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Insights into the menstrual cycle: exploring menstrual cycle patterns in healthy New Zealand women

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

In

Nutrition and Dietetics

Massey University

Auckland

New Zealand

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2025

Abstract

Background: Poor understandings of the female reproductive cycle and its variability within and between women have led to ambiguity when considering ‘normal’ characteristics and features of the menstrual cycle. While the evidence base in female reproductive health is growing, there is minimal research assessing the features and characteristics of the menstrual cycle in healthy, naturally menstruating, premenopausal women, particularly over numerous consecutive cycles. Therefore, the primary objective of this study was to assess the characteristics of the menstrual cycle in healthy New Zealand women across three to five cycles. A secondary aim was to determine the individual and lifestyle factors that may influence the presence of ovulatory disturbances.

Methods: This prospective cohort study assessed 97 healthy females over three to five consecutive menstrual cycles. A three-step method was used to collect menstrual cycle data, and included calendar-based counting, urinary luteinising hormone (LH) testing and plasma steroid hormone measurement. Presence of subclinical ovulatory disturbances (SOD), including anovulation and luteal phase defects (LPD), was assessed and classed using mid-luteal plasma progesterone thresholds. Women were assigned an ovulatory status based on whether they experienced two or more ovulatory cycles (ovulatory status) or two or more SOD cycles (SOD status). Individual and lifestyle data were collected using demographic, menstrual cycle history and physical activity questionnaires. Descriptive statistics were used to determine menstrual cycle features (menstrual cycle, menses, ovulation, follicular and luteal phase length). A linear mixed model was used to determine the within-woman and between-women variability of phase lengths across ovulatory status. A logistic regression including gynaecological age, total metabolic equivalent physical activity and percentage body fat was used to establish demographic and anthropometric associations with SODs.

Results: In this study, 74.1% of menstrual cycles were ovulatory, 13.5% were anovulatory, and 12.4% were LPD. In women who contributed at least two cycles of data, 34.9% experienced sporadic SOD cycles, and 8.1% experienced recurrent SOD cycles. In cycles in which ovulation occurred, the average day of ovulation was day 14, and day 13 for LPD cycles. The sporadic incidence of SOD cycles was found to be associated with age and gynaecological age, but not body composition, ethnicity, or level of physical activity. Within-woman variability of menstrual

features was found to be greater in SOD women than in ovulatory women. Significant differences in menstrual cycle and luteal phase length between ovulatory and SOD cycles were detected.

Conclusion: Overall, while the majority of menstrual cycles are ovulatory, the presence of SODs, including anovulatory and LPD cycles, are relatively common in healthy, naturally menstruating women. Of note, sporadic SOD cycles appear to be more common than recurrent SOD cycles. While ovulatory cycles tend to follow normative expectations of a menstrual cycle, women who frequently experience SOD cycles may experience more menstrual cycle variability. Future research should expand on the findings of the current study and examine the presence of SOD cycles within longitudinal studies in order to better capture the between-women and within-woman variability.

Acknowledgements

Completing a master's degree and thesis is not a solo journey. So, many thanks are required to everyone who kept tabs, lent a hand, and offered advice and support on my postgraduate journey.

To my supervisors: Claire Badenhorst, Robyn Lawrence, and Maria Casale. Writing a thesis is a steep learning curve, and I really couldn't have done it without your support and expertise (and endless (endless) feedback). Your passion for your fields is inspiring and consistently encouraged me to keep learning and exploring; I am no doubt a more critical thinker and researcher due to your invaluable advice, knowledge and encouragement.

To Silvan, my family and my friends: for checking in, for providing a steady presence and a cool and logical head when I was feeling anything but. In what was a busy season of life, your support enabled me to continue through with this journey with all the highs and lows and doubts that it brings. I feel privileged to have had many of you alongside me as I completed my thesis in the beautiful Bay of Plenty.

To Massey University and the Dietetics teaching team: for providing me the opportunity to grow my dietetics career and my knowledge, for continually challenging me and for introducing me to wonderful friends. While it was, certainly, challenging, the skills that I have learnt and developed over the past few years will serve me well, both professionally and personally.

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Abbreviations

Abbreviation	Meaning
BBT	Basal body temperature
BMI	Body mass index
FP	Follicular phase
LH	Luteinising hormone
LP	Luteal phase
LPD	Luteal phase defect
QBT	Quantitative basal temperature
SOD	Subclinical ovulatory disturbances

1 Purpose

1.1 Introduction

Despite around 50% of the world's population being biologically female (The World Bank, 2023), there is limited research in women's health, in particular, the female reproductive system and menstrual cycle (Mercuri & Cox, 2022; National Academies of Sciences, 2024). While a regular menstrual cycle is considered an indicator of good health particularly due to the protective effect of sex steroid hormones on factors such as bone health and cardiovascular function (Cignarella et al., 2024; Kathy et al., 2020; Liu et al., 2024; Manolagas et al., 2013; Nguyen et al., 1995), the literature reporting on menstrual bleeding, menstrual cycle and phase lengths demonstrates high variability (Fehring et al., 2006; Harlow, 2000; Najmabadi et al., 2020). In contrast, the timing of ovulation, frequency of ovulation and luteal phase defects (LPD) are not well recognised or detailed (Schliep et al., 2014; Schmalenberger et al., 2021; Wegrzynowicz et al., 2024). As a result, the definition of 'normal' characteristics and features of the menstrual cycle remains largely ambiguous in academic research, clinical practice, and wider society (Critchley et al., 2020; Harlow & Ephross, 1995; Persdotter, 2020).

The menstrual cycle is a physiological process that prepares the body for fertilisation and pregnancy and is regulated by the hypothalamic-pituitary-ovarian (HPO) axis (Beshay & Carr, 2017). Collectively, three key physiological processes occur within the menstrual cycle and include the growth of a mature follicle in the ovary and subsequent ovulation, the formation of the corpus luteum and, if fertilisation does not occur, the shedding of the endometrium (menses) (Hall, 2016). The cycle consists of two distinct phases, the follicular stage and the luteal phase, separated by ovulation. These phases can be further broken down into early (menstrual) and late follicular stages, ovulation, and the luteal phase (Elliott-Sale et al., 2021). While the cycle is typically described in this manner of sequential phases, as a physiological process, it functions within a continuum from no ovarian activity through to a fertile ovulatory cycle (Brown, 2011). Cycle variations along this continuum occur in response to environmental stressors (e.g., excessive physical activity) and life stage (e.g., adolescence or perimenopause) (Brown, 2011). These cycle variations result in different sex steroid hormone concentrations throughout the cycle and within phases. Subsequently, it is recognised that each of these phases and the cycle itself demonstrates high inter- and intraindividual variability, which may contribute to poorly defined hormonal phases within the literature (Schmalenberger et al., 2021).

The frequently acknowledged median menstrual cycle length is 28 days (Fehring et al., 2006; Harvey et al., 2009; Henry et al., 2024; Reed & Carr, 2018). However, research has demonstrated that a regular menstrual cycle can be anywhere from 21–35 days, and 80% of women may fall between 25–30 days (Chiazze et al., 1968; Creinin et al., 2004; Fehring et al., 2006; Grieger & Norman, 2020). Menstrual bleeding is commonly cited in the literature to last five days (Creinin et al., 2004; Dasharathy et al., 2012), an average follicular phase is described as lasting between 13 and 18 days (Cole et al., 2009; Fehring et al., 2006; Henry et al., 2024; Lenton, Landgren, Sexton, et al., 1984; Najmabadi et al., 2020), and the luteal phase is reported to be between 11 and 14 days (Cole et al., 2009; Crawford et al., 2017; Fehring et al., 2006; Henry et al., 2024; Lenton, Landgren, & Sexton, 1984; Najmabadi et al., 2020). These averages have served as a basis for a ‘textbook’ description of the menstrual cycle. While these values may reflect average menstrual cycle and phase lengths for premenopausal women, they do not detail the high inter- or intra-variability that exists between individuals. In reality, many women may deviate from these cycle and phase length averages, as evidenced by recent cycle app data, which demonstrated that only 15% to 16.3% of women had a median cycle length of 28 days (Grieger & Norman, 2020; Johnson et al., 2018).

In a 28-day cycle, ovulation is commonly considered to occur on day 14 of the menstrual cycle, approximately 14 days prior to menses (Thiyagarajan et al., 2024) however recent research has demonstrated that this may not always be the case (Bull et al., 2019). Additionally, knowledge surrounding the occurrence and prevalence of anovulation (lack of ovum release from the ovaries) in healthy premenopausal women would appear to be lacking. Studies examining women over two to twelve consecutive cycles suggest that up to 5% of regular cycles in healthy menstruating women may be anovulatory (Andrews et al., 2015; De Souza et al., 2010; Harvey et al., 2009). It has been proposed that anovulation may occur more frequently in women who exercise regularly, are in adolescence and during perimenopause (Brown, 2011; De Souza et al., 2010). These factors highlight the potential influence that stressors (e.g., psychological stress or excessive exercise) and reproductive life stage (e.g., adolescence, perimenopause) have on the variability of ovulation within the menstrual cycle.

In a similar manner to reports on anovulation, there is limited research that details the prevalence of LPD in healthy, regularly menstruating women. In the literature, LPD have been considered to

be due to disruptions to corpus luteum function and can reduce the ability of the endometrium to adequately support implantation and pregnancy (Bopp & Shoupe, 1993; Palomba et al., 2015). Clinically, diagnostic criteria for LPD are luteal phases that last 10 or fewer days, or a luteal phase over 10 days with insufficient progesterone production (Jones, 1976; Sonntag & Ludwig, 2012). The peak mid-luteal serum progesterone levels that define a deficient luteal phase (LP) vary between studies and has been reported to be between <3 ng/mL and <5 ng/mL (9.54 and 15.9 nmol/L) (Hammoud et al., 2012; Schliep et al., 2014). Notably, however, research with a random single time point blood test in the luteal phase has suggested that midluteal progesterone levels of ≥ 3.0 ng/mL (≥ 9.54 nmol/L) is indicative of an ovulatory cycle (Prior et al., 2015). Within the limited research that followed women for up to three consecutive menstrual cycles, the incidence of both short and deficient LPD in regularly cycling women is suggested to be between 5% and 10% (Andrews et al., 2015; De Souza et al., 2010; Schliep et al., 2014). Studies that followed women over a minimum of eight consecutive cycles suggest these rates may be higher at 25% and 26.1% (Harvey et al., 2009; Henry et al., 2024). Interestingly, LPD occurrence has been reported to be higher in women who regularly exercise, with one study suggesting an incidence of 29.2% (35 out of 120 cycles) in women who exceeded two hours of purposeful physical activity a week (De Souza et al., 2010). It is worth noting that both anovulation and LPD are considered to be subtle menstrual cycle disturbances that may not result in changes in menstrual cycle lengths (De Souza et al., 2010). Therefore, their presence in regularly menstruating women may go undetected. This lack of awareness is of particular concern as subtle menstrual disturbances may be associated with negative health outcomes such as infertility, early pregnancy losses and irregular menstrual bleeding (Arredondo & Noble, 2006; Pfister et al., 2019; Pluchino et al., 2014).

Much of the available research that has investigated menstrual cycle features and characteristics has frequently used retrospective or cross-sectional methods, collecting data from menstrual cycle trackers, apps and single time point surveys. While these study designs enable researchers to collect data from large cohorts of women, they require participants to accurately report data on their own menstrual cycle. As menstrual health literacy in educated women from developed countries has been found to be low (Fletcher, 2023), this may limit the reliability of self-reported menstrual cycle data, leading to an increased risk of recall and reporting bias within the literature (Bull et al., 2019; Grieger & Norman, 2020; Sohda et al., 2017). Moreover, few studies have examined menstrual cycle characteristics across more than a single cycle while also using objective measurements. As such, it is likely that these studies may not completely reflect the within-woman, day-to-day, or between-cycle variabilities that can occur in regularly

menstruating women. Therefore, more research is needed over consecutive cycles to help develop a greater understanding of variations in the menstrual cycle in healthy premenopausal women.

It has been considered that the menstrual cycle should be treated as an indicator of health and physiological wellbeing within women (Diaz et al., 2006; Itriyeva, 2022). A regular and ovulatory menstrual cycle has been suggested to have protective effects on the cardiovascular system, though the mechanisms behind this remain unclear (Gordon & Girdler, 2014; Wang et al., 2011). Additionally, associations between irregular cycles and type 2 diabetes mellitus, thyroid disorders and chronic stress have been reported in previous research (Lovkina et al., 2023; Solomon et al., 2002; Xiao & Ferin, 1997). However, without sufficient evidence to classify what a healthy and adaptive menstrual cycle looks like, it becomes exceedingly difficult to rely on it as a health metric. Collectively, these factors highlight the need for more evidence on menstrual cycle features and characteristics in women to better understand healthy and typical menstrual cycle variability.

1.2 Research aim and objective(s)

The primary aim of this research was to describe characteristics of the menstrual cycle in healthy New Zealand women over three to five cycles. A secondary aim was to determine the individual and lifestyle factors that may influence the presence of ovulatory disturbances.

Objectives:

1. To describe the average length and range of the menstrual cycle, menses, follicular and luteal phases in healthy naturally menstruating women.
2. To describe the frequency of ovulation, anovulation and luteal phase defects within healthy naturally menstruating women
3. To determine the average day and range of ovulation within the menstrual cycle in healthy naturally menstruating women.
4. To determine the association between demographic factors (ethnicity, age, gynaecological age, body composition, physical activity) and ovulatory status (e.g. ovulatory or subclinical menstrual cycle disturbance) in healthy naturally menstruating women.

Hypotheses:

1. The average length of the follicular and luteal phases in healthy naturally menstruating females will fall between 10 and 18 days and 12 and 15 days, respectively.
2. The average length of menstrual bleeding and menstrual cycles in healthy naturally menstruating females will fall between 5 and 7 days and 25 and 30 days, respectively.
3. The frequency of subtle menstrual disturbances (anovulation, luteal phase defects) observed in healthy, regularly menstruating women will be low (<10%).
4. The average day of ovulation will lie between day 12 and 16 of the menstrual cycle for healthy, naturally menstruating females.
5. Age and gynaecological age will be associated with the presence of subclinical ovulatory disturbances (SOD).

1.3 Structure of thesis

This thesis will begin with an introductory chapter outlining the purpose of this study. This will discuss the importance of understanding the patterns, features and characteristics of the menstrual cycle in healthy, regularly menstruating women. It will also outline the hypothesis, aims and objectives of the current study. The second chapter will then provide a review of the literature regarding the current knowledge of the menstrual cycle, its variations and previous research methods used when investigating this topic. Chapter three will provide a manuscript discussing the methodology and results of this study. The final and concluding chapter of this thesis will describe the outcomes and conclusions of the research, strengths and limitations, and provide future directions and recommendations for research in the field.

1.4 Researcher's contributions

Table 1.1: Researcher's contributions to study

Tyler Bowler (MSc Nutrition and Dietetics student)	Primary thesis author, completed statistical analysis, thesis writing and editing
Maria Casale (primary supervisor)	Provided thesis guidance and revisions, thesis editor
Claire Badenhorst (secondary supervisor)	Conceptualised this study, completed participant recruitment and data collection, completed and attained study ethics, provided thesis guidance and revisions, thesis editor
Robyn Lawrence (secondary supervisor)	Provided thesis guidance and revisions, thesis editor
Karen Mumme (statistician)	Provided assistance and confirmation with statistical analysis

2 Literature Review

2.1 Introduction

The menstrual cycle describes the cyclical physiological processes that occur within the female reproductive system that help to prepare the body for the possibility of fertilisation and pregnancy (Barbieri, 2014; Thiyagarajan et al., 2024). Commonly, day one of the menstrual cycle is determined by the commencement of menstrual bleeding (Thiyagarajan et al., 2024). The cycle is frequently described as two consecutive phases (follicular and luteal) which last approximately 14 days each, separated by ovulation (Beshay & Carr, 2017). These phases are characterised by the dynamic and biphasic fluctuation of sex steroid hormones (oestrogen and progesterone). The menstrual cycle is regulated by the hypothalamus, which coordinates key events of the menstrual cycle through the release of gonadotrophin-releasing hormone (GnRH), luteinising hormone (LH) and follicle stimulating hormone (FSH) via the pituitary gland (Barbieri, 2014; Beshay & Carr, 2017). The expected variations of these hormones across the cycle within a textbook 28-day menstrual cycle are described in Figure 2.1 (Janse De Jonge et al., 2019). Typically, females will, on average, begin to menstruate between 8.5 and 13 years of age (Biro et al., 2018; Punnonen et al., 1975), and will cease menstruating at menopause (defined by 12 months of no natural menses) at around 50 years of age (Aydos et al., 2005). Notably, sex steroid hormones, primarily oestrogen, have been reported in previous research to be associated with bone health, cardiovascular and chronic renal disease protection, outcomes that may be due to the sex steroid hormones influence on vasodilation and anti-inflammatory actions, as well as helping maintain bone mineral density (Cignarella et al., 2024; Gersh et al., 2024; Manolagas et al., 2013; Nguyen et al., 1995). Therefore, improving our understanding of sex steroid hormone variations within the menstrual cycle and between cycles in women, may be considered an important factor to for understanding women's health and health outcomes.

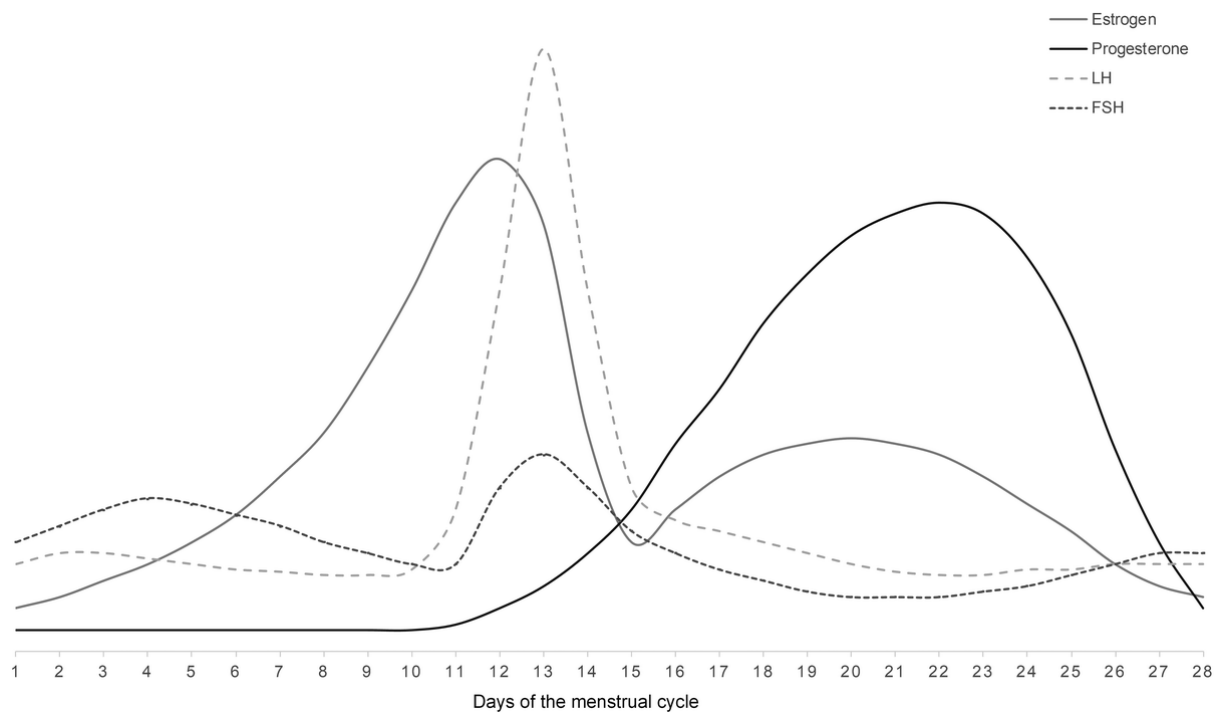


Figure 2.1: Expected hormonal fluctuations across a textbook 28-day menstrual cycle

As a physiological system, the menstrual cycle is in a constant state of flux and variations in cycle presentations and features have been reported throughout the literature. It has been suggested that the menstrual cycle status of a woman will sit along a continuum with a fertile and ovulatory cycle at one end, and amenorrhea (anovulation and low sex steroid hormones) at the other (Brown, 2011). Variations in features (e.g. presence of ovulation and menstrual bleeding) and characteristics (e.g. sex steroid hormone concentrations) of the menstrual cycle may occur more readily based on an individual's life stage, and have reportedly been found to be higher in women going through puberty or perimenopause (Al-Azzawi & Palacios, 2009; Deligeoroglou & Tsimaris, 2010). For adult premenopausal women (aged 18–40 years), variations within the menstrual cycle may still occur in response to multiple stressors including environmental stressors, exercise, energy deficiency, stress or illness (De Souza et al., 1998; De Souza et al., 2010; Huhmann, 2020; Liu et al., 2020; Poitras et al., 2024). The focus of this literature review is to detail the variations in features and characteristics of the menstrual cycle in healthy premenopausal women (aged 18–40 years) that have been reported across previous research.

2.2 Review methods

For the literature review, relevant articles were located through a systematic search conducted across online databases including Google Scholar and PubMed. It included papers published between 1960–2025. Article abstracts were screened for relevance. The following search criteria were used:

- “women” OR “females” OR “perimenopausal women” OR “premenopausal women” OR “healthy women” or “healthy female”
- “eumenorrheic” OR “normally/regularly cycling” OR “normally/regularly menstruating”
- “menstrual cycle” OR “menstruation” OR “menstruating”
- “luteal phase defect” OR “luteal phase deficiency”
- “ovulation” OR “anovulation”
- “oligomenorrhea” OR “irregular bleeding/menstruation” OR “infrequent bleeding/menstruation”
- “Amenorrhea” OR “secondary amenorrhea” OR “hypothalamic amenorrhea”
- “prevalence” OR “incidence” OR “frequency” OR “occurrence”

The structure of the literature review will summarise the published literature on the characteristics of an ovulatory, fertile menstrual cycle, including phase lengths, ovulation and overall cycle and bleeding length. It will explore the presentations of menstrual cycle features, variations and severe disturbances between and within women, including anovulation, luteal phase defects, amenorrhea, and oligomenorrhea.

2.3 The eumenorrheic cycle

In the literature, an eumenorrheic cycle is colloquially described as a ‘normal’ or ‘regular’ menstrual cycle (D’Souza et al., 2023). Characteristics of an eumenorrheic cycle include a menstrual cycle length between 21 and 35 days (Dawson & Reilly, 2009), occurrence of ovulation, a luteal phase lasting a minimum of 10 days, and a single random time point mid-luteal phase progesterone level of >9.54–10 nmol/L (Practice Committees of the American Society for Reproductive Medicine and the Society for Reproductive Endocrinology and Infertility, 2021; Prior et al., 2015). In this review, studies that described their cohort as eumenorrheic were included, as well as cohorts that were described as regularly cycling. Using this criterion, 17 articles were found that had measured and reported on eumenorrheic cycle features and characteristics.

Specific project details of these studies are presented in Table 2.1 and 2.2. Within the included studies, a mean cycle length of 28 and 29 days was reported in six studies (Dasharathy et al., 2012; Fehring et al., 2006; Harvey et al., 2009; Henry et al., 2024; Prior et al., 2015; Prior, Vigna, Schechter, et al., 1990), while two studies reported a mean cycle length of 27.7 and 30.3 days (Cole et al., 2009; Najmabadi et al., 2020).

In an eumenorrheic cycle, the start of menstrual bleeding, also referred to as menstruation, represents the beginning of the follicular phase. During the follicular phase, follicular development and maturation reportedly occur (Thiyagarajan et al., 2024). The follicular phase consists of the early follicular phase (menstruation) and mid-late follicular phase (from the end of menstruation until ovulation) (Elliott-Sale et al., 2021). Of the studies included in this review, the mean menstrual bleeding length of five to six days was reported (Dasharathy et al., 2012; Fehring et al., 2006; Najmabadi et al., 2020). Of the studies that reported on menstruation flow, the heaviest bleeding was reported to occur in the first two to three days following the start of menstruation (Dasharathy et al., 2012). The mean follicular phase lengths (from the start of menstruation through to ovulation) reported in the literature range between 12.9 and 18.5 days (Cole et al., 2009; Fehring et al., 2006; Henry et al., 2024; Lenton, Landgren, Sexton, et al., 1984; Najmabadi et al., 2020). Interestingly, previous research has noted that the substantial variability in the length of the follicular phase reported within the literature is a factor that will influence menstrual cycle length. Thus, the high variability in follicular phase length likely contributes to the high inter-individual variability in menstrual cycle length (Beshay & Carr, 2017; Bull et al., 2019; Waller et al., 1998).

Some research has reported on individual factors that may influence follicular phase length. As an example, age has been associated with variations in follicular phase lengths. Typically, studies report a decrease in follicular phase length with older age. For example, in a study by Lenton et al. (1984), the follicular phase in women aged 18–24 years was significantly longer than women aged between 40–44 years (14.2 days vs 10.4 days, respectively). Similarly, research in women aged 18–40 years reported that women aged ≥ 30 years vs < 30 years had shorter follicular phases (17.6 vs 19.6 days) (Najmabadi et al., 2020). Cumulatively, these results may suggest that premenopausal women over the age of 30, with a higher gynaecological age (defined as chronological age minus age of menarche (Kaplanoglu et al., 2015)), may have shorter follicular phase lengths compared to premenopausal women less than the age of 30 with a younger

gynaecological age. However, the relationship between age and follicular length requires additional research.

In an eumenorrhic cycle, the occurrence of ovulation concludes the follicular phase and is a key event of the menstrual cycle. Ovulation is commonly thought to occur around day 14 (Abo et al., 2022; Cole et al., 2009; Lenton, Landgren, Sexton, et al., 1984). However, within the literature, ovulation has been infrequently reported on day 14. Of the research that has recorded the day of ovulation in premenopausal women, it has been reported on day 16 (Fehring et al., 2006), day 17 (Henry et al., 2024), and day 18 (Najmabadi et al., 2020) of the menstrual cycle. However, of these studies, Henry et al. (2024) had a relatively small sample size of 53 women, whilst another study included women who may have discontinued hormonal contraceptives at any time prior to the study (Najmabadi et al., 2020). The results on the day of ovulation within a cycle may also be influenced by the number of cycles data that were collected, of which previous research has typically only examined a single cycle (Lenton, Landgren, Sexton, et al., 1984). Given this variability in cycle characteristics and study features, researchers examining menstrual cycle characteristics should avoid the assumption that ovulation consistently occurs on day 14. As such, more research over continuous cycles in healthy and regularly menstruating women is needed to examine the extent of variability in ovulation days.

Following ovulation and until the day preceding menstruation in the subsequent cycle, is the luteal phase. There are conflicting reports on the duration of the luteal phase within the available literature. Some research has stated that the lifespan of the corpus luteum is 14 days, and as a result, the luteal phase is consistently 14 days (Marieb & Hoehn, 2019). However, within the available literature that has collected menstrual cycle data, the length of the luteal phase in regularly menstruating women can range between 10.9 and 14.3 days (Cole et al., 2009; Fehring et al., 2006; Henry et al., 2024; Lenton, Landgren, & Sexton, 1984; Najmabadi et al., 2020). However, it should be noted that the duration of the luteal phase reported across studies appears to be dependent on the methods used. For example, in a study of 66 women, which defined luteal length from the quantitative basal temperature (QBT) shift to the day before menses, a mean luteal length of 10.1 days was reported (Prior, Vigna, Schechter, et al., 1990). Similar results were found in two studies where the same methodology was used, reporting luteal lengths of 10.7 and 10.9 days (Harvey et al., 2009; Henry et al., 2024). In contrast, a study following 293 women over one cycle defined luteal length from the day following the urinary LH peak to the day before the

next cycle menses (Lenton, Landgren, & Sexton, 1984). This study reported a longer luteal length of 14.3 days. These findings suggest that studies assessing QBT may report shorter luteal phases, as the assessment of temperature, which accompanies progesterone increase, may occur one to three days following the LH surge (Ecochard et al., 2015). As such, the usage of different methods should be considered when interpreting luteal lengths between studies. As methodological differences have been noted to affect the results of eumenorrheic cycle features and characteristics, the next section will provide a discussion on key methodological considerations in menstrual cycle research.

Table 2.1: Summary of menstrual cycle and phase lengths in studies examining naturally regularly menstruating women

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Methods used to classify ovulation, follicular and luteal phases	Results
Cole et al. (2009)	<i>n</i> =167 Aged 18–36 years No recent history of infertility or menstrual cycle disorders	Naturally menstruating	3–8 menstrual cycles	<ul style="list-style-type: none"> • Used calendar-based counting with urinary LH assessment • <i>Ovulation</i>: LH peak day from urinary samples (assessed via immunoassay) • <i>FP length</i>: first day of menses until LH peak • <i>LP length</i>: day of LH peak to the first day of the next menses. 	<ul style="list-style-type: none"> • Menstrual cycle length (mean): 27.7 days • Follicular phase length (mean): 14.7 days • Day of ovulation: day 14 • Luteal phase length (mean): 13.2 days
Fehring et al. (2006)	<i>n</i> =141 Aged 21–44 years No known fertility issues	Naturally regularly menstruating	3–13 menstrual cycles (mean 5.2)	<ul style="list-style-type: none"> • Used calendar-based counting with urinary LH assessment • <i>Ovulation</i>: LH peak day from urinary samples (measured via electronic fertility monitor) • <i>FP length</i>: first day of menses until and including ovulation 	<ul style="list-style-type: none"> • Menstrual cycle length (mean): 28.9 days, (median): 16 days • Menses length (mean): 5.8 days, (median): 6 days • Follicular phase length (mean): 16.5 days, (median): 16 days • Day of ovulation: day 16 • Luteal phase length (mean): 12.4 days, (median): 13 days

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Methods used to classify ovulation, follicular and luteal phases	Results
				<ul style="list-style-type: none"> • <i>LP length</i>: first day after ovulation to the day before the next menses 	
Dasharathy et al. (2012)	<p><i>n</i>=201 Aged 18–44 years</p> <p>No menstrual disorders, BMI >18 and <35</p>	Naturally regularly menstruating	Two consecutive cycles	<ul style="list-style-type: none"> • Used calendar-based counting with urinary LH tests and mid-luteal phase progesterone • <i>Ovulation</i>: day of positive LH result and mid-luteal phase progesterone > 5ml 	<ul style="list-style-type: none"> • Menstrual cycle length (mean): 28.8 days, (median): 28 days • Menses length (median): 5 days
Lenton, Landgren, Sexton, et al. (1984)	<p><i>n</i>=293 Aged 18–39 years</p>	Naturally regularly menstruating	One cycle	<ul style="list-style-type: none"> • Daily blood samples • <i>Ovulation</i>: day of blood serum LH peak + 0.69 days • <i>FP length</i>: first day of menses until but not including maximum LH concentrations 	<ul style="list-style-type: none"> • Follicular phase length (geometric mean and median): 12.94 days • Day of ovulation: 14.6 days
Lenton, Landgren and Sexton (1984)	<p><i>n</i>=293 Aged 18–39 years</p>	Naturally regularly menstruating	One cycle	<ul style="list-style-type: none"> • Daily blood samples • <i>Ovulation</i>: day of blood serum LH peak + 0.69 days • <i>LP length</i>: day following but not including the day of maximum LH 	<ul style="list-style-type: none"> • Luteal phase length (mean): 14.3 days

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Methods used to classify ovulation, follicular and luteal phases	Results
				concentrations, until the day before the next menses.	
Harvey et al. (2009)	<i>n</i> =66 Aged 22–43 years Healthy, non-smokers	Naturally regularly menstruating	One year	<ul style="list-style-type: none"> • Used calendar-based counting with QBT <i>Ovulation</i>: day of QBT shift • <i>FP length</i>: not outlined • <i>LP length</i>: day of QBT shift to the day before the next menses 	<ul style="list-style-type: none"> • Menstrual cycle length (mean): 28.4 days • Follicular phase length: 17.7 days • Luteal phase length: 10.7 days
Henry et al. (2024)	<i>n</i> =53 Aged 21–41 years Healthy, non-smoking, normal weight	Naturally menstruating	≥ eight cycles (mean 13)	<ul style="list-style-type: none"> • Used calendar-based counting with QBT • <i>Ovulation</i>: day of QBT shift • <i>FP length</i>: first day of menses until day before ovulation • <i>LP length</i>: day of QBT shift to the day before the next menses 	<ul style="list-style-type: none"> • Menstrual cycle length (mean): 28.1 days • Follicular phase length (mean): 17.6 days • Day of ovulation: day 17 • Luteal phase length (median): 10.9 days
Prior, Vigna, Schechter, et al. (1990)	<i>n</i> =66 Aged 20–42 years	Naturally regularly menstruating	One year	<ul style="list-style-type: none"> • Used calendar-based counting with QBT <i>Ovulation</i>: day of QBT shift 	<ul style="list-style-type: none"> • Menstrual cycle length (mean): 28.2 days • Luteal phase length: 10.1 days

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Methods used to classify ovulation, follicular and luteal phases	Results
	Stable weight, no eating disorders, non-obese			<ul style="list-style-type: none"> • <i>LP length</i>: day of QBT shift to the day before the next menses 	
Najmabadi et al. (2020)	<p><i>n</i>=581</p> <p>Aged 18–40 years</p> <p>No known subfertility</p>	Naturally regularly menstruating however included data from women who may have recently discontinued hormonal contraceptives oral contraceptives 1 – 2 menstrual bleeds prior to study	One year	<ul style="list-style-type: none"> • Used calendar-based counting with cervical mucus changes • <i>Ovulation</i>: peak day of cervical mucus • <i>FP length</i>: first day of menses until and including peak day of cervical mucus • <i>LP length</i>: first day after ovulation to the day before the next menses 	<ul style="list-style-type: none"> • Menstrual cycle length (mean): 30.3 days, (median): 29 days • Menses length (mean): 6.2 days, (median): 6 days • Follicular phase length (mean): 18.5 days, (median): 17 days • Day of ovulation: day 18 • Luteal phase length (mean): 11.7 days, (median): 12 days

Abbreviations: FP – follicular phase; LP – luteal phase; LH – luteinising hormone; QBT – Quantitative basal temperature

2.4 Methods used to classify the eumenorrhic cycle

Literature included in this review includes research that has been completed over a span of 60 years. In this timeframe, no consistent or standardised methods for classifying the features and characteristics of the menstrual cycle have been achieved (Allen et al., 2016; Hampson, 2020; Lenton, Landgren, Sexton, et al., 1984). Subsequently, a discussion on research methods and the strengths and limitations of these methods is required to comprehensively understand the menstrual cycle results reported.

When reviewing the literature that was included in this review, it is worth noting that the day of ovulation varies between studies, an outcome that is likely due to the research methods used. Commonly, studies have used calendar-counting methodology (e.g. counting days between menstrual bleeds) (see Table 2.1). This has allowed many studies to report on menstrual cycle length and cycle length variability between women. Based on the length of the cycle and the estimation of ovulation on day 14, the two phases (follicular and luteal) have been categorised as days 1–14 and day 14 until the start of the next menstrual bleed, respectively. Within these research studies, the duration of these phases has served as a proxy for hormonal fluctuations, yet sex steroid hormones have not been verified. In this review, five out of nine studies used calendar-based counting methods combined with an objective method to identify ovulation. The objective methods used to determine ovulation included measuring basal body temperature throughout the cycle to detect the temperature rise that is associated with the increase in progesterone that occurs following ovulation (Bedford et al., 2010; Harvey et al., 2009; Henry et al., 2024; Prior, Vigna, Schechter, et al., 1990) or monitoring for changes in cervical mucus (Najmabadi et al., 2020). Alternatively, studies have also used urinary LH testing, either completed as daily measurements throughout the whole cycle (Cole et al., 2009), or when advised by an electronic fertility monitor (starting testing from the first day of menses) or from cessation of menses until a positive LH test was returned (Fehring et al., 2006). Studies that used urinary LH testing have reported the day of ovulation on days 16 – 18 of the cycle (Cole et al., 2009; Fehring et al., 2006; Henry et al., 2024; Najmabadi et al., 2020). This demonstrates that in studies that used an objective measurement of ovulation in addition to calendar-based counting, a later day of ovulation has been reported than in studies that have only used calendar-based counting and assume ovulation to occur on day 14. Comparatively, four studies within this review have used a three-step method for confirming ovulation within the menstrual cycle. This method included using calendar-based counting of the length of the menstrual cycle, urinary LH tests

mid-cycle to confirm the day when ovulation may have occurred and assessment of mid-luteal phase progesterone to confirm ovulation, using thresholds of between 1 ng/mL and 5 ng/mL (Andrews et al., 2015; Dasharathy et al., 2012; Lynch et al., 2014; Schliep et al., 2014). Interestingly, in studies using this three-step method, the day of ovulation was not reported, therefore highlighting the need for further research into the variability and reporting of the day of ovulation.

Another key consideration of study design that can contribute towards variations in results is study duration. Of the nine studies that reported on menstrual features, four studies had a duration of more than eight cycles (Harvey et al., 2009; Henry et al., 2024; Najmabadi et al., 2020; Prior, Vigna, Schechter, et al., 1990). These studies reported the longest follicular phases of those examined (17.6–18.5 days), the latest days of ovulation (day 17–18), and the shortest luteal phases (10.1–11.7 days) (Harvey et al., 2009; Henry et al., 2024; Najmabadi et al., 2020; Prior, Vigna, Schechter, et al., 1990). Two studies that did not explicitly report the duration but appeared to only follow participants for one month (i.e. one menstrual cycle), reported the shortest follicular phase length at 12.9 days, the earliest day of ovulation on day 14 and the longest luteal phase of 14.3 days (Lenton, Landgren, & Sexton, 1984; Lenton, Landgren, Sexton, et al., 1984). Cumulatively, these results suggest that studies that collect data over one cycle may not be of adequate duration to collect intra-individual variability within the cycle compared to studies that collect data over numerous cycles.

The focus of this review is on healthy menstruating women, and research with a similar cohort of women has reported that external and internal factors are likely to contribute to the variability of menstrual cycle features and characteristics. For example, a one-year study with 581 participants reported the longest follicular phase and the latest day of ovulation (18.5 and 18, respectively). However, within this study, participants were included who may have recently discontinued hormonal contraceptives (i.e. one month) before study data collection (Najmabadi et al., 2020). In contrast, a study that did not include participants who had used oral or subdermal contraceptives in the previous three months reported a shorter follicular phase length of 16.5 days and an earlier day of ovulation on day 16 (Fehring et al., 2006). In women who use hormonal contraceptives, the provision of exogenous hormones suppresses ovulation (Sondheimer, 2008). Previous research has reported that when women stop using hormonal contraceptives, ovulation may be delayed and the follicular phase may be prolonged for up to nine months after

discontinuation (Gnoth et al., 2002; Nassaralla et al., 2011). Therefore, studies that include women who have recently stopped using hormonal contraception may have longer follicular phases and later days of ovulation.

Overall, the physiological features and characteristics of the eumenorrheic menstrual cycle have generally been well described. However, inconsistent methods across studies may contribute to high heterogeneity in the results between studies. While studies often utilise objective methodologies to accurately determine menstrual cycle features, in studies where methodologies include calendar counting or no sex steroid hormone measurement, subtle menstrual disturbances may go undetected. In research that has attempted to collect data on the eumenorrheic cycle but not confirmed ovulation through the measurement of sex steroid hormones in the luteal phase, the presence of undetected LPD and anovulation may be an additional factor that affects variability in results. Therefore, the following sections will provide a discussion of the literature on menstrual cycle variations.

2.5 Anovulation

Although the presence of menstruation is often used as an indicator of reproductive health, ovulatory function may be disrupted within women presenting with regular menstrual cycles (Prior et al., 2015). In this literature review, eleven studies were included that report on the occurrence of anovulation. Anovulation prevalence by cycle was reported to be between 2.7% and 37% (De Souza et al., 2010; Haiman et al., 2002; Harvey et al., 2009; Henry et al., 2024; Lynch et al., 2014; Prior et al., 2015). In studies that reported the percentage of women who experienced anovulatory cycles over the course of the study, the range varied from 3% to 61% (Bedford et al., 2010; Malcolm & Cumming, 2003; Prior, Vigna, Schechter, et al., 1990). The highest anovulation prevalence of 37% was reported in a single-cycle, cross-sectional study of 3709 participants (Prior et al., 2015), where the mean age of the participants was 41.7 years. Conversely, in two one-year observational studies where participants had a lower mean age of 33.9 years, the reported prevalence of anovulation was 2.6% and 3% (Harvey et al., 2009; Henry et al., 2024). The higher prevalence reported by Prior et al. (2015) is not unexpected, as women over the age of 45 years may be experiencing menstrual cycle variations associated with perimenopause (Burger et al., 2008; Van Voorhis et al., 2008). Therefore, studies with higher age cut-offs or mean ages of their participant cohorts may report higher prevalence rates of anovulatory cycles.

In studies including healthy menstruating women, the incidence of ovulation is sporadic, which may contribute to the highly variable prevalence rates of anovulation. For example, in a two year longitudinal study with 123 participants, 61% of women experienced at least one anovulatory cycle (Bedford et al., 2010). In contrast, studies with a shorter duration of data collection (one year or a minimum of eight cycles), a lower prevalence of 2.6% and 3% were reported (Harvey et al., 2009; Henry et al., 2024). Interestingly, the results from studies over one year are comparable to studies with a duration of only one or two cycles, where the reported prevalence was 2.7% (Andrews et al., 2015; Schliep et al., 2014). It is possible that studies with a longer data collection duration (e.g. more than a year) may capture more sporadic anovulatory cycles and, as a result, report higher prevalence. However, how studies report on the prevalence and incidence of anovulation may vary, and this may also contribute to the variable results reported in the literature. For example, in a recent study of 53 women by Henry et al. (2024), including more than eight cycles, the prevalence of anovulation across all cycles was 2.6%. In the same study, the number of women who experienced anovulation at any point during data collection was 17%. The higher percentage of women who experienced sporadic anovulatory cycles may suggest that more women may experience these cycle types inconsistently, rather than consistently, across consecutive cycles. Therefore, a lower prevalence may be observed across all cycles.

While features of anovulatory cycles are reported across the literature, they are not widely and consistently studied. Where reported, the mean cycle length of anovulatory cycles has ranged from 27.4–29 days (Henry et al., 2024; Prior et al., 2015). Within this previous research, no significant differences in cycle length have been reported between ovulatory and anovulatory cycles (Henry et al., 2024; Prior et al., 2015; Prior et al., 1990). Few studies have reported on the hormonal profiles between ovulatory and anovulatory cycles. Of the studies that have measured sex steroid hormones, Prior et al. (1990) reported that anovulatory cycles express oestrogen levels comparable to ovulatory cycles (271 ± 116 vs. 281 ± 103 pmol/L, respectively). However, a defining hormonal feature used to confirm the occurrence of anovulation is the measurement of mid-luteal serum progesterone. In a study by Prior et al. (2015), the researchers classified an ovulatory cycle by a mid-luteal serum progesterone measurement of 9.54 nmol/L or more. Of which, 37% of cycles within this study did not meet this mid-luteal progesterone threshold. However, a limitation of this study was that LPD and anovulation based on mid-luteal progesterone levels were not differentiated, likely resulting in the higher observed value of anovulation. Similarly, two studies using the same method reported a lower prevalence of

anovulation at 2.7% (Andrews et al., 2015; Schliep et al., 2014). It is worth noting that in these two studies, an anovulatory cycle was determined by a mid-luteal serum progesterone measurement of 1 ng/mL or 3.2 nmol/L. Using this threshold rather than combining anovulatory and LPD cycles may result in the lower prevalence of anovulation observed in these studies. As such, researchers may need to be mindful when interpreting anovulation rates and features between studies, as prevalence may be influenced by different mid-luteal phase cut-off values selected by the researchers.

In healthy menstruating women, there is some preliminary evidence that anovulation rates may vary between ethnicities. In a study completed in Los Angeles ($n=241$ participants) examining the incidence of anovulation across different ethnicities, Caucasian women were reported to have anovulatory cycles (14.3%) more frequently than African American (7.1%) or Latina women (6.9%). However, the researchers did note that these differences were not statistically significant (Haiman et al., 2002). As few studies have collected data across ethnic cohorts, more research is required to understand the prevalence and individual variability of the menstrual cycle in non-European cohorts.

Collectively, the findings across the literature indicate that anovulation prevalence rates may vary. Discrepancies in reporting techniques and methods used to determine anovulation prevalence result in variances across studies. As such, there is a need for consistent methodologies to be used in future research collecting menstrual cycle and ovulatory data.

2.6 Luteal phase defects

In a similar manner to anovulation, LPD are a subtle menstrual disturbance that have been inconsistently reported within the available literature. In the research included within this review that has examined the proportion of women who may experience a clinical LPD cycle (LP <10 days), studies reported a prevalence rate between 8.9% and 26.1% (Andrews et al., 2015; Hammoud et al., 2012; Harvey et al., 2009; Henry et al., 2024; Schliep et al., 2014). However, the frequency of this cycle type was higher in studies who reported the percentage of women, rather than the percentage of cycles, who experienced a LPD cycle over the duration of the study (60.6% to 88.7%) (Bedford et al., 2010; Henry et al., 2024; Prior, Vigna, Schechter, et al., 1990). Conversely, of the studies included in this review, three reported on biochemical LPD, reporting

a lower prevalence than clinical LPD cycles of 5% to 8.4% (De Souza et al., 2010; Schliep et al., 2014). Similar to clinical LPD cycles, the percentage of women who experience a biochemical LPD cycle (14%) was higher than the reported biochemical LPD cycle prevalence rates, but the overall percentage of women with biochemical LPD cycles appears to be less than a clinical LPD cycle (Hammoud et al., 2012). Yet it is worth noting that there are a limited number of studies reporting on biochemical LPD cycles, an outcome that may influence these prevalence results.

Within the available research, various methods of detecting LPD cycles have been utilised (see Table 2.2). Previously, LPD has been determined using an evaluation of endometrial maturation (Fritz, 2012; Pillet et al., 1990). However, these methods are no longer widely used due to the natural variation of endometrial maturation within and between women (Murray et al., 2004). In more recent research, mid-luteal phase progesterone levels have been used to define LPD cycles and report on clinical and biochemical LPD prevalence rates. For example, Schliep et al. (2014) reported that of all ovulatory cycles recorded over one to two menstrual cycles, 8.9% met the criteria for clinical LPD, 8.4% met the criteria for biochemical LPD, and 4.3% met both criteria. In the research by Hammoud et al. (2012), 38 cycles among 33 (14%) women had clinical LPD, 18 cycles among 15 (6%) women had biochemical LPD, and 11 cycles among nine (4%) women met both LPD criteria. Whilst these studies analysed the same data set, differences in the prevalence rates for each type of LPD cycle between the studies are likely due to different LPD classification criteria. Specifically, both studies use LP length <10 days for clinical LPD diagnosis, while for biochemical LPD, Schliep et al. (2014) used a mid-luteal progesterone concentration of <16 nmol/L, and Hammoud et al. (2012) used a mid-luteal progesterone concentration of <10 nmol/L. Thus, despite using similar methods, different LPD classification criteria (e.g. different mid-luteal phase progesterone levels) can contribute to differences in LPD prevalence rates reported between studies.

Currently, many studies provide information on both clinical and biochemical LPD prevalence, usually within a study duration of up to two menstrual cycles. The duration of available research may influence the reported incidence of LPD cycles and prevalence rates of LPD cycles over numerous or continuous cycles. Within studies, where data were available over one to two cycles, repeated biochemical and clinical LPD was observed in 2.1% and 3.4% of women, respectively (Schliep et al., 2014). In contrast, studies with a longer duration of one or two years reported that 60.6% and 82% of women experienced a clinical LPD cycle over the course of data

collection (Bedford et al., 2010; Prior, Vigna, Schechter, et al., 1990). Similarly, in a study following 53 participants over a minimum of eight cycles, the overall LPD cycle prevalence was 26.1%. However, 54.7% of women had more than one LPD cycle (Henry et al., 2024). Subsequently, studies with longer durations are more likely to capture the incidence of both sporadic and recurrent LPD cycles in naturally menstruating healthy women.

Cumulatively, the results from the limited number of studies reporting on LPD would suggest that there may be a poor understanding of the prevalence rates of both clinical and biochemical LPD cycles in regularly menstruating women. Differences in methods and study design across the literature (e.g., progesterone levels assessment in mid-luteal phase and the duration of the study) may be factors that affect our current understanding and reporting of LPD prevalence and incidence in healthy, naturally menstruating women.

2.7 Oligomenorrhea

Oligomenorrhea, or infrequent menstrual bleeding, is classified by a cycle length of more than 35 days or having between four to nine menstrual bleeds within a year (Prior, 2022; Riaz & Parekh, 2020). Within this review, six studies examined oligomenorrhea. One study reported a prevalence of 0% in sedentary women (De Souza et al., 2010), a further four studies reported rates of between 10% and 12.3% (Bachmann & Kemmann, 1982; Bedford et al., 2010; Goshtasebi et al., 2018; He et al., 2020), and one study reported a prevalence of 16% (Baranauskas et al., 2023). Despite the focus of this review being on healthy regularly menstruating women, only one examined study reported on oligomenorrhea in a similar population, where a prevalence of 12.3% was reported (Bedford et al., 2010). In three studies where the participant cohorts included hormonal contraceptive users or did not require regular cyclicity, prevalence was reported between 10% and 12.2% (Bachmann & Kemmann, 1982; Goshtasebi et al., 2018; He et al., 2020). While these results are similar to those of Bedford et al (2010) with regularly menstruating women, studies that include women with irregular cycles (e.g. hormonal contraceptive users) may capture more instances of oligomenorrhea, which may increase the prevalence rates of this type of menstrual cycle. This highlights the need for further research to examine oligomenorrhea incidence in regularly menstruating women.

Across the literature, differences in reported prevalence of oligomenorrhea may reflect the classifications chosen by the studies. Cycle intervals of greater than 35 but less than 90 days were used by five studies included in this review. Four of these studies reported a prevalence between 11.3% and 12.3% (Bachmann & Kemmann, 1982; Bedford et al., 2010; Goshtasebi et al., 2018; He et al., 2020). Here, the use of this classification for oligomenorrhea likely contributed to the consistent prevalence rates. In contrast, Baranauskas et al. (2023) reported on the number of women who had experienced ≤ 10 menses in the last year. In this study, the prevalence of oligomenorrhea was reported at 16%, the highest reported. It is possible that this definition used by Baranauskas et al. (2023) may have captured other menstrual disturbances (e.g. amenorrhea) in addition to oligomenorrhea, potentially elevating the prevalence rates reported. Consequently, when critically assessing the literature, attention should be drawn to the different criteria used between studies, as it is likely to impact reported prevalence rates.

While oligomenorrhea is widely defined by menstrual irregularity, methodological differences in its assessment, including study type and duration, are observed across the literature. Of the studies reporting on oligomenorrhea, four studies used cross-sectional survey-based methodologies (Bachmann & Kemmann, 1982; Baranauskas et al., 2023; Goshtasebi et al., 2018; He et al., 2020), and two were prospective observational/longitudinal (Bedford et al., 2010; De Souza et al., 2010). In the two-year longitudinal study, Bedford et al. (2010) reported the prevalence of oligomenorrhea at 12.3% in a regularly menstruating population. Comparatively, in a study following women for one to three menstrual cycles, De Souza et al. (2010) reported a lower rate of 0% in healthy sedentary women. This may suggest that the duration of a prospective cohort study will contribute to differences in prevalence rates for oligomenorrhea. Interestingly, these results differ from two cross-sectional studies that reported prevalence rates of oligomenorrhea as 10% and 12.2% (Bachmann & Kemmann, 1982; He et al., 2020). In these survey-based studies, women are asked to recall the number of menstrual bleeds they have had over the previous 12 months. Oligomenorrhea is then defined based on the self-reported number of cycles the women can recall in the last year, increasing the risk of recall bias. As such, when interpreting oligomenorrhea rates between studies, researchers may need to consider that these methodological differences may influence the reported prevalence rates.

In two cross-sectional studies reporting on oligomenorrhea, survey and objective measures were utilised. In a study of 12,964 women, the reported prevalence rate of oligomenorrhea was 12.2%

(He et al., 2020). Within this study, hormones were assessed during days two to four of menstruation in a random subset of participants, yet mid-luteal progesterone levels were not measured. The hormones that were measured during menstruation included follicle-stimulating hormone, oestradiol, and testosterone. However, these were not used to determine oligomenorrheic cycles, as this was defined by participant report of menstrual cycle intervals greater than 35 days. Conversely, in a study of 87 women, ovulatory disturbances were verified by measurement of daily urinary hormone metabolites, and a lower oligomenorrheic prevalence of 3.5% reported in regularly exercising women (De Souza et al., 2010). Within this study by De Souza et al (2010), at least one woman presented inconsistently with oligomenorrhea, i.e., experienced one oligomenorrheic cycle and one regularly ovulatory cycle. These results would suggest that some cycles may be ovulatory despite infrequent menstruation or being classified as oligomenorrheic. Although some studies have examined sex steroid hormonal levels within oligomenorrheic cycles, there is limited reporting on the prevalence of ovulation. Moreover, phase lengths within oligomenorrheic cycles have rarely been reported on. As such, future research may need to consider examining hormonal profiles, the occurrence of ovulation and the length of cycles and phases that oligomenorrheic cycles present with.

While oligomenorrhea prevalence rates are relatively consistent across studies, many studies use cross-sectional methodologies that rely on self-reported menstrual histories. In addition, few studies examine its incidence or sporadic occurrence of this cycle type in regularly menstruating women. This highlights the need for future research to incorporate observational studies with hormonal measurement over consecutive months to adequately capture the prevalence of oligomenorrheic cycles in healthy menstruating women.

2.8 Amenorrhea

Amenorrhea may be defined as the absence of menstruation and can be classified into either primary or secondary amenorrhea (Nawaz et al., 2024). Primary amenorrhea is the absence of menstruation in females aged 16 years or older who have already developed secondary sexual characteristics, or, in 14-year-olds who have yet to develop secondary sexual characteristics (Deligeoroglou & Tsimaris, 2010; West, 1998). Secondary amenorrhea, also known as functional hypothalamic amenorrhea, may be defined as the absence of menstruation for three or more months in a female who has menstruated previously (Kerns et al., 2022). The focus of the studies in this review is on secondary amenorrhea, and eight studies were included that reported on its

prevalence. Prevalence rates of secondary amenorrhea between studies varied from 0% to 16% (Bachmann & Kemmann, 1982; Baranauskas et al., 2023; Bedford et al., 2010; De Souza et al., 2010; Goshtasebi et al., 2018; Munster et al., 1992; Prior et al., 1990). These prevalence rates may be influenced by participant characteristics of the various studies included in this review. For example, in a two-year longitudinal study that recruited regularly menstruating women, Bedford et al. (2010) reported an amenorrhea prevalence rate of 0.8%. Comparatively, in a prospective cohort study that did not require regular menses, a higher prevalence of 8% was reported (Goshtasebi et al., 2018). Women who experience menstrual irregularities may do so recurrently (Song et al., 2022). Therefore, it may be proposed that studies that include women with non-regular cycles are more likely to capture instances of secondary amenorrhea, impacting prevalence rates reported within the literature.

Similar to much of the research on the menstrual cycle that has been reported already in this review, methodological differences across the reviewed studies were observed, which are likely to impact the prevalence rates of secondary amenorrhea. In a one-year prospective cohort study by Prior et al. (1990), a 0% prevalence of amenorrhea was reported in healthy regularly menstruating women. Similar results were found in a two-year longitudinal study by Bedford et al. (2010), who reported an amenorrhea prevalence rate of 0.8% in a similar cohort (healthy, regularly menstruating). Conversely, in two cross-sectional studies, slightly higher rates were recorded. In a group of college students, a self-administered questionnaire that examined amenorrhea incidence over their reproductive lifespan found an incidence of 2.6% (Bachmann & Kemmann, 1982). However, in a randomly selected group of female participants that examined amenorrhea incidence over the preceding year, a value of 4.6% was reported (Munster et al., 1992). As previously noted, cross-sectional studies such as the aforementioned studies rely on accurate self-reporting and recall of menstrual history from participants. Naturally, within these studies, the risk of recall bias may be increased. Since cross-sectional survey-based study designs enable larger sample sizes, an increase in recall bias may result in higher self-reported prevalence rates of secondary amenorrhea than observational prospective studies.

In addition to a range of methodologies used, a range in participant ages were observed across the studies. In a study assessing amenorrhea in a college student population of 991 women, where the mean age of the participants was 19.2 years, a secondary amenorrhea prevalence of 2.6% was reported (Bachmann & Kemmann, 1982). Conversely, in a study by Goshtasebi et al.

(2018) with 1532 women, the mean participant age was higher at 51.5 years, and a higher secondary amenorrhea prevalence rate of 8% was reported. As perimenopausal-related menstrual cycle changes may be present in women over the age of 45 years (Burger et al., 2008; Van Voorhis et al., 2008), the higher prevalence reported by Goshtasebi et al. (2018) is not unexpected. This demonstrates that studies with higher age (i.e. closer to menopause) may report higher prevalence rates of amenorrhea.

A potentially influential factor in the prevalence rates reported across studies is the classification of amenorrhea. In two studies where a classification of >180 days between menstrual bleeding was used, amenorrhea prevalence was reported at 0% and 0.8% (Bedford et al., 2010; Prior et al., 1990). Conversely, in a study where a lower criterion of >90 days between menstrual bleeds was used, a higher prevalence rate of 4.6% was reported (Munster et al., 1992). As such, researchers should be mindful when analysing amenorrhea rates between studies, as it is likely that the rates reported will be influenced by the criteria chosen by the researcher.

Collectively, these findings suggest that across the literature, the understanding of secondary amenorrhea prevalence in healthy women is largely lacking. Methodological differences, including study design (e.g., cross-sectional versus prospective), study duration and participant ages, are likely to influence the prevalence rates reported by studies. These findings highlight the need for further studies with both direct observational assessment and prolonged duration that can capture its incidence.

Table 2.2: Summary of studies reporting on the prevalence of subtle (anovulation, luteal phase defect) and severe (oligomenorrhea, amenorrhea) menstrual disturbances in naturally menstruating women

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Study design/methods	Definitions used to classify ovulation/phases	Results
Andrews et al. (2015)	n=259 Aged 18–44 years No menstrual or chronic disorders	Naturally regularly menstruating	<i>Biocycle study</i> : followed for one (n=9) or two (n=250) cycles.	<ul style="list-style-type: none"> • Prospective cohort study • Used electronic fertility monitor (LH) to time serum blood draws (menses, mid-follicular, late follicular, LH surge, expected ovulation and early, mid- and late luteal phases) 	<ul style="list-style-type: none"> • <i>Anovulation</i>: mid-luteal serum progesterone measurements <1 ng/ml • <i>LPD</i>: LP <10 days 	<ul style="list-style-type: none"> • Anovulation prevalence: 2.7% • Clinical LPD prevalence: 8.9%
Bedford et al. (2010)	n=123 Aged 19–35 years No menstrual or chronic disorders	Regularly menstruating – <i>some participants had current hormonal contraceptive use</i>	Two years	<ul style="list-style-type: none"> • Longitudinal study • Daily temperature measurements • Data collection (validated questionnaires) at baseline and two follow ups (mean 7 months and 2 years) after baseline 	<ul style="list-style-type: none"> • <i>Anovulation</i>: no significant increase in basal body temperature • <i>Clinical LPD</i>: LP <10 days • <i>Oligomenorrhea criteria</i>: >35 days, <90 days between menses • <i>Amenorrhea criteria</i>: >180 days between cycles 	<ul style="list-style-type: none"> • Anovulation prevalence: 61% of women had at least one anovulatory cycle • Clinical LPD prevalence: 82% of women had at least one LPD cycle • Oligomenorrhea prevalence: 12.3% • Amenorrhea prevalence: 0.8%

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Study design/methods	Definitions used to classify ovulation/phases	Results
De Souza et al. (2010)	<p><i>n</i>=87</p> <p>Aged 18–35 years</p> <p>Healthy, no menstrual or chronic disorders, non-smoker</p>	Naturally menstruating	One to three consecutive cycles (mean 2.3 cycles)	<ul style="list-style-type: none"> • Combined data sets of two studies • Daily ovarian steroid excretion measurements 	<ul style="list-style-type: none"> • Assessed hormones through urinary excretion • <i>Ovulation</i>: LH surge concentration >25 mIU/ml, E1G peak concentration > 35 ng/ml, peak PdG concentration >5 µg/ml during the luteal phase • <i>LPD criteria</i>: Short: <10 days Inadequate: sum of 3-day midluteal peak PdG <10 µg/ml and when the PdG peak concentration <5 µg/ml • <i>Oligomenorrhea criteria</i>: >35 days, <90 days between menses • <i>Amenorrhea criteria</i>: no menses >90 days between menses 	<ul style="list-style-type: none"> • <i>Sedentary women</i>: LPD: 5% Oligomenorrhea, amenorrhea: 0% • <i>Exercising women</i>: Anovulation: 25% LPD: 27% Oligomenorrhea: 3.5% Amenorrhea: 33.7%
Haiman et al. (2002)	<i>n</i> =241	Naturally regularly menstruating	Two cycles	<ul style="list-style-type: none"> • Cross sectional study 	<ul style="list-style-type: none"> • <i>Anovulation (blood cycle)</i>: serum 	<ul style="list-style-type: none"> • Anovulation prevalence in white women: 14.3%

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Study design/methods	Definitions used to classify ovulation/phases	Results
	Aged 18–34 years Nulliparous, free of underlying conditions			<ul style="list-style-type: none"> • Blood and urine hormone analysis completed • On blood cycle: samples taken on days 11 and 22, and on days 29, 36, and 42 until menses occurred (all ± 1 day) • On urine cycle: samples taken on day 10 and every subsequent 4 days until menses 	$P_4 < 3.0 \text{ ng ml}^{-1}$ at 3 – 11 days prior to menses <ul style="list-style-type: none"> • <i>Anovulation (urine cycle)</i>: Urinary Pdiol-3G level $< 1.25 \mu\text{g mg}^{-1}$ at 3 – 11 days prior to menses 	<ul style="list-style-type: none"> • Anovulation prevalence in African American women: 7.1% • Anovulation prevalence in Latina women: 6.9%
Harvey et al. (2009)	$n=62$ Aged 22–43 years Healthy, non-smokers	Naturally regularly menstruating	One year	<ul style="list-style-type: none"> • Daily temperature measurements 	<ul style="list-style-type: none"> • <i>Anovulation</i>: no significant increase in basal body temperature • <i>LPD</i>: LP < 10 days 	<ul style="list-style-type: none"> • Anovulation prevalence: 3% • Clinical LPD prevalence: 25%
Henry et al. (2024)	$n=53$ Aged 21–41 years	Naturally regularly menstruating	\geq eight cycles (mean 13)	<ul style="list-style-type: none"> • Prospective cohort study • Daily temperature measurements 	<ul style="list-style-type: none"> • <i>Anovulation</i>: no significant increase in basal body temperature • <i>LPD</i>: LP < 10 days. 	<ul style="list-style-type: none"> • Anovulation prevalence: 2.6% • Clinical LPD prevalence: 26.1% of cycles were LPD

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Study design/methods	Definitions used to classify ovulation/phases	Results
	Non-smoking, normal weight					<ul style="list-style-type: none"> • 17% of women had one LPD cycle, 54.7% of women had >one LPD cycle
Lynch et al. (2014)	<p><i>n</i>=259</p> <p>Aged 18–44 years</p> <p>BMI >18 and <35, no menstrual or chronic disorders</p>	Naturally regularly menstruating	<i>Biocycle study</i> : followed for one (<i>n</i> =9) or two (<i>n</i> =250) cycles.	<ul style="list-style-type: none"> • Prospective cohort study • Used electronic fertility monitor (LH) to time serum blood draws (menses, mid-follicular, late follicular, LH surge, expected ovulation and early, mid- and late luteal phases) 	<ul style="list-style-type: none"> • <i>Anovulation</i>: classified through 11 algorithms using serum progesterone thresholds ($\leq 3\text{--}5$ ng/mL), luteal-phase progesterone ratios, absence of mid-cycle LH surge, or a combination of progesterone + LH criteria 	<ul style="list-style-type: none"> • Prevalence of anovulation based on serum LH, E₂, and P4 level algorithms: 5.5%–12.8%. • Prevalence of anovulation based on urinary LH/ primary E₂ metabolite, estrone-3-glucuronide levels: 3.4%–18.6%
Malcolm and Cumming (2003)	<p><i>n</i>= 510</p> <p>Aged 24–46 years</p> <p>Couples attending the Fertility and Women’s Endocrine clinic for infertility</p>	Naturally menstruating	Retrospective study from Feb 1997–Dec 1999, follow up until Aug 2002	<ul style="list-style-type: none"> • Retrospective observational study • Reviewed midluteal serum progesterone levels of women presenting at a fertility clinic 	<ul style="list-style-type: none"> • <i>Anovulation</i>: mid-luteal serum progesterone ≤ 15 nmol/L at 7 days prior to menses 	<ul style="list-style-type: none"> • Anovulation prevalence: 3.7% of women had at least one anovulatory cycle

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Study design/methods	Definitions used to classify ovulation/phases	Results
Prior, Vigna, Schechter, et al. (1990)	<i>n</i> =66 Aged 20–42 years Stable weight, no eating disorders, non-obese	Naturally regularly menstruating	One year	<ul style="list-style-type: none"> • Daily temperature measurements • Serum samples taken on first and last menstrual cycles (early follicular and mid-luteal) – to assess LH, FS, oestrogen, progesterone 	<ul style="list-style-type: none"> • <i>Anovulation</i>: no significant increase in basal body temperature • <i>LPD</i>: LP <10 days • <i>Amenorrhea criteria</i>: no menstrual flow for ≥180 days 	<ul style="list-style-type: none"> • Anovulation prevalence: 19.7% of women had at least one anovulatory cycle • Clinical LPD prevalence: 60.6% of women had at least one LPD cycle • Amenorrhea prevalence: 0%
Prior et al. (2015)	<i>n</i> =3709 Aged 20–49.9 years No lactational amenorrhea or immediately postpartum, non-menopausal	Naturally regularly menstruating	One cycle	<ul style="list-style-type: none"> • Cross-sectional, population-based study • Single mid-luteal progesterone serum assessment, timed based on participants normal self-reported cycle characteristics 	<ul style="list-style-type: none"> • <i>Anovulation</i>: serum progesterone <9.54 nmol/L at 3 to 14 days prior to menses 	<ul style="list-style-type: none"> • SOD prevalence: 37% of women had at least one SOD cycle (anovulation and LPD were not differentiated)
Schliep et al. (2014)	<i>n</i> =259 Aged 18–44 years	Naturally regularly menstruating	<i>Biocycle study</i> : followed for one (<i>n</i> =9) or two (<i>n</i> =250) cycles.	<ul style="list-style-type: none"> • Prospective cohort study • Used electronic fertility monitor (LH) to time serum blood draws (menses, mid- 	<ul style="list-style-type: none"> • <i>Anovulation</i>: luteal serum progesterone <1ng/mL • <i>LPD confirmed by</i>: 	<ul style="list-style-type: none"> • Anovulation prevalence: 2.7% • Clinical LPD prevalence: 8.9% • Biochemical LPD prevalence: 8.4%

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Study design/methods	Definitions used to classify ovulation/phases	Results
	No menstrual or chronic disorders			follicular, late follicular, LH surge, expected ovulation and early, mid- and late luteal phases)	1) Clinical LPD: LP <10 days 2) Biochemical LPD: luteal serum progesterone 5 ng/mL or less	• 4.3% of cycles met both LPD criteria
Hammoud et al. (2012)	<i>n</i> =233 Aged 18–44 years No menstrual or chronic disorders	Naturally regularly menstruating	<i>Biocycle study</i> : followed for one (<i>n</i> =9) or two (<i>n</i> =250) cycles.	• Prospective cohort study • Used electronic fertility monitor (LH) to time serum blood draws (menses, mid-follicular, late follicular, LH surge, expected ovulation and early, mid- and late luteal phases)	• <i>LPD confirmed by</i> : 1) Clinical LPD: LP <10 days 2) Biochemical LPD: peak luteal serum progesterone <3 ng/mL	• Clinical LPD prevalence: 14% of women had at least one LPD cycle • Biochemical LPD prevalence: 6% of women had at least one LPD cycle • 11 cycles among 9 (4%) women met both LPD criteria
Bachmann and Kemmann (1982)	<i>n</i> =991 Aged 17–23 years College students	<i>Participants randomly selected – some participants had current contraceptive use</i>	Single, close-ended structured questionnaire	• Cross sectional • Self-administered questionnaire assessing menstrual history	• <i>Oligomenorrhea criteria</i> : >35 days, <90 days between menses	• Oligomenorrhea prevalence: 11.3% • Amenorrhea prevalence: 2.6%
Baranauskas et al. (2023)	<i>n</i> =3705 Aged >18 years	<i>Participants randomly selected –</i>	Single survey via STRAVA app	• Cross-sectional survey	• <i>Amenorrhea/oligomenorrhea criteria</i>	

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Study design/methods	Definitions used to classify ovulation/phases	Results
		<i>naturally menstruating</i>		<ul style="list-style-type: none"> • Self-administered questionnaire assessing menstrual history in past year 	<i>(combined):</i> ≤ 10 menses in the last year.	<ul style="list-style-type: none"> • Amenorrhea / oligomenorrhea prevalence: 16%.
Goshtasebi et al. (2018)	<p><i>n=1532</i></p> <p>Aged 30–60 years</p>	<i>Participants randomly selected – some participants had current contraceptive use</i>	Five years	<ul style="list-style-type: none"> • Cross-sectional study • Interviewer administered surveys distributed at baseline, year three and year five – lifetime incidence assessed 	<ul style="list-style-type: none"> • <i>Oligomenorrhea criteria:</i> >35 days, <90 days between menses • <i>Amenorrhea criteria:</i> no menses >90 days 	<ul style="list-style-type: none"> • Oligomenorrhea prevalence: 10% • Amenorrhea prevalence: 8%
He et al. (2020)	<p><i>n=12,964</i></p> <p>Aged 18–49 years</p> <p>Chinese ethnicity</p>	<i>Participants randomly selected – some participants had current contraceptive use</i>	Single standardised questionnaire	<ul style="list-style-type: none"> • Cross-sectional epidemiological study • Self-administered questionnaires • 8.8% of participants selected to provide blood samples for hormonal analysis on 2nd to 4th day of menses 	<ul style="list-style-type: none"> • <i>Oligomenorrhea criteria:</i> >35 days, <90 days between menses 	<ul style="list-style-type: none"> • Oligomenorrhea prevalence: 12.2%

Author	Participant characteristics	Menstrual cycle characteristics	Study duration	Study design/methods	Definitions used to classify ovulation/phases	Results
Munster et al. (1992)	n=3743 Aged 15–44 years	<i>Participants randomly selected – naturally menstruating</i>	Single questionnaire	<ul style="list-style-type: none"> • Cross sectional study • Postal questionnaires used, for non-responders, short telephone interviews were completed 	<ul style="list-style-type: none"> • <i>Amenorrhea criteria:</i> no menses for >3 months 	<ul style="list-style-type: none"> • Prevalence of secondary amenorrhea in women aged 15–24 (7.6%), 25–34 (3%), 35–44 (3.7%) • Amenorrhea prevalence: 4.6%

Abbreviations: FP – follicular phase; LP – luteal phase; LPD – luteal phase defect; LH – luteinising hormone; QBT – Quantitative basal temperature

2.9 Risk factors influencing menstrual disturbances

Amongst healthy menstruating women, there are a range of factors that may increase the risk of subtle or severe menstrual disturbances (De Souza et al., 2010; Metcalf et al., 1983; Metcalf, 1979). These can include physiological, environmental or behavioural factors which could occur at any stage during an individual's reproductive life (Brown, 2011). While major risk factors can be controlled for within studies, including hormonal contraceptive use, high or low body mass index (BMI), and hormonal disorders (e.g., polycystic ovarian syndrome), the lifestyle factors (e.g. exercise) that healthy, naturally menstruating females encounter in their day-to-day lives may be confounding factors that influence menstrual disturbance prevalence rates between studies.

Frequent or excessive exercise appears to be associated with both subtle and severe menstrual disturbances. In a study of 87 women with a median age of 26.3 years, De Souza et al (2010) reported the incidence of LPD and anovulation as 5% and 0% in sedentary women versus 27% and 50% in exercising women, respectively. This study also reported that 0% of sedentary women displayed either oligomenorrhoeic or amenorrhoeic cycles, whereas amongst exercising women, 7% demonstrated oligomenorrhoea and 37% displayed secondary amenorrhoea. These results are in alignment with previous research, which reported that undertaking high-intensity exercise of more than five hours per week was associated with 1.41 times greater odds of experiencing severe menstrual disturbances compared to those who exercise one to two hours per week (Baranauskas et al., 2023). Given the observed association between menstrual disturbances and exercise, it is important that studies that examine menstrual cycle features and characteristics collect data on participants' activity levels to understand how this lifestyle factor could be contributing to menstrual cycle inter-individual variability.

The relationship between body fat percentage and menstrual characteristics has not been thoroughly examined in the literature, thus remains unclear (de Liyis et al., 2024). In one cross sectional study, increases in body fat percentage was found to be correlated with prolonged menstrual cycles (de Liyis et al., 2024). Additionally, two studies have reported a relationship between lower body fat percentage and amenorrhoea in dancer populations (Liu et al., 2024; To et al., 1997). These findings may suggest that lower body fat percentage may be a risk factor for developing menstrual disturbances. However, further research should aim to examine its relationship with subtle menstrual disturbances, such as anovulation and LPD, in regularly menstruating populations.

Gynaecological age, or the years since a female's first menstrual period (menarche), has also been found to be associated with menstrual cycle regularity (Brown, 2011; Deligeoroglou & Tsimaris, 2010; Metcalf et al., 1983). For example, in a cross-sectional survey with 3743 women, Munster et al. (1992) reported that the prevalence of secondary amenorrhea in girls aged 15–24 years was 7.6%, which was higher than the prevalence rates reported for women aged 25–44 years (3% to 3.7%). Similarly, in a smaller prospective cohort study with 259 participants, Schliep et al. (2014) reported of cycles with clinical LPD, 65.9% occurred in women aged 18–22 years, compared to 17.1% for >22–30 years, and 17.1% for >31–44 years. Other cross-sectional studies have also found that a later age of menarche may be associated with higher rates of severe menstrual and subclinical ovulatory disturbances (Bachmann & Kemmann, 1982; Bedford et al., 2010; Schliep et al., 2014). Moreover, in studies investigating menstrual disturbances, it is frequently observed that higher prevalence rates may be reported in younger females (including adolescents and those with a younger gynaecological age) and in perimenopausal women who are experiencing declines in ovarian activity (Brown, 2011; Gambadauro et al., 2024; Itriyeva, 2022; O'Connor et al., 2001). As such, research examining menstrual cycle features and characteristics in regularly menstruating women should ensure that gynaecological age is considered and, where appropriate, accounted for within their selected cohort.

2.10 Conclusion

Variations within the menstrual cycle, both between and within women, are commonly reported (Brown, 2011; Fehring et al., 2006). While textbook definitions of menstrual cycle characteristics are often presented as a benchmark for cycle normality, results within current research in regularly menstruating women often may not align with these standards. Current research frequently examines menstrual cycle characteristics at a single time point in cross-sectional studies. Subsequently, there is limited prospective research on the natural variability in menstrual cycle characteristics in healthy women over consecutive cycles. Where prospective research is completed, objective hormonal measurement of sex steroid hormones is infrequently performed.

Despite years of menstrual research being performed, there are still poor classifications of the menstrual cycle. In addition, the research included within this review has highlighted that

evidence on the occurrence and prevalence of ovulatory disturbances within healthy premenopausal females is still an area in need of investigation. Furthermore, research methodologies used to classify ovulatory disturbances vary widely between studies. As a result, the menstrual cycle is not consistently defined, which contributes to the heterogeneity in results between studies. Further research in this area should aim to prioritise longitudinal studies to capture within-women and between-women changes in naturally menstruating women. In addition, the usage of consistent methodologies and criteria for cycle and population classification, including assessment of sex steroid hormones, would be beneficial. Taken together, research in this area can advance the understanding of the natural variability and presentations of the menstrual cycle in healthy women.

3 Research study manuscript

3.1 Abstract

Background: Currently, there is minimal research assessing the variability in features and characteristics of the menstrual cycle in healthy, naturally menstruating, premenopausal women, particularly over numerous consecutive cycles. The primary objective of this study was to assess the characteristics of the menstrual cycle in healthy New Zealand women across three to five cycles. A secondary aim was to determine the individual and lifestyle factors that may influence the presence of ovulatory disturbances.

Methods: This study followed 97 healthy females over three to five consecutive menstrual cycles. Menstrual cycle features and characteristics were determined using the three-step menstrual cycle tracking method (calendar-based counting, urinary LH testing for ovulation and plasma hormone measurements). Individual and lifestyle data were collected using demographic and validated menstrual cycle history and physical activity questionnaires. Key menstrual cycle features were described using descriptive statistics. A linear mixed model was used to determine the within and between-women variability of phase lengths, and a logistic regression including gynaecological age, total metabolic equivalent physical activity and percentage body fat was used to establish demographic and anthropometric associations with SODs.

Results: In this study, the most common cycle type was ovulatory (74.1%), followed by anovulatory (13.5%) and LPD (12.4%) cycles. Sporadic subclinical ovulatory disturbances (SOD) were more common (34.9%) than recurrent SOD cycles (8.1%). In cycles in which ovulation occurred, the average day of ovulation was day 14, and day 13 for LPD cycles. Age and gynaecological age were found to be associated with SOD cycles, but not body composition, ethnicity, or level of physical activity. Within-woman variability of menstrual features was found to be greater in SOD women than in ovulatory women.

Conclusion: The majority of menstrual cycles in healthy, regularly menstruating women are ovulatory. However, the presence of SOD is relatively common. Women who frequently experience SOD cycles may experience more menstrual cycle variability.

3.2 Introduction

The menstrual cycle has long been considered a vital indicator of both reproductive and overall health (Rosen Vollmar et al., 2025). Despite this, clinical research struggles to provide a clear understanding of what a 'typical' menstrual cycle may look like (Critchley et al., 2020; Harlow & Ephross, 1995; Persdotter, 2020). While current textbook definitions of a menstrual cycle assume a fertile and ovulatory cycle, recent research suggests that a variety of cycle presentations may too be considered part of a healthy and adaptive menstrual cycle (Brown, 2011). For example, while mean cycle length is often widely accepted as being 28 days long, it has been proposed that this may only be true in 15% to 16% of cycles (Grieger & Norman, 2020; Johnson et al., 2018). Moreover, previous research has reported that 42.5% of women will report intracycle variability of more than seven days (Fehring et al., 2006). In addition, evidence from observational studies suggests that in regularly menstruating women, between 2.7% and 37% of cycles may be anovulatory, and between 8.9% and 26.1% of cycles may be LPD (Andrews et al., 2015; De Souza et al., 2010; Haiman et al., 2002; Hammoud et al., 2012; Harvey et al., 2009; Henry et al., 2024; Lynch et al., 2014; Prior et al., 2015; Schliep et al., 2014).

Despite variations in the menstrual cycle being a common occurrence, research on both anovulation and LPD prevalence rates in healthy, regularly menstruating women is lacking. Research has demonstrated that anovulation and LPD cycles may be present despite regular cycles (Henry et al., 2024; Malcolm & Cumming, 2003; Prior, Vigna, Schechter, et al., 1990; Schliep et al., 2014). However, current literature frequently focuses on the prevalence of these cycle types within groups where cycle variations may be expected, for example, women with polycystic ovarian syndrome or adolescents. Subsequently, few studies document rates of anovulation and LPD within regularly cycling healthy women. In studies that have examined this population, research into the characteristics of the menstrual cycle demonstrates a heavy emphasis on single-time point surveys or single-cycle observations, rather than examining the variability of the menstrual cycle and its features and characteristics over consecutive cycles (Gloe et al., 2023; Schmalenberger et al., 2021). Additionally, few studies have completed an assessment of sex steroid hormones across consecutive cycles, despite this measurement being required to accurately report on cycle type, and variability across cycles. As the presentation of anovulation and LPD may be sporadic, such methodologies mean the intra-individual variability within the cycle is may not be adequately captured. Therefore, the current study aims to describe the characteristics and features of the menstrual cycle over three to five consecutive cycles.

3.3 Methods

This study was a prospective cohort study conducted in Auckland, New Zealand. Data across five consecutive menstrual cycles were contributed by 97 participants. Data collection occurred between September 2023 and April 2025. This study received ethics approval from the Massey University Southern Human Ethics Committee (SOA 22/56).

3.3.1 Recruitment and study participants

Study participants were recruited through snowball recruitment methods. This study was advertised through social media (e.g. researcher personal accounts and community groups), email, physical flyers distributed across Massey University Albany Campus and word-of-mouth. Potential participants were screened for study eligibility through the completion of an online screening questionnaire, hosted by survey software Qualtrics. Women were eligible to take part after meeting the following inclusion criteria: biologically female and menstruating, aged 18–40 years, no hormonal contraceptive use in the previous six months (including oral tablets, intrauterine devices, injections or implants), were not pregnant or breastfeeding (presently or in the past year), and no known health issues (including inflammatory bowel disease, coeliac disease, history of gastric ulcers, blood in urine, malaria, polycystic ovarian syndrome). Participants were also required to have a self-reported regular (21 – 35 days) menstrual cycle.

3.3.2 Study procedures and protocols

Data collection was completed at Massey University (Auckland) campus. Participants who met the inclusion criteria were invited to attend a familiarisation session, where they completed three baseline questionnaires. These included a demographic questionnaire which examined participant ethnicity, age and menstrual history data, the New Zealand Short Form Physical Activity questionnaire (Moy et al., 2008), and a menstrual history survey founded on recommendations from Schmalenberger et al. (2021). Participants were instructed on the use of Qualtrics to record their menstrual cycle information.

Details of menstrual cycle features were recorded from the first menstrual cycle. The first day of the menstrual cycle was identified as the first day of menstrual bleeding and was self-reported

via electronic message. Duration of menstrual bleeding was also captured by daily self-report. To determine the day of ovulation and to time mid-luteal progesterone draws, urinary LH testing was completed. Participants were provided Baby4You urinary ovulation test kit strips and were required to test daily from day eight of their menstrual cycle until a positive test was returned or until cycle day 21/22. For participants with cycle lengths longer than 30 days, ovulation testing began on day 10 and ended when there was a positive result or when day 24 to 25 of their cycle was reached (see Figure 3.1). Responses were recorded in a central Excel document.

Reproductive hormones (oestrogen and progesterone) were measured to assess sex steroid hormonal profiles for each cycle phase, and to characterise cycle types. To assess these, venous blood samples were taken at various points across the menstrual cycle during the menstrual bleeding phase, mid-follicular phase, and mid-luteal phase. Further details on the timing of blood sample collection are provided in Figure 3.1.

3.3.3 Body composition assessment

Body composition assessments were completed at baseline and the mid-luteal phase of the fifth cycle. Height (cm) was measured using a stadiometer. Body composition of participants was determined via bioelectrical impedance analysis (InBody 230). Body composition variables of interest for this study included body mass (kg), fat mass (kg) and percentage (%), lean muscle mass (kg) and BMI (kg/m^2). Of these, body fat percentage was selected as a primary variable for analysis as it may be an alternative indicator of correlations of body composition and SOD than BMI or weight, which does not distinguish between fat mass or lean mass.

3.3.4 Blood analysis

For analysis of plasma sex steroid hormones (oestrogen (pmol/L) and progesterone (nmol/L)), venous blood samples were collected over cycles three to five (see Figure 3.1). Blood draws were completed by a trained phlebotomist from the antecubital vein. Participants were in a seated position as blood draws were collected, using a 23G butterfly needle into two 8ml plasma separator tubes. Samples were slowly inverted eight times immediately after collection and centrifuged at 4° C, 2000 RFC for 10 minutes within 30 minutes of collection. The separated plasma supernatant was removed, placed into 1 mL and 2 mL aliquots, and stored at -80° C until analysis. Batches of plasma samples were sent to Auckland LabPlus for analysis. For clinical

interpretation, ovulatory cycles were defined as peak mid-luteal progesterone >10 nmol/L, anovulatory cycles were defined as peak mid-luteal progesterone <3 nmol/L, and biochemical LPD cycles were defined as peak luteal progesterone >3 nmol/L and <10 nmol/L. These thresholds were based off previous population research and based on advice from reproductive health researchers as described by Prior et al. (2015). In instances where participants were unable to attend blood collection, additional cycle assessments were completed i.e., cycle six.

3.3.5 Sample size

The sample size for this study was calculated to accurately reflect the prevalence of ovulatory and SOD cycles in a wider population, It was determined *a priori* using a statistical power calculation of $n = (Z^2 p(1-p)) / d^2$, based on an expected prevalence of 48% for SOD cycles as estimated by previous research (De Souza, 2003), a two-sided significance level of 0.05 (Z=1.96) and an absolute error of 10%. A minimum sample size of 96 participants was required to ensure adequate power to detect effect. A total of 97 participants completed this study, and 86 participants provided sufficient data to be assigned an ovulatory status.

Sample collection

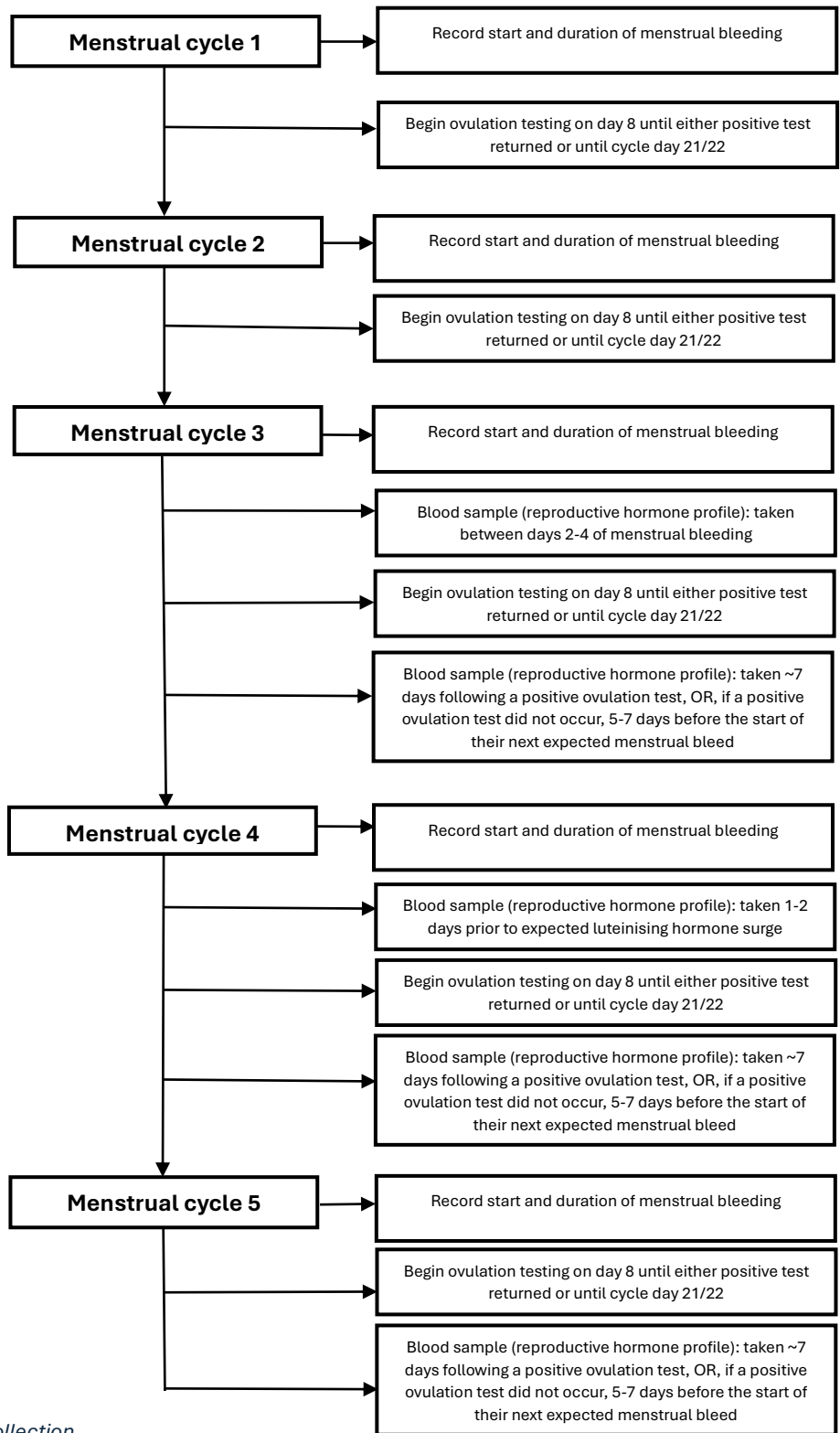


Figure 3.1: Flow chart of data collection

3.3.6 Statistical method for data analysis

Collected data were examined and cleaned before being imported into statistical software (IBM SPSS Statistics, version 27.0) for analysis. Data were checked for normality using the Shapiro-Wilk test, with a statistical significance of $p < 0.05$. Participants were grouped by ovulatory status (ovulatory and SOD) where sufficient data were available. Participants with two or more ovulatory cycles were classed as ovulatory, and participants with two or more SOD cycles were classed as SOD. This grouping was based off previous work done by De Souza et al. (2010) where women were categorised into a single category based off the predominant cycle presentation of a woman. Level of physical activity was calculated based on duration and intensity of exercise, and classed as Low, Medium or High as per the International Physical Activity Questionnaire scoring criteria (Craig et al., 2003).

For analysis of participant characteristics, independent t-tests were used to assess differences between ovulatory groups for scale variables with normal distribution (gynaecological age, muscle mass), and Mann Whitney U for non-normally distributed (age, weight, fat mass, body fat percentage). Chi Squared Test of Independence was used for categorical variables (BMI, number of children, priority ethnicity, level of physical activity).

For analysis of the primary aim, descriptive statistics were used. For normally distributed data, mean and standard deviation were reported, and for non-normally distributed data, median and interquartile range were reported. Where separation of cycles by ovulatory status was required for analysis, only cycles with a confirmed ovulatory status (e.g. mid-luteal phase measurements) were included. For participants who contributed data across multiple cycles, their data was first aggregated to provide participant-level means. To compare differences in menstrual cycle, menses, follicular and luteal length across ovulatory status, the non-parametric Kruskal-Wallis test was used, and Mann-Whitney U tests were utilised for post hoc analysis. The day of ovulation was completed using aggregated data for cycle type per woman and was compared using a one-way ANOVA. A linear mixed model was used to determine the between-women and within-woman variance of the menstrual cycle.

For the secondary aim, a multiple logistic regression was used to determine the association between demographic factors and ovulatory status, and included gynaecological age, percentage of body fat, and physical activity as continuous variables.

Within this study, menstrual characteristics were defined as the following. Menstrual cycle length was defined as the number of days from the first day of menstrual bleeding until the last day before the next menses. Menstrual period/menses was defined as the number of days from the first day of menstrual bleeding until the last day of menstrual bleeding. The follicular phase was defined as the number of days from the first day of menstrual bleeding up to and including the day of ovulation, and the luteal phase as the number of days from the day after ovulation until the day before the next menses.

3.4 Results

3.4.1 Participant characteristics

A total of 97 women were included in the final analysis, of which 86 participants (88.7%) provided sufficient data to determine ovulatory grouping. Participant characteristics are presented in Table 3.1. The majority of participants identified as New Zealand European (72.2%).

3.4.2 Subclinical ovulatory disturbances

Hormonal data were collected for 259 cycles. Of these cycles, 192 (74.1%) were ovulatory, 35 (13.5%) were anovulatory, and 32 (12.4%) were LPD. A total of 25 cycles were determined to be anovulatory by mid-luteal progesterone assessment despite returning a positive urinary LH test. At least one ovulatory cycle was experienced by 82 women (84.5%), whilst 25 women (25.8%) experienced at least one anovulatory cycle, and 26 women (26.8%) experienced at least one LPD cycle. Additionally, of the women who contributed at least 2 cycles of data, 49 women (57%) experienced only ovulatory cycles, 7 women (8.1%) experienced only SOD cycles, and 30 women (34.9%) experienced both ovulatory and SOD cycles.

Two factors were found to be associated with the presence of SOD cycles: gynaecological age ($p=0.009$) and age ($p=0.030$). Each additional year of gynaecological age was associated with a 14% (95% CI 23%, 4%) decrease in the likelihood of the occurrence of an SOD cycle ($p<0.01$).

However, body fat percentage ($p=0.324$) and amount of physical activity ($p=0.479$) were not found to have an association.

Table 3.1: Participant characteristics by ovulatory status

Variable		All Participants N=97	Ovulatory [†] N=68	SOD [§] N=18	p-value [†]
Age (years) ^a		31.0 (25.5 – 35.0)	31.5 (27.0 – 36.0)	29.5 (23.0 – 31.3)	0.030
Weight (kg) ^a		68.4 (60.3 – 75.6)	68.4 (60.6 – 75.6)	67.7 (58.7 – 72.9)	0.665
Fat mass (kg) ^a		20.0 (14.5 – 24.1)	20.3 (15.3 – 24.1)	18.8 (14.8 – 28.5)	0.909
Gynaecological age (years) ^b		17.7 (5.7)	18.7 (5.2)	15.2 (5.9)	0.014
Muscle mass (kg) ^b		26.6 (4.1)	26.8 (3.9)	25.3 (3.6)	0.166
Percentage body fat (%) ^b		29.5 (8.7)	29.5 (8.8)	30.6 (9.0)	0.633
Priority ethnicity (n (%))	European	70 (72.2%)	52 (76.5%)	12 (66.7%)	0.779
	Māori	7 (7.2%)	4 (5.9%)	2 (11.1%)	
	Pacific Peoples	1 (1%)	1 (1.5%)	-	
	Asian	18 (18.6%)	10 (14.7%)	4 (22.2%)	
	MELAA	1 (1%)	1 (1.5%)	-	
BMI (n (%))	Underweight (<18.5)	1 (1%)	-	1 (5.9%)	0.244
	Normal (18.5 – 24.9)	50 (51.5%)	36 (55.4%)	10 (58.8%)	
	Overweight (25 – 29.9)	28 (28.9%)	23 (35.4%)	5 (29.4%)	
	Obese (>30)	7 (7.2%)	6 (9.2%)	1 (5.9%)	
Number of children (n (%))	0	75 (78.1%)	50 (73.5%)	16 (88.9%)	0.336
	1 – 2	16 (16.7%)	14 (20.6%)	2 (11.1%)	
	3+	5 (5.2%)	4 (5.9%)	0	

Variable		All Participants N=97	Ovulatory [‡] N=68	SOD [§] N=18	p-value [†]
Level of physical activity (n (%))	Low	10 (10.3%)	8 (11.8%)	1 (5.6%)	0.333
	Medium	32 (33.0%)	24 (35.3%)	4 (22.2%)	
	High	55 (56.7%)	36 (2.9%)	13 (72.2%)	

^aVariables reported as median and 25th – 75th quartiles due to non-normal distribution, ^bvariables reported as Mean + SD due to normal distribution

[‡]Participants were classified as ovulatory if they experienced two or more ovulatory and luteal sufficient cycles (mid-luteal progesterone >10 ng/mL)

[§]Participants were classified as SOD if they experienced two or more anovulatory cycles (mid-luteal progesterone <3ng/mL) or LPD cycles (mid-luteal progesterone 3 – 10ng/mL or LP length <10 days)

[†]Significant differences between ovulatory and SOD groups ($p < 0.05$). Mann Whitney U used for scale variables with non-normal distribution, t-test used for scale variables with normal distribution, Chi Squared Test of Independence used for categorical variables.

Abbreviations: MELAA – Middle Eastern, Latin American and African; BMI – body mass index

3.4.3 Presentation of menstrual cycle features and cycle types

The average length and range of the menstrual cycle, menses bleed, follicular and luteal phases across consecutive cycles are reported in Table 3.2. For continuity, cycle data from cycles five and six have been grouped. Overall, the median length of all ovulatory menstrual cycles was 28.5 days, menses length 5.0 days, follicular phase 14.0 days and luteal phase 14.0 days. Significant differences in overall menstrual cycle and luteal phase length were detected across ovulatory vs anovulatory and LPD cycles ($p < 0.001$). Differences in menstrual cycle, menses, follicular and luteal phase lengths across ovulatory status are described in Table 3.3. The average day of ovulation in both ovulatory and anovulatory cycles was day 14, and the day of ovulation for LPD cycles was day 13. No significant differences were detected between cycle types ($p = 0.391$).

Further analysis using a linear mixed model revealed that within-woman variance was greater than between-women variance for all menstrual cycle factors (menstrual cycle, menses, follicular and luteal phases) (see Table 3.4). SOD women were found to have significantly greater variability in within woman cycle length (6.7 days vs 2.0 days, $p = 0.003$), follicular phase (3.2 days vs 2.0 days, $p = 0.006$) and the luteal phase (8.0 days vs 2.0 days, $p < 0.001$). Menses length variability was also greater in SOD women (0.8 days vs 0.7 days) however this was non-significant ($p = 0.377$).

Table 3.2: Menstrual cycle, menses, follicular and luteal phase lengths by cycle type across consecutive cycles

Length (days)	All women	Ovulatory	Anovulatory	LPD
CYCLE 3				
Cycle length	N=90 28.0 (26.0 – 31.0)	N=56 28.0 (26.0 – 30.0)	N=13 34.0 (29.0 – 38.0)	N=11 24.0 (22.0 – 25.0)
Menstrual bleed	N=94 5.0 (4.0 – 6.0)	N=58 5.0 (4.0 – 6.0)	N=13 5.0 (3.0 – 5.5)	N=12 3.5 (2.0 – 5.75)
Follicular phase	N=82 14.0 (12.0 – 15.0)	N=52 13.5 (12.0 – 15.0)	N=9 12.0 (9.5 – 19.0)	N=10 13.0 (11.8 – 14.5)
Luteal phase	N=76 14.0 (12.0 – 16.8)	N=49 15.0 (13.0 – 16.0)	N=9 22.0 (15.5 – 23.5)	N=10 10.5 (8.3 – 15.5)
CYCLE 4				
Cycle length	N=83 28.0 (27.0 – 31.0)	N=65 28.0 (27.0 – 30.5)	N=8 34.0 (29.3 – 37.5)	N=5 23.0 (21.0 – 27.5)
Menstrual bleed	N=89 5.0 (4.0 – 5.0)	N=68 5.0 (4.0 – 5.8)	N=9 4.0 (3.0 – 4.5)	N=5 4.0 (3.5 – 5.5)
Follicular phase	N=78 15.0 (13.0 – 16.0)	N=60 15.0 (13.0 – 16.0)	N=7 12.0 (10.0 – 15.0)	N=5 14.0 (12.0 – 17.5)
Luteal phase	N=73 14.0 (13.0 – 15.0)	N=58 14.0 (13.0 – 15.0)	N=6 19.5 (15.5 – 24.5)	N=5 9.0 (6.0 – 13.0)
CYCLE 5 and 6				
Cycle length	N=12	N=3	N=0	N=4

Length (days)	All women	Ovulatory	Anovulatory	LPD
	27.5 (25.0 – 30.8)	30.0 (27.5 – 30.5)		23.0 (18.0 – 30.5)
Menstrual bleed	N=98 5.0 (4.0 – 6.0)	N=59 5.0 (4.0 – 5.0)	N=12 5.0 (4.0 – 5.8)	N=10 4.5 (4.0 – 6.0)
Follicular phase	N=80 14.0 (12.0 – 15.0)	N=55 14.0 (12.0 – 15.0)	N=9 15.0 (13.5 – 16.5)	N=8 11.5 (10.3 – 13.0)
Luteal phase	N=9 14.0 (12.0 – 15.0)	N=3 14.0 (14.0 – 16.0)	N=0	N=3 9.0 (7.5 – 13.0)

Variables reported as median and 25th – 75th quartiles

All women were included in 'all women' however only cycles with a confirmed ovulatory status were included in ovulatory, anovulatory and LPD columns

Table 3.3: Estimated differences (days) in length of menstrual cycle, menses, follicular and luteal phase across cycle types

	Ovulatory vs anovulatory cycles – difference (days) [§]	CI	SE	P value [†]	Ovulatory vs LPD cycles – difference (days) [§]	CI	SE	P value [†]
Menstrual cycle	4.5	2.4 – 6.6	1.1	0.001	-4.7	-6.9 – -2.6	1.1	0.001
Menses	-0.3	-0.71 – -0.10	0.21	0.142	-0.6	-0.99 – -0.18	0.20	0.004
Follicular phase	-0.6	-1.7 – -0.52	0.56	0.298	-0.3	1.7 – -0.53	0.56	0.313
Luteal phase	5.2	2.7 – 7.7	1.3	0.001	-4.4	-6.8 – -2.0	1.2	0.001

[§]Ovulatory cycle used as reference value

[†]Differences between ovulatory and SOD groups calculated with a linear mixed model and a significance level of $p < .05$. Significant values are bolded.

Abbreviations: CI – confidence interval; SE – standard error

Table 3.4: Calculation of between women and within woman variability across menstrual cycle, menses, follicular and luteal phase lengths

	Between women variability	SD	Within woman variability	SD
Menstrual cycle	8.213	2.866	13.203	3.634
Menses	0.612	0.782	0.681	0.826
Follicular phase	2.871	1.694	4.371	2.091
Luteal phase	3.433	1.853	17.989	4.241

Values calculated using a linear mixed model and reported in variance units (days²) with standard deviation (SD) (days)

3.5 Discussion

The current study aimed to describe the characteristics of the menstrual cycle in healthy New Zealand women over three to five cycles. Additionally, this study explored the individual and lifestyle factors that may influence the presence of ovulatory disturbances. Our results showed that while the majority of cycles assessed were ovulatory, over a third of women experienced at least one anovulatory or LPD cycle over the duration of this study. Within our cohort of healthy women, a higher incidence of sporadic SOD occurrence was observed than recurrent SOD. In this study, age and gynaecological age were identified to be significantly associated with the occurrence of SOD cycles. Finally, our results demonstrated greater within-woman than between-women variability for all menstrual cycle features (menstrual cycle, menses, follicular and luteal phases). The within-woman variation for cycle length, follicular phase length and luteal phase length was significantly greater in SOD women.

3.5.1 Subclinical ovulatory disturbances

The results of this study suggest that the majority of cycles within this cohort of healthy, naturally menstruating women were ovulatory (74.1%). This outcome is consistent with results from the available literature that has previously reported the prevalence of ovulatory cycles in healthy menstruating women to be between 63.3% and 72% (Harvey et al., 2009; Henry et al., 2024; Prior et al., 2015). Cumulatively, this would suggest that in healthy, regularly menstruating premenopausal women, the majority of their cycles may be expected to be ovulatory. Comparatively, the incidence of SOD reported by the current study appears to be dissimilar to the results reported in previous research. Within this study, the overall prevalence of anovulatory and LPD cycles was 13.5% and 12.4%, respectively. This is contrary to our hypothesis that SOD would be rare (<10%). The prevalence of these cycles is higher than what was reported by Schliep et al. (2014) and Andrews et al. (2015), who both observed an ovulation prevalence of 2.7% and clinical LPD prevalence of 8.9%. However, Schliep et al. (2014) and Andrews et al. (2015) did not differentiate between clinical (<10 days) or biochemical LPD cycles and, as such, may have misclassified biochemical LPD cycles as ovulatory, leading to lower rates of LPD cycles. In addition, both Henry et al. (2024) and Harvey et al. (2009) reported notably higher rates of LPD cycles (<10 days) than the current study at 25% and 26.1%. A result that may be due to these studies using QBT for menstrual cycle tracking, which does not enable researchers to differentiate between clinical or biochemical LPD cycles, and, as such, they may have misclassified biochemical LPD cycles as ovulatory. Additionally, as there is a delay in basal body

temperature increases following ovulation (~1–3 days) (Prior, Vigna, Schulzer, et al., 1990), therefore, there is a higher likelihood of ovulatory luteal phases being classified as less than 10 days. Logically, our criterion, which included both clinical (<10 days) and biochemical (<10 nmol/L mid-luteal progesterone) LPD definitions, is more likely to capture reliable rates of LPD cycles in healthy naturally menstruating women. However, further research is needed to validate these findings.

Previously, it has been suggested in the literature that SOD cycles may intermittently occur in healthy, naturally menstruating women (Prior et al., 2015) and that ovulatory function may vary in response to everyday stressors such as weight changes or psychological stress (Brown, 2011; Vigil et al., 2022). A finding in this study was that a small percentage of women may experience SOD cycles continuously over consecutive cycles (8.1%), while a larger percentage of women experienced them sporadically amongst otherwise ovulatory cycles (34.9%). These results align with Prior, Vigna, Schechter, et al. (1990), who reported that within their cohort of 66 women over a one year period, 18% of women had one SOD cycle, specifically a short luteal phase, while a further 42% had more than one SOD cycle (more than one short luteal phase). Additionally, in previous research of at least eight cycles, the number of women who experienced zero SOD cycles was less than half of that reported by the current study (53.6%) (Bedford et al., 2010; Henry et al., 2024; Prior, Vigna, Schechter, et al., 1990). Taken together, these findings may suggest that study duration contributes to the differences in prevalence rates between studies, wherein the probable occurrence of sporadic SOD cycles within studies of a longer duration (e.g. eight cycles or one year) is more likely to be captured compared to the current study, which collected data over three to five cycles. Interestingly, despite a frequent rhetoric of SOD cycles being classed as ‘abnormal’ (Brown, 2011), the sporadic occurrence of these cycles within this study and previous research results may suggest otherwise. Alternatively, the collective results from this research may suggest that sporadic anovulatory and LPD cycles may be normal menstrual cycle presentations in a woman’s reproductive lifespan and may instead reflect a healthy and adaptive reproductive system (Prior, 2022). However, the general lack of research examining the incidence of SOD over continuous cycles and the lifestyle factors that may contribute to the occurrence of SOD cycles suggests that this area needs further research.

3.5.2 Features of the menstrual cycle

In the current study, 73 women (88%) had cycle length differences of ≤ 7 days and 10 women (12%) had cycle length differences of >7 days. Similarly, Fehring et al. (2006), have also previously reported that the majority (57.5%) of 141 women observed in their study over three to 13 menstrual cycles had cycle length differences of ≤ 7 days. While this statistic does not specify the frequency of normal-length cycles, it does suggest menstrual cycle length consistency within women regardless of whether cycles are regular (21–35 days) or irregular (<21 , >35 days). Recognition of relative cycle length consistency within a woman may enable women to develop an understanding of their personal menstrual cycle patterns, better educating them to be able to detect deviations as a symptom of deeper reproductive health issues. Specifically, previous research has shown that when women are able to track their menstrual cycle length and recognise individual patterns, menstrual health literacy may improve (Zhaunova et al., 2023). Improved menstrual health literacy may encourage healthcare-seeking behaviours when necessary and earlier diagnoses of underlying reproductive disorders known to impact menstrual cycle regularity, such as polycystic ovarian syndrome (Gill & Hall, 2009). However, further research on the relative menstrual length consistency across ovulatory statuses is required.

Across the literature, the textbook menstrual cycle is described as a 28-day cycle, with the follicular and luteal phases each considered to be 14 days long and separated by ovulation (Reed & Carr, 2018). The results from the ovulatory cycles within the current study align closely with these values, with the median menstrual cycle length being 28.5 days, follicular length and luteal length at 14 days, and ovulation occurring on day 14. Interestingly, within our study, the variation in cycle features was found to be much higher within-woman than between-women. This contrasts with previous research, which found that between-women variance in menstrual cycle features was greater than within-woman (Cole et al., 2009; Henry et al., 2024). For example, the median between women variance reported in a one-year study by Henry et al. (2024) for menstrual cycle length, follicular phase length and luteal phase length was 3.1 days, 5.2 days and 3.0 days, respectively. The between-women variance in menstrual cycle features reported in this previous research is greater than the present study, where a variance of 2.0 days was noted for ovulatory cycles. It is likely that these differences occur due to the longer length of time during which women were examined (Cole et al., 2009; Henry et al., 2024), enabling increased variances between women to be captured compared to the current study. Previous research has also suggested that the occurrence of ovulation is not necessarily synonymous with regular-length

cycles (Henry et al., 2024; Prior et al., 2015). This finding is supported by the results of the current study, where 13.2% of participants who were classed as ovulatory did not demonstrate consistently regular length cycles. Interestingly, within SOD women, only 22.2% had a regular menstrual cycle length of between 21 and 35 days. Moreover, SOD cycles demonstrated much higher within-woman menstrual cycle feature variability than ovulatory cycles. This suggests that women with SOD cycles may display less menstrual cycle regularity than ovulatory cycles. When this variability between cycle types was explored further, significantly longer cycle lengths were found in anovulatory cycles than in ovulatory cycles. In addition, the length of LPD cycles was found to be significantly shorter than both ovulatory and anovulatory cycles. Within our dataset, the luteal phase had higher variability than the follicular phase, which contradicts previous research that has suggested that the follicular phase, rather than the luteal phase, is the primary driver for menstrual cycle variability (Francis & Keay, 2024). It should be noted that within the study by Francis and Keay (2024), cycles were confirmed ovulatory by ultrasound, as is recognised as gold standard for ovulation detection, and daily hormones. However, analysis was only done over one cycle which is therefore unable to examine whether women consistently or inconsistently experience cycle types. As such, differences in results may be due to length of study duration, or sample size ($n=20$). To further our understanding of menstrual cycle characteristics in naturally menstruating women, future longitudinal research should look to examine the relative frequency of ovulation and phase lengths in women who consistently experience regular length cycles.

3.5.3 Demographic associations with SOD

The occurrence of anovulation and LPD have been found to be associated with a variety of factors across the literature. In the present study, ethnicity was not found to be associated with either anovulatory or LPD cycles. However, as only a small number of individuals identified as either Pacific Person ($n=one$) or Māori ($n=seven$), and as participants were classed through a singular priority ethnicity, this finding may need to be interpreted with caution. Despite this, a similar lack of association was also reported by Haiman et al. (2002), who found no significant differences in anovulation rates between African American, Caucasian and Latina women. It is possible that, as this study and the previous study required regular menstruation for participation, ethnic groups who may have a propensity for irregular menstruation may have been inadvertently excluded, therefore leading to the lack of association between ethnicity and SOD prevalence. In contrast to these results, Hammoud et al. (2012) reported that clinical LPD was associated with

Caucasian women. However, the participant cohort within this study included a significant number of African American women, which may have enabled associations between ethnic cohorts and SOD prevalence to be determined. However, as only two studies have detailed any associations between SOD cycles and ethnicity (Haiman et al., 2002; Hammoud et al., 2012), there is a need for further research in this area. Within the context of Aotearoa New Zealand, further research should aim to include diverse cohorts of women which may include higher rates of Māori and Pacific Peoples.

No evidence of associations between body weight or composition were found in our study. However, Bedford et al. (2010) reported that a higher BMI was found to be associated with SOD cycles. Interestingly, this cohort had a lower mean BMI than the current study (21.8kg/m^2 vs 24.8kg/m^2). As BMI is often regarded as an inaccurate measurement of body composition (Wu et al., 2024), these differences may instead be attributable to differences in lean muscle mass or fat mass, which were analysed in the current study. Interestingly, neither BMI nor body fat mass appeared to be associated with SOD cycles in the present study. Often, previous research has placed more emphasis on how body composition may be associated with severe menstrual cycle disturbances (Klein et al., 2019; Podfigurna-Stopa et al., 2012; Stokić et al., 2005). For example, lower BMI, which is often associated with a lower fat mass, has been identified as a contributor to menstrual cycle disturbances (e.g. amenorrhea and oligomenorrhea) (Liu et al., 2024). It may be suggested that estimations of body fat may provide more accurate correlations to the menstrual cycle than alternative body composition measures, however, there is little research that has been completed on examining associations between specific body composition and SOD occurrence in healthy, naturally menstruating women. As such, further research into body composition associations with SODs may be required.

Our study did not find any associations between the level of physical activity and the presence of SOD cycles. Studies with a healthy and recreationally active study population, such as Bedford et al. (2010), Schliep et al. (2014) and Henry et al. (2024) have all reported similar results. In contrast to much of this previous research, Andrews et al. (2015) reported an association between vigorous exercise and LPD cycle occurrence, with the likelihood of LPD cycles increasing as the level of vigorous exercise increased. However, this previous research did investigate the association between a single occurrence of an LPD cycle with individuals' levels of physical activity. This may partially explain the different results from the current study that classified

women as SOD if they presented with at least two LPD or anovulatory cycles. Single cycle analysis may be more susceptible to random variation as opposed to grouping women based on numerous cycle disturbances, potentially contributing to the differences in results between longitudinal and cross-sectional studies. In addition, De Souza et al. (2010) assessed the rates of SOD cycles in sedentary versus exercising populations and found that the presence of both anovulatory and LPD cycles was associated with higher levels of exercise. These results are interesting, considering the exercising group reported similar levels of exercise per week to the cohort in the current study, despite physical activity not being a prerequisite for participation in the current study. However, in this study by De Souza et al. (2010) exercising women were not only classified based on them meeting the minimum amount of exercise per week, as per the current study, but also on their VO₂ max (the maximum amount of oxygen that can be utilised by the body during physical activity). As VO₂ max is a more objective measure of fitness than hours of exercise a week, which is often subjectively reported, it is probable that associations between SOD and physical activity levels and fitness were more accurately determined compared to the current study. Moreover, the participants within the study by De Souza et al. (2010) also demonstrated a lower cohort age (26.3 vs 31 years) compared to the current study. As it is widely recognised that younger age may be associated with SODs (Bedford et al., 2010; Brown, 2011; Schliep et al., 2014), this may have been a confounding factor in the analysis between exercise and SOD cycle occurrence. Therefore, future research is still needed in longitudinal and healthy cohorts of women to determine the association between exercise and SOD.

Only two demographic factors, age and gynaecological age, were found to be associated with the presence of SOD cycles in the present study. Specifically, SOD cycles were significantly associated with younger gynaecological age. Within the current study population, each additional year of gynaecological age was associated with a 14.2% decrease in the odds of being SOD. These findings may not be unexpected, as across the literature, age has been widely found to be associated with a range of ovulatory disturbances. Previously, Bedford et al. (2010) reported that SOD cycles were associated with both younger age and younger gynaecological age. Similarly, Schliep et al. (2014) reported a significant association between clinical LPD and younger age. Interestingly, Henry et al. (2024) did not find any significant differences in LPD prevalence between age groups. This may be due to the majority of their premenopausal cohort being between the ages of 32 and 36 years. Of note, these findings may be supported by the present study, suggesting that amongst this age group of mid-thirties premenopausal healthy naturally menstruating women who have a higher gynaecological age, SOD cycles may not be a

regular occurrence and instead may only occur sporadically. To develop our understanding of the association between gynaecological age and SOD occurrence in naturally menstruating women, further research should consider capturing a wide age range of participants in order to best report on these differences.

3.6 Conclusion

The findings from this study have highlighted that within regularly menstruating women the sporadic occurrence of SOD was found to be higher than the consistent occurrence of SOD. The majority of women may have cycle lengths that vary by less than seven days, and in ovulatory cycles, ovulation may occur mid-cycle around day 14. In women with SOD cycles, variability in menstrual cycle features and characteristics increases. Variability in menstrual cycle length in SOD cycles was associated with increased luteal phase length variability. Within this cohort of healthy, naturally menstruating women, only age and gynaecological age were associated with the occurrence of SOD. Overall, this study adds to the growing body of research literature that describes menstrual cycle features and characteristics and the natural variability between and within naturally menstruating women.

4 Conclusion

4.1 Research outcomes

The current study aimed to describe the characteristics of the menstrual cycle in healthy New Zealand women across three to five cycles. A secondary aim was to determine the individual and lifestyle factors that may influence the presence of ovulatory disturbances. Within this study, the following key findings were identified.

Overall, the majority of cycles in healthy, regularly menstruating women were ovulatory. However, SOD cycles were not uncommon, with over a third of women experiencing at least one SOD cycle over the course of this study. Sporadic SOD cycles appeared to be more frequently experienced by women than recurrent SOD cycles. Few demographic and lifestyle factors appeared to be associated with SODs in the present study. Specifically, only age and gynaecological age were associated with the occurrence of SOD cycles. Despite research that has suggested that high levels of physical activity may be associated with the occurrence of SOD cycles (De Souza et al., 2010), the present study did not share these results. Similarly, no associations were found for ethnicity or body composition (fat mass percentage, lean muscle mass, weight and BMI).

Our findings suggested that in ovulatory cycles, the average length of the menstrual cycle may be 28.5 days, menses length is five days, the follicular and luteal phases are 14 days long, separated by ovulation occurring on day 14. While these values align closely with other research, the same findings are not necessarily reported in anovulatory or LPD cycles, as significant differences are observed. In this study, SOD cycles tended to demonstrate higher within-woman variability. However, as these cycles were often experienced sporadically, it is likely that 'normal' cycle length and phases may not be regularly and consistently experienced across all women.

It may be important to recognise that the findings of this study suggest that variability of the menstrual cycle, both in ovulatory status and presentation of menstrual cycle, menses, follicular and luteal length, are common occurrences. Previous researchers have proposed that this variability in menstrual cycle features and characteristics may be representative of an adaptive menstrual cycle (Prior, 2022). These findings are important as menstrual health education may

need to consider integrating information on healthy menstrual cycle variability to increase menstrual health literacy among premenopausal women.

4.2 Strengths and limitations

A major strength of this study was the use of the three-step method for menstrual cycle classification, including mid-luteal plasma progesterone assessment following urinary LH test to confirm cycle's ovulatory status. This reduced the reliance on self-reported cycle characteristics and is in alignment with current methodological recommendations for improving accuracy in menstrual health research (Schmalenberger et al., 2021). Subsequently, this has improved the reliability, accuracy and validity of the results reported within this research.

A second strength of this study was its ability to assess menstrual characteristics and features over three to five consecutive menstrual cycles per woman. In doing so, this study was able to capture between-women and within-woman variations. This enabled the analysis of both recurrent and/or sporadic incidence of SOD cycles to be identified. As such, the present study is more likely to reflect the true nature of menstrual cycle variability within a population of healthy naturally menstruating women.

It is a limitation of this study that our cohort was predominantly Caucasian with relatively low representation from Māori and Pacific Peoples, despite these ethnicities comprising over 20% of the New Zealand population (Stats NZ, 2024). Therefore, these ethnicities may not be adequately represented by the data reported by this study. For results to be generalised to the wider population here in New Zealand, further research of a similar manner is required in these populations.

While the present study met the required sample size, we did have relatively small numbers of women who were classed as consistently SOD (e.g. two or more SOD cycles). This provides a possible limitation as the data analysis for SOD cycles may have an increased risk of Type II errors and reduced statistical power. As such, it may be beneficial for further research to employ similar methods, which may then be gathered into a larger data set for statistical analysis.

4.3 Recommendations and future directions

The menstrual cycle is considered an integral part of women's health and can be treated as an indicator of physiological well-being within women (Rosen Vollmar et al., 2025). The findings of this study demonstrate that the majority of menstrual cycles in healthy, naturally menstruating women may be ovulatory and last between 21 and 35 days. However, there is a high sporadic occurrence of anovulation and LPD cycles, suggesting that these SOD cycles may be a relatively common occurrence with healthy naturally menstruating women. Previously, research has suggested that ovarian activity exists on a continuum and that in response to internal and external stressors, ovulatory function may move between fully fertile and infertile activity (Brown, 2011; Prior, 2022). Taking into consideration this proposal, the deviations of ovulatory functioning within naturally menstruating women reported within this study may be considered natural biorhythms of the menstrual cycle. However, more research is required to support the findings of this study.

Key findings of this study, including the sporadic incidence of LPD and anovulation and the variability of the menstrual cycle and phase lengths, may be beneficial to include in menstrual health education. The primary application for these findings may be for healthcare providers and patients. As previous research has suggested that increased menstrual health literacy in women may benefit overall wellbeing (Kurpanik et al., 2024; Long et al., 2022; Mahajan, 2019), dissemination of these findings may encourage and improve self-tracking of menstrual cycles. This may increase women's understanding of their own menstrual cycle variability, which may be important to them for conception planning, or avoidance of conception. It may also increase engagement with healthcare professionals as previous research has shown that when women are empowered with information about their menstrual cycles, they may be more likely display health seeking behaviours (Armour et al., 2022; Mahajan, 2019). Notably, as sex steroid hormones may be associated with protective effects on cardiovascular and bone health (Cignarella et al., 2024; Gersh et al., 2024; Manolagas et al., 2013), large within-woman fluctuations in menstrual cycle patterns may be considered a risk factor for associated health issues (Yi et al., 2024). However, future research in longitudinal cohort studies investigating the implications of menstrual cycle and sex steroid hormone variability on health outcomes is still required. Conversely, educating women about natural menstrual cycle variability may provide reassurance for those who perceive their own cycles as abnormal, as some women believe that irregularities and inconsistencies in their menstrual cycle from month to month is considered

abnormal (Wood et al., 2007). This may help women to distinguish natural cycle variability from matters that require clinical attention.

Finally, the findings of this study regarding cycle variation may have implications for future researchers. Due to the lack of female representation within physiology and wider health-based research, these results may contribute to furthered understandings of the importance of sex steroid hormone assessment within studies that consider the menstrual cycle as part of the study design. In particular, research that investigates factors that are impacted by the fluctuation of sex steroid hormones, such as osteoporosis or cardiovascular factors, should consider the need to accurately quantify plasma hormone levels in female populations. As the current study has demonstrated, menstrual cycle variability may be evident in healthy regularly menstruating women across small timeframes of three to five cycles, studies may need to be designed to understand how eumenorrheic menstrual cycles and SOD cycles influence health outcomes. Therefore, considering and accounting for this sex steroid hormone variability within research using quantifiable measures such as mid-luteal serum progesterone may be crucial for accurate and nuanced research into female health and wellbeing.

Based on the findings of this study and assessment of previous research, some recommendations for future research in this area are outlined below.

- The current study does not accurately reflect the ethnic proportions within New Zealand. Therefore, future research in this field should aim to capture higher numbers of Māori and Pacific Peoples. This may be used to better understand how the presentation of the menstrual cycle and its variability differ across ethnic cohorts within New Zealand. For international research, a focus on capturing ethnically diverse cohorts with adequate numbers of non-European women should be applied.
- Current research examining menstrual cycle variability, when considering body composition, often places a focus on weight and BMI. However, these factors do not account for body composition. Women who may have high muscle mass may be misclassified as overweight or obese. Conversely, women with low muscle mass but high fat mass may be classed as 'normal' (Buss, 2014). Therefore, additional anthropometric factors, including muscle mass and fat mass, should be analysed when considering the presence of SOD cycles in future research.

- Differences between the current study and other research in the field suggest that higher rates of menstrual disturbances may be captured in studies of longer duration. Therefore, future research should continue to undertake longer study durations to examine the consecutive cyclic differences between and within women.

5 References

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