
Body fat (%)	32.1±4.5 (30.9-34.4)	32.3±5.3 (30.0-34.6)	34.2±4.6 (31.9-36.4)	28.1±4.7 (24.2-32.0)*$^{\#}$
Cycle length (days)	28±0 (28-28)	31±4 (30-33)$^{\#}$	30±2 (29-31)	34±5 (31-38)*$^{\#}$
Positive ovulation test (day)	NA	15±2 (14-16)	15±2 (14-16)	15±3 (12-17)
Test day 1 (day)	17±4 (16-19)	22±2 (21-23)$^{\#}$	22±2 (21-23)$^{\#}$	22±3 (19-24)$^{\#}$
Test day 2 (day)	20±4 (18-22)	25±2 (24-25)$^{\#}$	25±2 (24-26)$^{\#}$	24±3 (22-26)$^{\#}$

MC_{ALL}: menstrual cycle group; **MC_{NORM}:** menstrual cycle participants meeting normal mid-luteal phase criteria; **MC_{LPD}:** menstrual cycle participants exhibiting luteal phase deficiency; **NA:** not applicable. Parametric data are presented as mean±SD (95%CI); non-parametric data are presented as median [IQR] (95%CI).

* $p<0.05$ vs. MC_{NORM}; $^{\#}$ $p<0.05$ vs. OC

Table 2: Participant hormone characteristics

	Oral contraceptive group (OC; n=28)	Menstrual cycle group (MC; n=26)		
			MC_{NORM} (n=18)	**MC_{LPD} (n=8)**
Oestradiol ($pg.mL^{-1}$) Day1	10.3±9.3 (6.7-14.4)	137.6±74.5 (105.6-169.9)#	144.2±53.7 (117.5-170.9)#	120.5±116.3 (12.9-228.1)#*
Day 2	9.0±5.2 (7.1-11.3)	114.6±50.4 (93.3-135.9)#	132.4±32.8 (116.1-148.7)#	68.7±51.8 (25.4-112.1)#*
Progestogen ($ng.mL^{-1}$) Day 1	0.6±0.3 (0.5-0.7)	10.4±8.1 (7.0-13.8)#	14.2±5.9 (11.3-17.2)#	0.9±0.5 (0.4-1.3)#*
Day 2	0.6±0.3 (0.5-0.7)	6.9±5.5 (5.0-9.6)#	9.6±4.4 (7.5-11.8)#	0.9±0.3 (0.6-1.1)*
Total testosterone ($ng.mL^{-1}$) Day 1	0.2± 0.1 (0.2-0.3)	0.2±0.2 (0.1-0.3)	0.2±0.2 (0.1-0.3)	0.3±0.2 (0.1-0.5)
Day 2	0.2±0.1 (0.1-0.2)	0.2±0.2 (0.1-0.3)	0.2±0.2 (0.1-0.3)	0.3±0.2 (0.1-0.5)#
SHBG ($pg.mL^{-1}$) Day 1	196.2±89.4 (160.1-232.3)	64.6±35.3 (51.4-81.1)#	64.2±29.9 (49.3-79.0)#	65.7±49.6 (19.9-111.6)#
Day 2	196.3±93.1 (160.7-236.6)	64.1±30.4 (52.8-78.7)#	62.7±26.6 (49.5-75.9)#	67.2±39.5 (34.2-100.3)#
Free androgen index Day 1	0.2± 0.1 (0.1-0.2)	0.5±0.6 (0.2-0.8)#	0.3±0.3 (0.2-0.5)#	0.8±1.1 (0.0-1.8)#*
Day 2	0.1± 0.1 (0.1-0.2)	0.4±0.6 (0.2-0.7)#	0.3±0.4 (0.1-0.5)#	0.7±0.9 (0.0-1.4)#

MC_{NORM}: menstrual cycle participants meeting normal progesterone characteristics for mid-luteal phase criteria; **MC_{LPD}:** menstrual cycle participants exhibiting progesterone characteristics for luteal phase deficiency; **SHBG:** sex-hormone binding globulin. Data are presented as mean±SD (95%CI).

# $p<0.05$ vs. **OC**; * $p<0.05$ vs. **MC_{NORM}**

Table 3: Binary logistic regression examining the prediction of luteal phase deficiency in regularly menstruating women.

	Unadjusted			Adjusted		
	Odds ratio	**95% confidence interval**	**p-value**	**Odds ratio**	**95% confidence interval**	**p-value**
Age (years)	0.762	0.587-0.989	0.041	0.175	0.001-27.560	0.500
Cycle length (days)	1.695	1.104-2.602	0.016	0.391	0.073-79.321	0.391
Body mass (kg)	0.869	0.749-1.009	0.065	NA	NA	NA
Fat mass (kg)	0.999	0.999-1.000	0.028	NA	NA	NA
Body fat (%)	0.444	0.209-0.943	0.035	0.385	0.003-9.880	0.385

NA: not applicable