Menstrual Fluid Loss and Its Association with Body Composition, Sociodemographic and Lifestyle Factors, and Menstrual Health in Healthy Menstruating New Zealand Women

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

Master of Science

In

Nutrition and Dietetics

at Massey University, Auckland,

New Zealand

Gemma McDougall

2025

Abstract

Background: Despite menstruation occurring regularly for most females during their reproductive years, empirical understanding of the volume and variation of menstrual fluid loss (MFL) remains limited. This gap limits our understanding of normal and abnormal menstrual patterns and the biological and sociodemographic factors that may influence MFL. The primary objective of this study was to determine the daily and total MFL over one or up to three consecutive menstrual cycles (MCs). The secondary objectives were to explore the associations between total MFL and sociodemographic and lifestyle factors (including age, ethnicity, and physical activity (PA)), body composition, and menstrual health, including gynaecological age, parity, and hormonal contraceptive (HC) use.

Methods: Menstruating females (n=40), aged 18–45 years, were recruited from Auckland, New Zealand, to participate in a prospective observational study collecting MFL. Participants used either a menstrual cup or disc for one or up to three consecutive MCs to measure daily and total MFL. Participants completed a baseline questionnaire that captured sociodemographic data, PA levels, and menstrual and general health history. Anthropometric measurements (height and weight) and body composition (fat mass, fat-free mass, body fat percentage) were collected in a laboratory setting. Descriptive statistics were used to summarise participant characteristics and MFL. Participants were grouped into light-medium and medium-heavy groups based on the sample medium. Associations between total MFL and sociodemographic and lifestyle, body composition, and menstrual health factors were explored using independent samples *t*-tests, Mann-Whitney *U*, Fisher-Freeman-Halton exact tests, Friedman's *ANOVA*, and multivariate linear regression. Statistical analyses were conducted using IBM SPSS Statistics (version 30.0) and significance was set at $p < 0.05$.

Results: A statistically significant difference was observed in median MFL across MCs one, two, and three (χ^2 (1) = 768.0, $p < 0.001$) and days one to eight (χ^2 (1) = 167.3, $p < 0.001$) of menstruation. Significant differences in both objective and subjective MFL were observed between light-medium and medium-heavy MFL groups. Specifically, the medium-heavy group had higher objective MFL, which aligned with a higher subjective rating of MFL on days of menstruation. No significant associations were observed between MFL and sociodemographic and lifestyle ($F(4,30) = 0.17$, $p = 0.95$, $R^2 = 0.03$), body composition ($F(3,31) = 0.06$, $p = 0.98$, $R^2 = 0.01$) or menstrual health factors ($F(3,31) = 0.61$, $p = 0.61$, $R^2 = 0.06$).

Conclusion: Menstrual fluid loss exhibited substantial inter- and intra-individual variability. The MFL of this cohort of healthy women appeared to be within the physiologically normal ranges reported in previous literature. No significant associations were found between MFL and sociodemographic and lifestyle, body composition, or menstrual health factors. This highlights the multifactorial nature of menstrual physiology and the need for larger, more diverse studies using objective measurement methods.

Acknowledgements

I would like to say a massive thank you to Claire Badenhorst. You have provided such invaluable support throughout this journey, and I am so grateful for your feedback and inspiring passion for the Female Health Research Programme. Thank you also to Cathryn Conlon for your guidance and invaluable insights, and to Karen Mumme for your expertise and support during the statistical analysis.

I would also like to acknowledge my family and partner for their unwavering support, encouragement, and patience throughout this journey; it has not gone unnoticed. To the friends I have made along the way in dietetics, you have brought so much joy and support to this experience, I am very lucky to have met you all.

Lastly, I would like to thank Massey University and the Dietetics programme for providing the resources and learning environment that made this journey possible. This Master's programme has allowed me to grow both academically and personally, and I am deeply grateful for the opportunity and journey in becoming a Dietitian.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Term
AUB	Abnormal Uterine Bleeding
B	Beta
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CI	Confidence Interval
cm	Centimetre
FHQ	Female Health Questionnaire
FIGO	International Federation of Gynaecology and Obstetrics
g	Gram
HC	Hormonal Contraceptive
HMB	Heavy Menstrual Bleeding
IQR	Interquartile Range
IUD	Intrauterine Device
kg	Kilogram
kg/m^2	Kilogram per square metre
L	Litre
LH	Luteinising Hormone
LNG-IUD	Levonorgestrel-Releasing Intrauterine Device
Max	Maximum
MBL	Menstrual Blood Loss
MC	Menstrual Cycle
MF	Menstrual Fluid
MFL	Menstrual Fluid Loss
Min	Minimum
mins	Minutes
mL	Millilitres
n	Number of patients
nm	Nanometres
OC	Oral Contraceptive
p	Predictive value
PA	Physical Activity
PBAC	Pictorial-Based Assessment Chart
R^2	Multiple coefficients of determination
SD	Standard Deviation
SE	Standard Error
VIF	Variance Inflation Factor
yrs	Years

Chapter 1: Introduction

Menstruation is a natural and recurring physiological process experienced by most females throughout their reproductive lifespan (Rohatgi & Dash, 2023). It is marked by the shedding of the uterine lining and the loss of menstrual fluid (MF) through the vagina (Allen et al., 2016). Globally, approximately 1.8 billion females menstruate every month (UNICEF, n.d.), and on any given day, an estimated 800 million are menstruating, representing about 26% of the global population (Rohatgi & Dash, 2023).

In Aotearoa New Zealand, education about menstruation has been taught from a scientific and medical perspective, with an emphasis on reproduction and hygiene (Diorio & Munro, 2000). This narrow framing has often excluded comprehensive education on menstrual health and menstruation (Diorio & Munro, 2000; Fletcher, 2023). As a result, a notable gap in menstrual health literacy remains, particularly around the understanding of menstrual fluid loss (MFL) and menstrual blood loss (MBL) between women and menstrual cycles (MC). Menstrual fluid is a complete biological mixture, while menstrual blood is one component of this fluid.

Recent research has highlighted low levels of MFL awareness (Fletcher, 2023). Findings from an online questionnaire assessing MC knowledge among New Zealand females aged 16–40 years revealed that most participants could correctly identify the definition of a prolonged menstrual bleed (96.1%) and the components of MF (99%); however, knowledge gaps persisted in key clinical areas (Fletcher, 2023). For example, over half (51.7%) of the participants were unaware of the volume of MBL that constitutes heavy menstrual bleeding (HMB), and only 57.6% could identify what is considered a normal amount of MBL during one menstrual bleed.

Similar levels of menstrual health literacy have been observed internationally. Damian et al. (2019) conducted an online questionnaire assessing the knowledge of 19,415 Polish females on the physiology of the MC. In this study, less than half (47.5%) were able to identify that menstruation typically lasts 3–5 days and normal MBL was around 30–70 mL. A further 31.0% selected “5–7 days and less than 30 mL”, 21.0% selected “7–10 days and less than 30 mL”, and 0.41% selected that “it doesn't matter how long the menstruation lasts". These findings highlight the substantial variability in women's understanding of both the duration and volume of menstrual bleeding.

Limited understanding or knowledge of MBL and MFL is not a recent issue. Evidence of women's low menstrual health literacy can be found in research completed in the 1960s. Hallberg, Hôgdahl, et al. (1966) compared subjective perceptions of MBL with objectively measured volumes in 475 Swedish participants. In this study, over 40% of women with an MBL above 80 mL, thus exceeding the clinical threshold for HMB, perceived their menstruation as "moderate" or even "small". Conversely, 14% of women with an MBL below 20 mL considered their bleeding to be "heavy". These discrepancies demonstrate the unreliability of subjective assessments of MBL and point to a broader lack of understanding around MBL and MFL volume norms that appears to have persisted over time.

Over the past six decades, MFL has been assessed using both subjective and objective methods. Research that has objectively measured MFL has employed a range of collection and quantification methods. The early objective studies predominantly focused on measuring or determining MBL, rather than total MFL. As a result, in research conducted between the 1960s and the early 2000s, sanitary pads and tampons were widely used in combination with the alkaline hematin method to measure MBL. However, over time, the measurement of MFL has evolved significantly, shifting from the alkaline haematin method toward more practical approaches such as gravimetric and menstrual cup methods. This indicates a shift toward measuring MFL rather than MBL alone.

To date, Donoso et al. (2019) are the only study to use menstrual cups to objectively measure MFL. As a result, clinical guidance for interpreting MFL measurements obtained via menstrual cups remains limited. Additionally, research studies have not yet established how volumes of MFL measured using menstrual cups correspond to the thresholds for HMB or MBL as quantified using the alkaline hematin method. This gap highlights the need for further research to validate mensural cup measurements and define clinically meaningful thresholds for MFL.

During a female's reproductive years, MFL can vary significantly among and between individuals (Atsuko et al., 2024). Both inter- and intra-individual variations in MFL and MBL are influenced by a range of factors, including lifestyle, nutrition, physical characteristics, and health-related factors (Mena et al., 2021; Reavey et al., 2021). While improving knowledge and awareness of menstrual health and MFL is essential, it is equally important to understand the various factors that contribute to this variability to support accurate interpretation and personalised menstrual and health care for women. For example, understanding MFL variations for women is important for identifying potential impacts on iron status, as HMB can lead to substantial iron losses. As such, increased MFL for premenopausal women can increase their risk of iron deficiency and iron deficiency anaemia (Napolitano et al., 2014).

Previous research has reported on many factors that may influence MFL. These include age, number of pregnancies, contraceptive use, body mass index (BMI), underlying health conditions and disease states, and stress (Atsuko et al., 2024; Jahanfar et al., 2024; Poitras et al., 2024; Yi et al., 2023). For the purpose of this thesis, we will explore the association among sociodemographic and lifestyle factors, body composition, and menstrual health factors.

Sociodemographic and lifestyle factors such as age, physical activity (PA), and ethnicity, and their influence on menstruation and MFL have been explored to a limited extent. The existing literature suggests that age is associated with increasing MFL across the reproductive lifespan. Physiologically, lower MBL among adolescents may be due to an immature reproductive axis, a younger gynaecological age, and low parity (Hallberg, Hôgdahl, et al., 1966; Rybo, 1983). In contrast, the higher prevalence of HMB in older women may be attributed to age-related uterine conditions such as fibroids, polyps, and endometrial hyperplasia, which are more common during the perimenopausal years and can contribute to increased MFL (Hallberg, Hôgdahl, et al., 1966; Rybo, 1983; Van Voorhis et al., 2008). While ethnicity and PA have been scarcely researched, some studies suggest potential correlations, and others remain inconclusive (Bruinvels et al., 2016; Dasharathy et al., 2012; Mena et al., 2021).

While nutrition influences body composition, body composition itself independently affects various health outcomes, including menstrual health, making it an important variable in understanding MFL. Research on body composition and anthropometry in relation to menstruation has primarily focused on changes in body composition across the MC. While most research suggests statistically significant positive correlations between height, weight, BMI, and MBL (Hahn et al., 2013; Mena et al., 2021; Reavey et al., 2021; Santos et al., 2011), others report no clear associations (Atsuko et al., 2024; Dasharathy et al., 2012; Donoso et al., 2019). Currently, there are few studies examining the relationship between objectively measured MFL and more precise body composition metrics, such as fat mass, lean body mass, or body fat percentage.

Within the existing research, factors such as parity (Atsuko et al., 2024; Donoso et al., 2019) and contraceptive use (Fraser et al., 1985; Israel et al., 1974) have been reported to have a significant influence on MFL, whereas age of menarche does not (Ji et al., 1981; Napolitano et al., 2014). Research consistently indicates that parity is positively associated with MFL (Atsuko et al., 2024; Donoso et al., 2019). Hormonal contraceptives (HC), particularly oral contraceptives (OC) and hormonal intrauterine devices (IUDs), which are widely used to manage HMB and regulate MCs (Bradley & Gueye, 2016), are often associated with a significant reduction in MBL and, in many cases, can lead to lighter or absent menstrual bleeding (Fraser et al., 1985). Within the available evidence, it is suggested that MFL may vary depending on the type of contraceptive used (Andrade et al., 1979; Fraser et al., 1985). However, the majority of the existing literature on contraceptive use and MBL was conducted prior to the 2000s. Consequently, while historical research offers valuable insights, there is a clear need for research examining the effects of modern contraceptive methods on MFL and MBL.

In summary, while menstruation is a universal experience for most females throughout their reproductive lifespan, significant gaps remain in our understanding of MFL. Particularly regarding the objective measurement of MFL as compared to MBL, and what sociodemographic and lifestyle, body composition, and menstrual health-related factors may influence both inter- and intra-individual variability in MFL. Addressing these gaps is essential to advancing menstrual health research, informing evidence-based guidelines, and supporting informed clinical care, particularly in relation to the influence of body composition on MFL.

1.1 Aims

To determine the daily and total menstrual fluid loss variations in menstruating women and their association with sociodemographic factors, body composition, and menstrual health.

1.1.1 Objectives

1. To determine the daily and total menstrual fluid loss variations over 1–3 consecutive menstrual cycles.
2. To explore the associations between total menstrual fluid loss and sociodemographic and lifestyle factors, including age, ethnicity, and physical activity.
3. To explore the associations between total menstrual fluid loss and lean muscle mass and body fat percentage.
4. To explore the associations between total menstrual fluid loss and menstrual health, including gynaecological age, parity, and contraceptives.

1.1.2 Hypothesis

1. There will be a significant difference between daily MFL in menstruating New Zealand women.
2. There will be a positive association between age and total MFL in menstruating New Zealand women.
3. Women with higher levels of PA will experience lower MFL compared to women with low levels of PA.
4. There will be a positive association between MFL and body fat percentage in menstruating New Zealand women.
5. Parous women will experience significantly higher MFL than nulliparous women.
6. Hormonal contraceptive (HC) users will experience significantly lower MFL than non-HC users.

1.2 Structure of Thesis

This thesis begins with an introductory chapter discussing the purpose of this study, with a focus on menstruation as a key part of the MC. This chapter discusses the limited knowledge of MFL, particularly its inter- and intra-individual variability and the factors that contribute to this variability among women. This first chapter will conclude with the study aims, objectives, and hypothesis. Chapter two presents an in-depth review of current literature on MFL and MBL, including measurement methodologies and their associations with sociodemographic and lifestyle factors, body composition, and menstrual health. Chapter three will include a manuscript detailing the methodology and results of this research. Chapter four will summarise the implications of this research, discuss strengths and limitations, and outline recommendations to guide future research in this area.

1.3 Researcher's Contributions

Table 1.1 Researcher's Contributions

Researchers	**Contributions**
Gemma McDougall MSc Nutrition and Dietetics Student	Primary author of thesis and manuscript Statistical analysis Interpretation of results
Dr Cathryn Conlon Primary supervisor Professor of Nutrition, School of Sport, Exercise and Nutrition	Primary supervisor Provided advice and revised thesis
Dr Claire Badenhorst Co-supervisor Associate Professor School of Sport, Exercise and Nutrition	Co-supervisor Designed the present study Ethics application Data collection Provided advice and revised thesis
Rebecca Paul Research Officer	Research Officer Data collection and management
Dr Karen Mumme Statistician	Statistician Preparation and cleaning of data Assisted with statistical analysis
Dr Beatrix Jones Associate Professor Statistics	Statistician Assisted with statistical analysis

Chapter 2: Literature Review

2.1 Introduction

Menstruation is a vital aspect of female health, yet our understanding of the factors that are associated with intra- and inter-individual variations in menstrual fluid loss (MFL) remains insufficiently explored in scientific literature. In the absence of this information, this chapter will critically review the available literature on menstruation, specifically MFL and its association with various sociodemographic and lifestyle factors, body composition metrics, and menstrual health factors. The first section of this review will focus specifically on studies that have objectively quantified MFL and, therefore, will exclude studies that have used subjective measures of MFL. The remaining three sections will focus on factors that may be associated with MFL, and given the limited research on this topic, will review studies that have included both subjective and objective measures of MFL.

To provide additional context for this area of research, this review will present a discussion on the methodologies used in previous research to measure MFL. A critical discussion will be presented on the effectiveness and limitations of various measurement methods, including menstrual cups, sanitary pads, tampons, and gynaeseals. By synthesising current knowledge on MFL and factors that may be associated with its variability between and within women, this review aims to highlight gaps in the literature and suggest future research directions to improve our understanding of this under-discussed aspect of women's health.

Review Methods:

To investigate the current understanding of MFL, this review focused on (a) methodologies to quantify MFL and factors that previous research has suggested may be associated with MFL variability between and within women. These factors include (b) sociodemographic and lifestyle factors, (c) body composition metrics, and (d) menstrual health factors.

This literature review search was conducted in electronic databases including PubMed, Google Scholar, Scopus, and Discover (through the Massey University Library), to identify studies published between 1960 and 2024.

The search terms used included combinations of the following keywords:

- "Menstrual bleed*" OR "menstrual fluid" OR "menstrual blood"
- Menstrua* OR menses
- "Blood flow" OR "blood loss" OR "Blood Volume" OR *"fluid los*"* OR "fluid volume"
- Measur* OR estimat* OR volume
- Ethnic* OR race
- Sociodemograph* OR "socio demograph*" OR demograph* OR ethnic* OR race
- "Physical activit" OR exercise* OR fitness*
- "Body composition" OR BMI OR "body mass index" OR "body weight" OR "body fat" OR height

Abstracts, full-text articles, and methodologies were screened for relevance and were critically reviewed if they investigated the association between MFL and sociodemographic and lifestyle, body composition, and menstrual health factors. The studies that objectively measured MFL were further assessed, with their methodology evaluated based on their effectiveness at collecting MFL.

2.2 Menstrual Cycle and Menstrual Fluid Loss

2.2.1 Menstrual Cycle and Menstruation

The menstrual cycle (MC) is a natural, recurring process that is regulated by the neuroendocrine system. For most females, the duration of the MC typically lasts 21 to 35 days. The MC is broadly divided into two main phases, the follicular and luteal phases (Allen et al., 2016). These two phases can then be further subdivided into early-, mid-, and late-follicular phases, as well as early-, mid-, and late-luteal phases. Each phase and subphase of the MC is characterised by the variations in concentrations of the two main sex steroid hormones involved in the MC, oestrogen and progesterone (Allen et al., 2016; Sukhija & Katiyar, 2024).

Throughout the MC and within different phases, variations in oestrogen and progesterone will drive physiological changes of the endometrium. For example, when oestrogen and progesterone are low in the early-follicular phase, menstruation and the shedding of the endometrial lining occur (Allen et al., 2016; Salamonsen et al., 1999). During the mid-follicular phase, oestrogen levels increase as a mature follicle develops in the ovary, with levels of this hormone peaking in the late-follicular phase (Allen et al., 2016). This rise in oestrogen stimulates the proliferation of the endometrial lining, rebuilding the functional layer that was shed during menstruation. Progesterone is low throughout the follicular phase. At the end of the follicular phase, a surge in luteinising hormone (LH) occurs, triggered by high oestrogen levels (Allen et al., 2016). The LH surge signals ovulation (the rupturing of the mature follicle in the ovary and release of the ovum into the fallopian tubes), after which the remaining cells of the mature follicle in the ovary will form a temporary endocrine structure known as the corpus luteum. The corpus luteum secretes both progesterone and oestrogen, and its formation marks the start of the early-luteal phase (Allen et al., 2016). In the mid-luteal phase, progesterone concentrations will peak, and oestrogen concentrations will have a secondary peak within the MC. During the luteal phase, progesterone has been shown to act on the oestrogen-primed endometrium to promote its differentiation into a secretory, decidualised tissue that is capable of supporting implantation (Gellersen & Brosens, 2014). During the late-luteal phase, if no pregnancy occurs, the corpus luteum will break down, leading to a drop in progesterone and oestrogen levels. The decline in progesterone concentrations triggers spontaneous decidualisation and subsequently, the breakdown and shedding of the decidualised endometrial layer, resulting in menstruation (Gellersen & Brosens, 2014). The commencement of menstruation at the end of the late luteal phase signals the start of the early-follicular phase of the subsequent cycle (Allen et al., 2016). For this literature review, the author will focus on the menstrual/early follicular phase, when menstruation occurs, and MF is lost.

Women typically experience menstruation for approximately four decades of their lives, which is more than 50% of their average lifespan (Rohatgi & Dash, 2023). The first menstruation a woman experiences is known as menarche and typically occurs 2–3 years after the onset of puberty (American Academy of Pediatrics et al., 2006). In developed countries, such as New Zealand, menarche generally occurs between 12 and 13 years (American Academy of Pediatrics et al., 2006). At the end of their reproductive years, women will experience menopause. Menopause marks the cessation of menstruation, typically around 50 years of age (Lobo, 2022).

During their reproductive years, between menarche and menopause, the duration of menstruation and volume of MFL can vary greatly across individuals. Typically, the duration of menstruation is between 2 and 8 days (Atsuko et al., 2024). Within the available research, MFL has been measured both subjectively and objectively. The research studies that have objectively measured MFL have categorised menstrual flow as abnormally light (≤15.6 g); light (15.7-36.4 g); normal (36.5-96.8 g); heavy (96.9-166.4 g), and abnormally heavy (≥166.5 g) (Atsuko et al., 2024; Hallberg, Hôgdahl, et al., 1966). From the initial research in this area, heavy menstrual bleeding (HMB), also previously known as menorrhagia, was defined as menstrual blood loss (MBL) ≥ 80 mL per MC (Hallberg, Hôgdahl, et al., 1966). However, few studies have clearly distinguished between MBL, a component of MF, and total MFL, and as a result, the volumes of MBL reported in the literature have often become synonymous with MFL. In contrast, subjective assessments of MFL use qualitative terms such as “light”, “moderate”, or “heavy” based on an individual's perception and estimation of their MFL.

2.2.2 How Has Menstrual Fluid Loss Been Measured in Previous Research

The measurement of MFL has been a subject of ongoing research since the 1960s, with various methods used throughout the research field. Previous research on MFL has emphasised measuring MBL, with a focus on understanding HMB. Studies have either specifically recruited women presenting with HMB (Fraser et al., 2001; Gleeson et al., 1993; Gudmundsdottir et al., 2009; Wyatt et al., 2001) or the research has aimed to investigate the assessment, diagnosis, or treatment of HMB (Gannon et al., 1996; Hurskainen et al., 1998; Rees, 1991; Reid, 2006; Reid et al., 2000).

Following a review of the available literature, 20 studies were identified that objectively measured MFL or MBL. Their methodologies are outlined in Table 2.1, which is ordered according to the method of fluid collection. Various methods have been used within previous research to collect and determine MFL volumes. Specifically, one study has used menstrual cups, one gynaeseals, and 18 used pads and/or tampons to collect menstrual fluid (MF). The primary methods that have been used to quantify volumes of components (e.g. blood) in MFL include gravimetric measurements and the alkaline haematin method (Hallberg & Nilsson, 1964).

Initial studies predominantly focused on measuring or determining MBL. Subsequently, the use of sanitary pads and tampons to collect MFL, combined with the alkaline hematin method first described by Hallberg and Nilsson (1964), became the most common method used to quantify the volume of MBL from the 1960s through to the early 2000s. For context, the alkaline hematin method involves extracting dried MF from used sanitary products and measuring haemoglobin concentration in these samples of MF using alkaline hematin analysis or spectrophotometry (Hallberg & Nilsson, 1964). This method provides an estimate of MBL based on the haemoglobin concentration in the extracted sample.

For the measurement of MF, researchers have used gravimetric methods where the weight of pads and tampons before and after use was recorded, assuming MF had a specific gravity similar to that of water (1 g = 1 mL). Between 1985 and 2024, gravimetric measurements were increasingly used by researchers instead of the alkaline hematin method, indicating a shift toward measuring MFL rather than MBL alone. Reported mean total MFL volumes across these studies ranged from 48.1 mL to 350.1 mL per menstrual period. In these studies, the risk of fluid evaporation was a common limitation of the gravimetric method used to determine MFL, especially when pads and tampons were used for MF collection, potentially reducing the reliability of reported MFL results. However, the gravimetric method was evaluated by Fraser et al. (1985), who found that although fluid loss due to evaporation or to loss on the inner surface of the bag was highly significant, it would be negligible relative to MFL. Despite this, in order to minimise evaporation, most studies that utilised the gravimetric method stored the used sanitary products in airtight, sealed bags. Interestingly, to help mitigate the risk of evaporation of collected samples, Gudmundsdottir et al. (2009) converted MFL into a gel prior to their gravimetric measurement of MF. However, in their study, they reported an unusually low mean MFL per menstrual period (51.0 g) compared with previous research, suggesting that this method may underestimate MFL and may not be a reliable approach for objectively quantifying MFL. More recently, Donoso et al. (2019) have utilised menstrual cups, a readily available reusable menstrual product, to provide a direct measurement of MFL. The menstrual cup method eliminates the need for storage of used sanitary products, reducing the risk of evaporation, but does not provide an estimate of MBL; it only provides a measurement of the entire MF. This limitation means that direct volume measurements cannot be compared with those from studies that measured MBL using alkaline hematin methods.

Clear and comprehensive participant instruction is critical for ensuring the accuracy and consistency of MFL collection. Studies that failed to mention whether instructions were provided leave uncertainty around data reliability and participant compliance (Gleeson et al., 1993; Gudmundsdottir et al., 2009; Hallberg & Nilsson, 1964; Higham & Shaw, 1999; Rees, 1991; Reid, 2006; Van Eijkeren et al., 1991). In contrast, studies that implemented detailed verbal and written instructions demonstrated higher methodological rigour and participant cooperation. For example, Fraser et al. (2001), Hurskainen et al. (1998), and Mansfield et al. (2004) provided thorough instructions, including guidance on the simultaneous use of tampons and pads, storage guidelines, and the use of menstrual diaries. These methods are likely to have minimised any loss of MF during data collection and, as a result, would have improved data accuracy and reliability. Similarly, several studies emphasised direct guidance through verbal or written materials, helping participants avoid common mistakes such as improper product storage or fluid loss during bathing (Chimbira et al., 1980; Donoso et al., 2019; Fraser et al., 1985; Gannon et al., 1996; Hallberg, Hôgdahl, et al., 1966; Napolitano et al., 2014; Reid et al., 2000; Wyatt et al., 2001). In contrast, studies such as Levin and Wagner (1986), which instructed participants to follow their usual habits, may have introduced greater variability into their MBL results. Overall, 15 out of 20 studies specified standardised instructions, reducing the risk of data collection and measurement errors. This enhances the reliability and validity of the findings in these studies that have quantified MFL and MBL.

It should be noted that there are various strengths and limitations of MFL quantification methods. In a clinical setting, the alkaline haematin method is recognised as impractical for quantifying MBL due to the multiple steps required to collect and test MF, the length of the process, as well as the invasiveness and discomfort associated with storing and analysing used sanitary products. Similarly, a standardised wash method outlined in Gannon et al. (1996) used for the measurement of MBL may not be appropriate for a clinical setting, as it requires several additional steps than the alkaline hematin method, making it more challenging and time-consuming. In a clinical setting, measuring MFL using the gravimetric or menstrual cup method is simpler, quicker, and less labour-intensive than the alkaline haematin or standardised wash method. Moreover, these methods can be carried out in the privacy of the participant's home, providing real-time results. In addition, these methods may be considered relatively non-invasive, as they could eliminate the need for MF to be handled or viewed by researchers. Instead, participants can record their own fluid volume, which further enhances the method’s convenience. However, no research to date has quantified MBL within complete samples of MFL, limiting our understanding of what volumes of MFL should be considered normal or heavy. Although the use of menstrual cups is a promising alternative for measuring MFL in a clinical setting, the absence of clear clinical recommendations based on MFL measurements underscores a significant gap in both research and patient care.

Within the research that has quantified MFL and MBL, leakage of sanitary products was a common limitation within many studies (Table 2.1). For example, Gleeson et al. (1993) used Gynaeseals, with their results suggesting that this method was ineffective for MFL collection, particularly for women with HMB. Specifically, women within their study who presented with HMB and who tried to extract MF from the Gynaeseal experienced spillage. It should be noted that Wyatt et al. (2001) were the only study to quantify extraneous blood loss by asking women to report slight, moderate, and severe fluid loss when changing sanitary products using three pictograms that were representative of these volume losses. They reported that after accounting for extraneous fluid loss, the median MBL for women presenting with HMB and women in the non-HMB group increased by 60% and 33%, respectively.

Subsequently, these results may suggest that MBL in previous studies that have not considered extraneous fluid loss during data collection may be underestimated. Interestingly, both Donoso et al. (2019) and Hurskainen et al. (1998) included subjective assessments of leakage within their data collection of MFL. In both studies, no statistically significant differences in MFL were found between women reporting significant versus little or low leakage while using a menstrual cup (Donoso et al., 2019). Similarly, Stewart et al. (2010) found that the menstrual cup users experienced half as many leakage episodes per menstrual bleed than pad users. These results could reinforce that menstrual cups, especially more recent products, may be effective for quantifying MF volumes in both research, clinical, and practical settings. Regardless, to ensure that the reported MFL is accurate and not underestimated, researchers need to monitor leakage throughout data collection. Insights from the available research would suggest that best practice methods for the MFL include thorough participant education on collection methods, including sanitary product changes, clotting and flooding episodes, as well as accurate subjective assessment of leakage. During data collection, maintaining a daily menstrual diary may be recommended to support this subjective reporting of MFL and leakage. The addition of these methodological considerations within research is a good practice for ensuring reliable and accurate data collection.

The measurement of MFL has evolved significantly since the 1960s, shifting from the alkaline haematin method toward more practical approaches such as gravimetric and menstrual cup measurements. While gravimetric methods are simpler than the alkaline haematin method, they still present challenges to MFL determination due to the fluid evaporation and leakage that can occur during data collection. The use of menstrual cups provides direct measurement of MFL, and more recent products, when worn correctly, may have low leakage. However, as noted throughout this section, clinical recommendations for women who measure their MFL using menstrual cups remain limited. To date, no research has established what volumes of MFL, measured via menstrual cups, correlate with HMB or MBL as quantified using the alkaline hematin method. Nevertheless, research studies that aim to quantify MFL should include participant education and accurate leakage assessments to reduce the risk of underreporting and improve the reliability of the results.

Table 2.1: Objective Menstrual Fluid Loss Studies

Menstrual Fluid Loss					
Reference	**Population** *n*, mean age (years) ± SD or range	**Study Design**	**Methodology for Fluid Collection**	**# Menstrual Cycles**	**Main Outcome**
Donoso et al. (2019)	*n* =28 24-49 years	Prospective observational study	- Daily measurement of MFL using a menstrual cup. - A continuous measurement approach, day and night, removing the menstrual cup every 6–8 hours. - Contents emptied into a graduated measuring tube and the volume was recorded on a daily chart.	3	Mean MFL: 86.7 mL (range 15-271 mL) Mean MFL for nulliparous: 45.9 mL (15–71 mL) Mean MFL for multiparous: 99.1 mL (41–271 mL)
Gannon et al. (1996)	*n*=372 40, 24-54 years	Prospective observational study	- MF extracted from used pads and tampons. - The sanitary products from one menstrual period were processed together. - The used sanitary products were wrapped in aluminium foil before sealing in a plastic specimen bag and dropped at the laboratory. - The sanitary products were washed in a Smallboy benchtop washing machine with 10 L of tap water and a non-ionic detergent, iso-octylphenoxypolyethoxyethanol.	1	Median MBL: 63.0 mL (range 2–808 mL)
Fraser et al. (1985)	*n*=28	Descriptive, observational,	- Gravimetric measurement of pads and tampons.	1	Mean MFL: 74.4 ± 10.3 mL

Menstrual Fluid Loss					
Reference	**Population** *n*, mean age (years) ± SD or range	**Study Design**	**Methodology for Fluid Collection**	**# Menstrual Cycles**	**Main Outcome**
		Cross-sectional study	- The sanitary products were stored in self-sealing polyethylene bags before and after use. - Analysis was performed within two-three days of the end of the menstrual bleed.		Mean MBL: 30.6 ± 6.1 mL Women who only used pads mean MFL per day: 17.4 ± 4.1 mL Women who only used tampons mean MFL per day: 15.6 ± 1.5 mL
Fraser et al. (2001)	*n*=53	Prospective cohort study	- Gravimetric measurement of pads and tampons assuming specific gravity equals one. - The sanitary products were stored in self-sealing polythene bags before and after use. - Analysis was performed within three days of the end of the menstrual period.	2	Mean MFL for normal menses: 48.1 mL (SE 2.3, range 14–81.6 mL) Mean % blood content for normal menses: 39.4% (SE 1.8, range 12–80%)
Mansfield et al. (2004)	*n*=31 47.6, 38-52 years	Prospective, observational validation study	- Gravimetric measurement of pads and tampons assuming specific gravity equals one. - Used sanitary products were placed in zippered bags immediately after use and collected by the research assistant as soon as menstrual bleeding ceased.	3	The mean menstrual blood/fluid loss was not reported in this study
Reid (2006)	*n*=115	Prospective observational study	- Gravimetric measurement of pads and tampons.	1	Mean MFL: 183.5 mL (range 33.6–611.9 mL)

Menstrual Fluid Loss					
Reference	**Population** *n*, mean age (years) ± SD or range	**Study Design**	**Methodology for Fluid Collection**	**# Menstrual Cycles**	**Main Outcome**
			- The modified alkaline haematin method of Newton et al. (1977) was used to determine MBL. - Used sanitary products were collected in plastic bags, treated with 5% sodium hydroxide and homogenised using a Stomacher Lab-Blender. - The optical density was determined at 550 nm and the volume of menstrual blood was calculated.		
Gudmundsdottir et al. (2009)	*n*=113 16-49 years	Developmental study of an objective method	- Gravimetric measurement of pads and tampons. - The pads converted liquid menstrual discharge into a gel to prevent evaporation. - Each pad was placed in a plastic, airtight collection bag and returned to the laboratory immediately following the menstrual bleed. - The total number of used pads in the protective cover were counted and weighed in the collection bags.	1	Mean MFL: 51.0 g (median 44, range 5–144) Normal MFL: <110 g/menses
Napolitano et al. (2014)	*n*=105 29.9, 20–45 years	Prospective observational study	- Gravimetric measurement of pads and tampons. - Used sanitary products were vacuum sealed in plastic bags within 2 hours post use.	1	Mean MFL for normal menses: 65.1 ± 52.4 mL (range 9.5–188.4 mL)

Menstrual Fluid Loss					
Reference	**Population** *n*, mean age (years) ± SD or range	**Study Design**	**Methodology for Fluid Collection**	**# Menstrual Cycles**	**Main Outcome**
					Mean MFL for HMB: 350.1 ± 138.1 mL (range 189.6–575.2 mL)
Atsuko et al. (2024)	*n*=167 18–49 years	Prospective observational study	- Gravimetric measurement of menstrual napkins.	3	Mean MFL: 77.6 ± 99.6 g (range 15.7–166.4 g)
Hallberg and Nilsson (1964)	*n*=2	Prospective observational study	- MBL was measured using both pads and tampons following the alkaline haematin method. - 5% sodium hydroxide was added to the sanitary products, the sanitary products were squeezed, filtered and optical density was measured at 546 nm.	12	Mean MBL for participant 1: 9.6 ± 2.2 mL Mean MBL for participant 2: 39.8 ± 7.8 mL
Hallberg, Hôgdahl, et al. (1966)	*n*=476	Prospective observational study	- MBL was measured using both pads and tampons following the alkaline haematin method of Hallberg and Nilsson (1964). - The used sanitary products were collected at home in a plastic container with a tight cover. - For MBL extraction, 5% sodium hydroxide was added, the sanitary products were squeezed, filtered and optical density was measured at 546 nm.	1	Mean MBL: 43.4 ± 2.3 mL
Chimbira et al. (1980)	*n*=92	Prospective observational study	- MBL was measured using both sanitary towels and a tampons following the	2	34% of participants self-reported as light bleeders

Menstrual Fluid Loss					
Reference	**Population** *n*, mean age (years) ± SD or range	**Study Design**	**Methodology for Fluid Collection**	**# Menstrual Cycles**	**Main Outcome**
			alkaline haematin method of Hallberg and Nilsson (1964). - For MBL extraction, 5% sodium hydroxide was added, the sanitary products were squeezed, filtered and optical density was measured at 546 nm.		had a median MBL: 63.0 mL (range 1–283.0 mL) 47% of participants self-reported as heavy had a median MBL: 97.0 mL (range 27–758.0 mL) 55% of participants self-reported as medium bleeders had a median MBL: 99.0 mL (range 8–493.0 mL)
Levin and Wagner (1986)	*n*=8 22-24 years	Cross-sectional, experimental, descriptive study	- MBL was measured using the alkaline haematin method described by Hallberg and Nilsson (1964) and Shaw Jr et al. (1972). - Participants were instructed to maintain their usual tampon usage. - After removal, tampons were returned to their numbered containers, sealed, and brought to the lab the next day for weighing. - The tampons were cut into small pieces and placed into individual beakers. - Blood was extracted using a plastic multiperforated disc plunger, as much fluid volume as possible was expressed	3 days	Mean MBL: 0.76 g/h

Menstrual Fluid Loss					
Reference	**Population** *n*, mean age (years) ± SD or range	**Study Design**	**Methodology for Fluid Collection**	**# Menstrual Cycles**	**Main Outcome**
			from the tampon into a graduated cylinder by a strong manual pressure. - The expressed volume was centrifuged, and a sample was analysed for haematin content.		
Rees (1991)	*n*=17 30-45 years	Pilot study	- MBL was measured using the alkaline haematin method described by Hallberg and Nilsson (1964). - For MBL extraction, 5% sodium hydroxide was added, the sanitary products were squeezed, filtered and optical density was measured at 546 nm.	2	All participants had MBL of < 80.0 mL (range 15–60.0 mL)
Van Eijkeren et al. (1991)	*n*=11 < 45 years	Prospective study	- MBL was measured using the alkaline haematin method described by Hallberg and Nilsson (1964). - For MBL extraction, 5% sodium hydroxide was added, the sanitary products were squeezed, filtered and optical density was measured at 546 nm.	1 day	Mean MBL: 98.0 mL
Gleeson et al. (1993)	*n*=22	Observational study	- MBL was measured in two MCs using the alkaline haematin method described by Hallberg and Nilsson (1964). - For MBL extraction, 5% sodium hydroxide was added, the sanitary products were squeezed, filtered and	3	Median MBL from the first two cycles (not using the gynaeseal) in the normal group: 26.0 mL (range 15–43.0 mL)

Menstrual Fluid Loss					
Reference	**Population** *n*, mean age (years) ± SD or range	**Study Design**	**Methodology for Fluid Collection**	**# Menstrual Cycles**	**Main Outcome**
			optical density was measured at 546 nm. - Gynaeseals were used in the third cycle. - Women with HMB measured their losses at home by aspirating the fluid from the collection chamber into a wide nozzle syringe.		Median MBL from the first two cycles (not using the gynaeseal) in the HMB group: 112.0 mL (range 83–460.0 mL)
Hurskainen et al. (1998)	*n*=156 35-49 years	A multicentre randomised controlled trial across five Finnish university hospitals	- MBL was measured using both pads and a tampons following the alkaline haematin method of Hallberg and Nilsson (1964). - Sanitary products were collected in tight plastic bags and returned after the last day of menstrual bleeding. - For MBL extraction, 5% sodium hydroxide was added, the sanitary products were squeezed, filtered and optical density was measured at 546 nm.	1	Mean MBL per cycle: 125.0 mL (range 14–724.0 mL)
Higham and Shaw (1999)	*n*=254	Prospective study	- MBL was measured using both pads and tampons following the alkaline haematin method. - 5% sodium hydroxide was added to the sanitary products, the sanitary products were squeezed, filtered and optical density was measured at 546 nm.	1–3	Median MBL for women who complained of HMB: 79.0 mL (8–616.0 mL) Median MBL for women with normal menses: 36.0 mL (2.5–288.0 mL)

Menstrual Fluid Loss					
Reference	**Population** *n*, mean age (years) ± SD or range	**Study Design**	**Methodology for Fluid Collection**	**# Menstrual Cycles**	**Main Outcome**
Reid et al. (2000)	*n*=103 16-47 years	A prospective, validation study	- MBL was measured using both pads and tampons following the alkaline haematin method of Hallberg and Nilsson (1964). - All sanitary products were put in polythene bags and returned to the clinic. - For MBL extraction, 5% sodium hydroxide was added, the sanitary products were squeezed, filtered and optical density was measured at 546 nm.	1	Mean MBL: 110.1 mL (range 10.2–389.4 mL)
Wyatt et al. (2001)	*n*=108 25-51 years	Prospective study	- MBL was measured using both pads and a tampons following the alkaline haematin method of Hallberg and Nilsson (1964). - For MBL extraction, 5% sodium hydroxide was added, the sanitary products were squeezed, filtered and optical density was measured at 546 nm.	1	Median MBL for women presenting with HMB: 68.0 mL (range 8–606.0 mL) Median MBL for the self-defined normal controls: 36.0 mL (range 4–184.0 mL) Median MBL for the HMB group: 42.0 mL (range 8–78.0 mL) Accounting for extraneous blood loss, the median total MBL in the HMB

Menstrual Fluid Loss					
Reference	**Population** *n*, mean age (years) ± SD or range	**Study Design**	**Methodology for Fluid Collection**	**# Menstrual Cycles**	**Main Outcome**
					group: 109.0 mL (range 15–836.0 mL) Accounting for extraneous blood loss, the median total MBL in the control group: 48.0 mL (range 4–211.0 mL)
Abbreviations: g, grams; HMB, Heavy Menstrual Bleeding; L, litre; MC, Menstrual Cycle; mL, millilitres; MBL, Menstrual Blood Loss; MF, Menstrual Fluid; MFL, Menstrual Fluid Loss; nm, nanometres; SD, Standard Deviation; SE, Standard Error.					

2.2.3 What We Know About Menstrual Fluid Loss

Menstrual fluid is composed of peripheral blood, cervicovaginal secretions, and endometrial tissue (Yang et al., 2012). Early research by Fraser et al. (1985) determined that blood, a component of the sample, accounted for approximately 48% of the MFL. A similar finding was reported in a more recent study by Fraser et al. (2001), with approximately 48% of MF being comprised of blood for women who were reported to have moderately heavy blood loss (>60 mL), and approximately 50% for women with excessive blood loss (>100 mL) during menstruation.

To date, there are no official clinical recommendations defining what constitutes light, moderate, or heavy MFL or MBL in grams or millilitres. However, based on the objective measurements reported by Atsuko et al. (2024), MFL has been categorised per menstrual period as follows: abnormally light (≤15.6 g); light (15.7–36.4 g); normal (36.5–96.8 g); heavy (96.9–166.4 g), and abnormally heavy (≥166.5 g). The upper normal limit of MBL is typically situated between 60–80 mL, with MBL exceeding 80 mL classified as abnormal uterine bleeding/HMB and is considered pathological (Hallberg, Hôgdahl, et al., 1966). A recent study has attempted to determine the normal limits of MFL during reproductive life using menstrual cups. Their study proposed that the upper normal limit of MFL should be the 95th percentile, which in this study was equivalent to 162.0 mL for all samples and 169.0 mL for multiparous women (Donoso et al., 2019).

Across studies, there is variation in the mean MFL reported among women. Donoso et al. (2019), Fraser et al. (1985), and Atsuko et al. (2024) reported comparable MFL means of 86.7 mL, 74.4 mL, and 77.6 g, respectively. Assuming that 1 mL of MF is equivalent to 1 g of MF, these results would suggest a good correlation between the gravimetric method and direct measurement of MF using a menstrual cup for determining MFL volumes. In contrast, Reid (2006) reported a higher mean MFL (183.5 mL), likely due to the study's focus on recruiting women who presented or were diagnosed with HMB. Meanwhile, Gudmundsdottir et al. (2009) reported a low mean MFL (51.0 g), and as previously mentioned, this may suggest that their methods, which included the conversion of dried MF to a gel, may not have been an effective way to objectively measure MFL.

Despite collecting MF, many studies have predominantly focused on defining the volume of MBL during menstruation. Interestingly, the median MBL from 372 participants in Gannon et al. (1996) was 63.0 mL, which is similar to the blood loss reported for light bleeders in Chimbira et al. (1980) (63.0 mL) and for 61 women presenting with HMB in Wyatt et al. (2001) (68.0 mL). This previous research highlights notable variability in MBL between women. It is likely that a range of lifestyle, physical, and health-related factors may contribute to both inter- and intra-individual variations in MBL and MFL that have previously been reported.

2.3 Sociodemographic and Lifestyle Factors and Menstrual Fluid Loss

2.3.1 Age

Age has been investigated as a potential factor associated with MFL, although findings remain mixed. The most common finding across studies has been that MBL and MFL do not change significantly according to the age of the participant (Atsuko et al., 2024; Cole et al., 1971; Donoso et al., 2019; Gudmundsdottir et al., 2009; Hallberg, Hôgdahl, et al., 1966; Hallberg, Högdahl, et al., 1966; Hefnawi et al., 1974; Ji et al., 1981; Ji et al., 1987; Reavey et al., 2021; Rybo, 1966a, 1966b). While other studies indicate age-related trends, particularly at the extremes of the reproductive lifespan (Hallberg, Hôgdahl, et al., 1966; Rybo, 1966a, 1983). However, a lack of adolescent representation in many studies limits a comprehensive understanding of how MFL varies across the full reproductive lifespan.

Among premenopausal women, Hallberg, Högdahl, et al. (1966) found no statistically significant differences in MBL between the age groups ≤ 25 (28.9 mL), 26–35 (35.4 mL), and 36–45 years (34.1 mL). Although there was a trend toward higher MBL in the > 45-year group (75.0 mL). However, this trend may have been influenced by two participants in this older age group presenting with HMB (114.0 mL and 153.0 mL). Regardless, the results from this study did demonstrate a shift toward higher MBL values in women in the 26–35 and 36–45 year age groups compared to the ≤ 25 year age group. The results of a recent prospective cohort study of 10,618 participants aged 18–23 years, who were followed up every 3–4 years, between 1996 to 2015, support the previous results and reported that the prevalence of HMB almost doubled over the 15-year study period as the participants moved into the 37–42 year age range (Mena et al., 2021). Similarly, in a study of 117 healthy women aged 18–50 years, Janssen et al. (1997) also reported significantly higher MBL in women aged 41–49 years (54.5 mL) compared to younger women < 36 years (31–35 years, 31.1 mL; 26–30 years, 27.0 mL; 19–25 years, 29.8 mL). Additionally, these authors noted that women with HMB were significantly older than women who did not report HMB (mean age 35.3 and 30.3 years, respectively). Similarly, Dasharathy et al. (2012) found that women with heavier MBL were significantly older than those with light MBL, while Hefnawi et al. (1974) and Higham and Shaw (1999) reported a positive, statistically insignificant association between age and MBL. Contrary to this trend, a study of 167 Japanese women aged 18–49 years observed a trend toward a lower MBL in participants within their 40s, though they too found no significant differences in menstrual flow volume by age (Atsuko et al., 2024).

At both ends of the reproductive age spectrum, studies have observed that mean MBL is lowest in the younger age groups and highest in the older age groups. While showing no statistically significant differences between the age groups and MBL, Hallberg, Hôgdahl, et al. (1966), Rybo (1966a), and Rybo (1983) reported that 15-year-old participants had the lowest mean MBL or prevalence of HMB, while 50-year-olds had the highest. However, it has been reported that these findings may have been influenced by methodological issues. For example, Hallberg, Hôgdahl, et al. (1966) noted that many 15-year-olds did not use tampons, which may have contributed to greater leakage and underestimated blood loss in that group. Physiologically, lower MBL among adolescents may be due to an immature reproductive axis, a younger gynaecological age, and low parity (Hallberg, Hôgdahl, et al., 1966; Rybo, 1983). In contrast, the higher prevalence of HMB in older women may be attributed to age-related uterine conditions such as fibroids, polyps, and endometrial hyperplasia, which are more common during the perimenopausal years and can contribute to increased MFL (Hallberg, Hôgdahl, et al., 1966; Rybo, 1983; Van Voorhis et al., 2008).

While the association between age, MBL, and MFL remains inconclusive, several studies have observed a consistent trend of increased MBL with advancing age, particularly during the perimenopausal years. Both physiological and methodological factors may contribute to these age-related differences. Therefore, further standardised research is needed to confirm these findings and better understand the association between age and MFL.

2.3.2 Ethnicity

The association between ethnicity and MFL has been scarcely researched. To the best of the author's knowledge, only one prospective cohort study, the BioCycle study, has investigated the association between ethnicity (e.g. Caucasian, Black, and Other) and MFL. Results from the BioCycle study demonstrated no significant association between MFL and ethnicity (Dasharathy et al., 2012). Additional research in this area is required to provide a holistic understanding of MFL variations between women globally.

2.3.3 Physical Activity

The association between physical activity (PA) and MFL has been scarcely researched, with existing studies focusing primarily on subjective MBL rating, self-reported HMB, and menstrual dysfunctions such as amenorrhoea rather than variations in MFL. The research conducted by both Dasharathy et al. (2012) and Hahn et al. (2013) found no significant association between the amount of vigorous PA or sedentary time and MBL or HMB. Contrary to these findings, a prospective cohort study by Mena et al. (2021) reported that the prevalence of HMB varied according to PA level, with the highest prevalence observed among women who reported no PA (19.9%), compared to those who reported low, moderate, or high PA (15.5%, 15.3% and 15.6%, respectively). Over the study's 15-year data collection and monitoring period, HMB was consistently highest in women in the lowest PA category, and lowest for those in the highest PA category. Additionally, the cohort of highly active women had 10% lower odds of experiencing HMB than those who reported no PA. However, body mass index (BMI) was a confounding factor in this relationship (Mena et al., 2021).

In contradiction to the previously discussed results, a study conducted by Bruinvels et al. (2016) using the developed 'Female Health Questionnaire' (FHQ) found that HMB was common among exercisers and elite athletes. The FHQ consisted of twelve questions, including a combination of free-text and yes/no items. Within the FHQ, HMB was identified using a four-symptom definition, where participants were considered to have HMB if they met two or more of the following criteria: 1) passing of large blood clots, 2) requiring double sanitary protection (both towels and tampons), 3) needing to change sanitary products frequently (changes every two hours or less, or using ≥12 sanitary items per menstrual period), and 4) experiencing flooding through to clothes or bedding (Fraser et al., 2015). Data were collected in two phases: first, through an online survey of 789 participants recruited via social media, and second, through face-to-face interviews with 1,073 female runners registered for the 2015 London Marathon. The results from the study suggested that 35.5% of female London Marathon runners self-reported HMB (Bruinvels et al., 2016). Runners who reported experiencing HMB were, on average, older than those who did not experience HMB (Bruinvels et al., 2016). A sub-analysis examining elite athletes from both recruitment streams found that over one-third (36.7%) met the criteria for HMB. In a further analysis of HMB and 5 km personal best times, a significant difference in HMB prevalence was observed, with faster runners being less likely to report HMB symptoms (Bruinvels et al., 2016). However, no statistically significant association was found between average weekly exercise volume and HMB status, suggesting a complex relationship that warrants further investigation.

Interestingly, similar results have been found in a recent cross-sectional study evaluating 101 high-performance Peruvian athletes, all of whom represented the Peruvian sports delegation in national and international events. All athletes participated in an online questionnaire to assess the presence of abnormal uterine bleeding (AUB) (Querevalú-Pancorbo et al., 2024). The questionnaire included items to identify AUB according to the International Federation of Gynaecology and Obstetrics (FIGO) guidelines (Munro et al., 2018), including self-reported MFL volumes, categorised as “light”, “normal”, or “heavy”. Among the athletes, 25.8% reported experiencing heavy MFL, while 69.3% and 4.9% reported normal and light MFL, respectively. Additionally, the study found that among the various parameters used to define AUB, the most frequently reported abnormalities were the presence of intermenstrual bleeding and the volume of MFL.

The finding that 25 to 36% of elite athletes may meet the criteria for HMB is somewhat surprising, given that previous literature typically focused on the prevalence of amenorrhea and oligomenorrhea in this population, which is often associated with energy imbalance and high training loads (Nattiv et al., 2007; Nazem & Ackerman, 2012). The absence of a significant association between average weekly exercise volume and the presence of HMB further complicates this understanding. Amenorrhea, defined as the absence of menses for three or more consecutive months, is most prevalent in sports that emphasise leanness, aesthetics, weight categories, or endurance, such as gymnastics, ballet, figure skating, and running (De Souza & Williams, 2004; Egan et al., 2003; Nattiv et al., 2007; Nazem & Ackerman, 2012). Rather than supporting the assumption that elite athletes are more susceptible to menstrual suppression, the findings from Bruinvels et al. (2016) and recent research suggest that they may also be vulnerable to a wider range of menstrual disturbances and AUB, including HMB. More research in this area is needed to better understand the menstrual health challenges faced by both recreational and elite athletes.

Overall, the association between PA and MFL remains unclear and inconsistent across the literature. Much of the existing research relies on self-reported experiences of HMB rather than objectively measured MFL, which limits reliability and introduces potential bias. This contributes to a significant gap in our understanding of how MFL may vary with different levels and types of PA. Moreover, methodological inconsistencies and a lack of control for potential confounding variables, such as menstrual status, further obscure this relationship. For instance, reduced MFL in highly active women may reflect menstrual suppression (e.g. amenorrhea or oligomenorrhea), rather than a direct relationship between exercise and MFL. As a result, the association between PA and MFL remains poorly defined. Future research should prioritise objective measurement methods, account for confounding factors, and consider MC status to clarify this association, particularly in active and athletic women.

2.4 Body Composition and Anthropometric and Menstrual Fluid Loss

2.4.1 Body Composition Metrics

Body composition refers to the distribution of fat, bone, muscle, and other tissues in the body (Duren et al., 2008). It can be measured using direct, criterion, and indirect methods such as anthropometry and bioelectrical impedance analysis (BIA), which estimate body composition based on the results from direct or criterion methods (Duren et al., 2008).

Research findings on the association between self-reported MBL and various physiological factors, including body composition and anthropometric metrics such as height, weight, hip size, and BMI, are mixed, with some studies identifying weak but statistically significant correlations, while others report no clear associations. Most evidence to date has explored the relationship between BMI categories and MBL, with BMI categorised as follows: underweight < 18.5 kg/m², normal weight 18.5–24.9 kg/m², overweight 25.0–29.9 kg/m², obese class I 30.0–39.9 kg/m², and obese class II for BMI > 40.0 kg/m².

Within the studies that have explored associations between BMI and MBL, Tang et al. (2020) used a menstrual pictorial-based assessment chart (PBAC) to estimate MBL and found that MBL varied significantly across BMI categories. Specifically, they reported that women with obesity had 2.28 times higher odds of having HMB compared to women with normal weight. However, the odds of higher MBL were not statistically different between overweight and normal-weight women. In contrast, underweight women had significantly lower odds of HMB in comparison to women of normal weight. Furthermore, a 15-year prospective cohort study revealed a positive relationship between BMI and HMB (Mena et al., 2021). In this research, women classified as overweight had 15% higher odds of experiencing HMB, while women with obesity had 37% higher odds compared to those in the under/normal weight BMI category. The study suggested that women with a higher BMI (> 30 kg/m²) tend to have elevated oestrogen levels due to peripheral aromatisation of androgens in adipose tissue. These unopposed and elevated levels of oestrogen are thought to contribute to increased proliferation of the endometrium, which subsequently is thought to contribute to heavier MBL (Seif et al., 2015). Additionally, research has proposed that women with obesity who have higher insulin levels, a common presentation seen in women with polycystic ovarian syndrome (PCOS), will also present with lower levels of sex hormone-binding globulin compared to women who have a normal BMI. The lower levels of sex hormone-binding globulin have been associated with increased ovarian androgen production, particularly increased testosterone. This hormonal disruption can interfere with follicle development and lead to increased rates of anovulation. In the absence of ovulation, oestrogen from the ovaries may be unopposed by progesterone, promoting excessive endometrial growth and heavier, more prolonged bleeding (Seif et al., 2015). Inference from this research would suggest that women with obesity are more likely to experience menstrual abnormalities, including AUB as defined by the updated FIGO guidelines (Munro et al., 2018).

Additional studies have further reinforced the association between BMI and MBL. Van Voorhis et al. (2008) identified that a BMI ≥ 30 kg/m² contributed to HMB, while Hahn et al. (2013) found that HMB prevalence increased consistently across higher BMI categories compared to women with a normal BMI. In this study, obesity was associated with a two-fold increase in the prevalence of HMB. Although these findings should be interpreted with caution, as the participants subjectively reported their menstrual flow based on the number of sanitary products used per cycle. Santos et al. (2011) found that, after adjusting for age and education, women with obesity had a 29% higher probability of reporting HMB compared to women with a BMI ≤ 24.9 kg/m². Similarly, Reavey et al. (2021) using a PBAC reported a weak positive association between BMI and MBL, though BMI's influence remained borderline significant when adjusting for the presence of fibroids. Subsequently, these authors proposed that their findings support a mechanism whereby obesity promotes a pro-inflammatory endometrial environment during menstruation, which may delay endometrial repair and thereby increase MBL (Reavey et al., 2021).

In comparison to these previously discussed results, Stoegererhecher et al. (2012) reported that amenorrhea (the absence of menstrual bleeding) was more common in women with a lower BMI. Within their study that investigated MBL in women using the levonorgestrel-releasing intrauterine device (LNG-IUD), women with a mean BMI of 27.6 ± 6.5 kg/m² reported a subjective increase in MBL, while those with a mean BMI of 24.4 ± 4.4 kg/m² were more likely to experience amenorrhea. Additionally, within the study, the number of women who experienced amenorrhea after LNG-IUD insertion increased with the duration of use and varied by BMI category. Within the first 6 months of LNG-IUD use, amenorrhea was scarce, with only a few cases reported, mostly among women with a normal weight and BMI. However, between six months and two years of use, the number of amenorrhoeic women increased gradually, first among those with a normal BMI, with smaller numbers seen in women who were overweight or classified in obesity class I. After two years of use, amenorrhea became more common across all BMI categories, with the most notable increases among women with a normal weight, peaking between 4.1 and 5 years of use. Women who were overweight or in the obese class I also showed rising numbers of amenorrhea in the later years of LNG-IUD use, although not to the same extent as women with normal BMI. In contrast, underweight and obese class II women consistently exhibited very low to no levels of amenorrhea throughout the entire 5-year observation period. These results suggest that while amenorrhea is common and occurs earlier in women with a normal weight using the LNG-IUD, it can also develop in women with a higher BMI, given a longer duration of LNG-IUD use.

Other studies have individually compared height and weight to MBL. Cole et al. (1971) revealed a weak but statistically significant association between height and weight and MBL. Since height remains unaffected by age and parity, while weight fluctuates, the study focused on height as a comparative factor, revealing that across all groups, short women, under 160 cm, had the lowest MBL. Even after adjusting for parity, a weak but statistically significant association between height and MBL remained. The study reported minimal differences between medium-height and tall women and their volume of MBL. Similarly, Hefnawi et al. (1979) reported statistically significant positive associations between height, weight, and body surface area with MBL, suggesting that taller and heavier women had heavier MBL. Ji et al. (1987) also observed a statistically significant, weak, positive correlation between MBL and both height and weight among their 421 participants, suggesting that women with greater height or weight may tend to have higher MBL. Similarly, Higham and Shaw (1999) also found a weak association between height and MBL; however, within their cohort of women, weight was not significantly associated with MBL.

Conversely, Hartz et al. (1979) identified a statistically significant difference in weight between women who self-reported heavy MFL. After adjusting for age and parity, the mean weight difference between the heavy and normal MFL groups remained statistically significant (3.95 kg). A weak but statistically significant association was also observed between obesity and self-reported MFL (normal versus scant flow) among younger age groups, though this relationship was not evident in older age groups, suggesting that age may confound the association between obesity and subjective MFL. Furthermore, Hartz et al. (1979) investigated the correlation between past and present weight and MFL, with findings suggesting that prior weight gain, independent of current weight, may be associated with an increased risk of HMB. In addition, the study explored individual menstrual abnormalities in relation to obesity and found a weak association between obesity and self-reported heavy MFL. While scant menstrual flow was only associated with obesity when it occurred as part of a syndrome of menstrual disorders, such as oligomenorrhea, oligohypermenorrhea, oligohypomenorrhea, polycystic ovaries, menorrhagia, or polymenorrhea.

Contrary to most findings, Atsuko et al. (2024), Donoso et al. (2019), Janssen et al. (1997), and Dasharathy et al. (2012) all found no significant association between MFL/MBL and height, weight, or BMI. These conflicting results may be partly explained by the use of self-reported anthropometric data, which can introduce reporting bias. Notably, Atsuko et al. (2024) and Janssen et al. (1997) did not specify how anthropometric measurements were obtained, leaving the reliability of their data uncertain.

While some research suggests weak but statistically significant correlations between height, weight, BMI, and MBL, other studies have found no clear associations. These inconsistencies may be partly attributed to methodological variability and the frequent reliance on self-reported menstrual data, which can compromise the accuracy and comparability of findings. Moreover, to the best of the authors' knowledge, the majority of existing research measured MBL, with most focusing predominantly on HMB. Within this research, it is noted that women with obesity are at greater risk of experiencing HMB, possibly due to hormonal imbalances, increased oestrogen levels, and endometrial dysfunction (Mena et al., 2021; Seif et al., 2015). However, the absence of research investigating MFL in relation to body composition highlights a critical gap in the literature and limits our understanding of how MFL may vary across women within the population.

Relying solely on anthropometric measures such as height, weight, and BMI may be misleading, as these metrics can be significantly altered by changes in muscle and fat mass proportions (Duren et al., 2008). Instead, methods that offer a more accurate estimation or measurement of metrics such as body fat percentage, lean body mass, fat mass, BMI, and total body weight may help improve our understanding of how body composition influences MFL. Therefore, future research is needed to investigate these associations using objective measurements of MFL and comprehensive body composition.

2.5 Menstrual Health and Menstrual Fluid Loss

2.5.1 Age of Menarche

Menarche marks a significant milestone in female reproductive development, yet its association with MFL throughout the reproductive years remains understudied and poorly understood. Existing research findings are inconsistent, and most studies have not identified statistically significant associations, leaving the association between age of menarche and MFL uncertain.

Prospective observational studies have attempted to explore the association between age of menarche and MBL, though results have been mixed. The BioCycle study by Dasharathy et al. (2012), which used validated self-report questionnaires to objectively measure MBL, found that heavier MBL was associated with marginally earlier menarche (mean 12.5 years), while lighter MBL was associated with a later menarche (mean 12.9 years). Similarly, Hefnawi et al. (1979), who objectively measured MBL in a large cohort of 745 participants aged 14–49 years, found a weak negative correlation between age of menarche and MBL. While not statistically significant, both studies suggest that earlier menarche might be associated with higher MBL later in life. Conversely, Napolitano et al. (2014), who objectively measured MFL, reported a small difference in the mean age of menarche between women with normal menses, who were marginally older than those with HMB (12.2 and 11.9 years, respectively). Similarly, Ji et al. (1981) also found no meaningful difference in mean MBL between women whose menarche occurred before or after the age of 15 years.

Interestingly, and in contrast to previous research discussed above, Rybo (1966b) reported that both early (< 12 years) and late (> 15 years) menarche were associated with higher mean MBL compared to those with a typical age of menarche between 12 and 15 years. However, it is worth noting that these differences in age of menarche and MBL were not statistically significant. These findings might suggest a non-linear relationship between age of menarche and MBL; however, this proposition remains to be investigated.

Notably, another factor related to menarche that may influence menstrual characteristics is gynaecological age, an indicator of physiological maturity. Research suggests that gynaecological age may affect MC regularity, with individuals of higher gynaecological age typically experiencing more regular cycles, potentially resulting in more consistent MFL (Harley et al., 2024; Järvelaid, 2005; Van Hooff et al., 1998). Gynaecological age (in years) is calculated by subtracting the age of menarche from the participant's age at the time of data collection. Although not directly reported by Dasharathy et al. (2012) and Napolitano et al. (2014), estimates of mean gynaecological age can be derived from their reported mean age of menarche and mean age at data collection. For instance, based on the reported mean ages in the BioCycle study (Dasharathy et al., 2012), light bleeders had a lower gynaecological age (12.7 years) than heavy bleeders (16.6 years). Similarly, using data from Napolitano et al. (2014), women with normal menstrual bleeding had a younger gynaecological age (17.7 years) than those with HMB (21.7 years). These findings may suggest that MFL may increase with advancing gynaecological age, though additional research is needed to verify this proposition.

Overall, while some studies suggest an association between earlier menarche and higher MFL, the current evidence remains inconclusive. Differences in study results may be due to differences in methodology, with the more recent studies tending to use subjective self-report of HMB presence or gravimetric methods, whereas older studies often relied on the alkaline haematin method, which estimates the quantity of MBL, a sub-component of MFL. Therefore, future research should objectively measure MFL and examine the association with age of menarche and or gynaecological age. Such research would add to the literature and provide information on whether these menstrual health variables influence total MFL.

2.5.2 Parity

Parity, defined as the number of pregnancies resulting in live or stillbirths, has been extensively studied as a factor associated with MFL. Research consistently indicates that parity is a significant factor associated with MFL (Atsuko et al., 2024; Cole et al., 1971; Dasharathy et al., 2012; Donoso et al., 2019; Hallberg, Högdahl, et al., 1966; Hefnawi et al., 1979; Higham & Shaw, 1999; Janssen et al., 1997; Santos et al., 2011). Subsequently, researchers have suggested that increased endometrial surface area and uterine volume after pregnancy may contribute to higher MFL in parous women (Atsuko et al., 2024).

Several cross-sectional studies have explored the association between parity and MFL or MBL. Santos et al. (2011) used a standardised pre-coded questionnaire to collect a subjective assessment of MFL and reported that after adjusting for age, education, and BMI, women with four or more pregnancies were almost 60% more likely to experience heavy MFL in comparison to nulliparous women. Conversely, a more recent study by Reavey et al. (2021), which used a subjective PBAC to estimate MBL, found no significant association between parity and MBL after adjusting for BMI and fibroids.

Within prospective observational studies, parity has consistently been found to be associated with MBL and MFL. In a study that used validated questionnaires to measure MBL, over two MCs, older, married, and parous women were more likely to report heavier MBL than younger, non-married nulliparous women (Dasharathy et al., 2012). In contrast, Hefnawi et al. (1979) found strong correlations between parity and MBL in all age groups, aside from the 21–25 year age group, where a weak and statistically insignificant positive correlation was reported. Another prospective observational study by Donoso et al. (2019), which directly measured MFL with a menstrual cup, found that multiparous women had significantly higher MFL than nulliparous women; however, the volume of MFL did not vary significantly with increasing number of children. Additionally, Atsuko et al. (2024) also observed that parous women had significantly heavier MFL in all age groups compared to nulliparous women. Similar to the work of Donoso et al. (2019), the authors found that additional factors related to parity (e.g. postpartum years) did not influence MFL variations among multiparous women. In contrast to much of this previous research, Gudmundsdottir et al. (2009) found no significant differences in MFL between parous and nulliparous women. Interestingly, both Ji et al. (1987) and Ji et al. (1981) also found no significant differences in MFL between parous and nulliparous women.

The lack of significant differences in MFL between parous and non-parous women observed in these three studies may be attributed to methodological limitations. For example, Gudmundsdottir et al. (2009) converted MFL into a gel before gravimetrically measuring it, which resulted in an unusually low mean MFL of 51.0 g, and may have underestimated the MFL of their participants. In contrast, the studies by Ji et al. (1987) and Ji et al. (1981) are older and likely used dated sanitary products, which may have had reduced absorption capacity or increased susceptibility to evaporation. Notably, participants in the study by Ji et al. (1981) used only sanitary pads and not tampons, which could further affect fluid collection and measurement accuracy. Given these methodological issues, the findings from these studies should be interpreted with caution.

Within the research that has found parity to be associated with MFL and MBL, age may need to be considered a confounding factor. In research that accounted for women's age at data collection, parity no longer contributed to HMB. However, when the age of the individual was not considered a confounding factor, a positive association between parity and MFL had been reported (Janssen et al., 1997). A prospective cohort study that objectively measured MBL using the alkaline hematin method found that, after adjusting for age, the association between parity and MBL was statistically significant only in women aged 25 years or younger. In this subgroup, parous women had a mean MBL of 42 mL, compared with 27.4 mL in nulliparous women (Hallberg, Högdahl, et al., 1966). A similar trend was observed in a study by Rybo (1966b), which also objectively measured MBL using the alkaline hematin method. Within their prospective cohort study, parous women had a higher mean MBL (45.5 mL) than nulliparous women (38.0 mL), although this difference was not statistically significant. However, when comparing the age groups, 23-year-old parous women had a significantly higher mean MBL (47.3 mL) than nulliparous women (33.5 mL). No significant differences in MBL between parous and nulliparous women were found in the older age-groups (30, 40, and 45 years). Additionally, Rybo (1966b) found no significant associations between MBL and factors such as childbirth weight, proximity to delivery, number of births, or age of the youngest child. Cumulatively, these results from previous research would suggest that the influence of parity on MBL may be more pronounced in younger women.

Further analysis of HMB prevalence within the study by Rybo (1966b) indicated a higher proportion of HMB among parous women (12.8%) compared to nulliparous women (9.8%), though this difference was not statistically significant. Additionally, a separate prospective cohort study by Rybo (1966a) that used a twin study design (Paper II) to explore hereditary influences on MBL found that twin pairs with differing parity statuses exhibited greater variability in MBL than twins with the same number of children. The greatest variance was observed when one twin was parous and the other nulliparous. The findings from these studies may suggest that both the number of childbirths and parity itself could influence MBL.

As with many other factors that have been found to influence MFL, differences in study design likely contribute to the inconsistent findings regarding the association between parity and MFL. The cross-sectional studies rely on subjective assessments of MFL, which are more prone to bias and less reliable than objective methods. In contrast, prospective observational studies that use direct (menstrual cup) or laboratory-based measurements (alkaline haematin technique) offer more accurate estimates, yet even among these studies, the results vary, highlighting the need for further research. Despite this, overall, the majority of studies indicate that MBL increases with parity. It is evident that age is a common confounder of the association between parity and MBL and MFL, with several studies reporting that the association weakens once age is accounted for. Therefore, adjusting for age is essential when examining the impact of parity on MFL and should be considered in future research. Furthermore, some evidence indicates that the effect of parity on MFL and MBL may be more prominent in younger women, suggesting a potential age-parity interaction. However, this proposal remains to be clarified in future research studies.

2.5.3 Contraceptives

Hormonal contraceptives (HC) such as oral contraceptives (OC) and hormonal intrauterine devices (IUDs) suppress endogenous hormone levels, which affect MFL patterns and volumes (Bradley & Gueye, 2016). These contraceptives can reduce MBL through several physiological mechanisms, including suppression of ovulation, thinning of the endometrial lining, reduced endometrial vascularity, and hormonal stabilisation (Bradley & Gueye, 2016). As a result of these effects, certain HC methods, such as combined OCs, can be used to treat HMB.

Conversely, multiple studies have found that women with non-hormonal plastic and copper IUDs may experience increased MBL and MFL. Previously, Hefnawi et al. (1974) compared MBL across different contraceptive methods and found that plastic, non-hormonal IUDs significantly increased MBL in the first year of use (mean: 78.0 mL/cycle) compared to copper IUDs (mean: 49.8 mL/cycle), OCs (mean: 20 mL/cycle), and a control group not using any form of contraception (mean: 37.0 mL/cycle). Within their study, OCs were associated with significantly lower MBL in the first year of use, and copper IUDs had a similar MBL in their first year of use compared to the control group. Cole et al. (1971) also observed similar significant differences in MBL based on contraceptive type. These authors reported that women using an IUD (coil) had a significantly higher mean MBL (56.3 mL) than those using OCs (12.7 mL). These mean values were also significantly different from the rest of the population (37.9 mL). Additionally, a cross-sectional study that subjectively assessed MFL found a higher prevalence of HMB among women not using contraception (53.9%) and those using an IUD (56.8%), whereas women using a contraceptive pill or injectable hormones were less likely to report HMB (24.5% and 22.9%, respectively) (Santos et al., 2011). Furthermore, researchers have noted that the prevalence of HMB may be higher among women not taking OCs (20.0%) compared to those who do (12.5%) (Mena et al., 2021).

Multiple studies have investigated pre- and post-IUD insertion on MBL and have reported that MBL tends to increase immediately post-insertion (Andrade et al., 1979; Guttorm, 1971; Israel et al., 1974; Liedholm et al., 1975). Guttorm (1971) measured MBL in 20 women pre- and post-plastic IUD insertion, revealing that the average MBL more than doubled post-insertion, increasing from 35.5 mL to 73.1 mL. Interestingly, the increase in MBL was found to be greater in those who had normal or scanty loss prior to the IUD insertion. Additionally, this increase in MBL appeared to persist over the first 6 months post-insertion, with all menstrual periods having a significantly higher MBL than the pre-insertion. Furthermore, Israel et al. (1974) found that various plastic and copper IUDs resulted in increased MBL at 6-, 12-, and 18-month post-insertion. Similarly, Liedholm et al. (1975) found that MBL was significantly increased post-copper IUD insertion, and remained increased for up to 12 months post-insertion. These findings suggest that the change and increase in MBL is likely to occur with non-hormonal forms of contraception, particularly plastic and copper IUDs, and may not return to the women's pre-insertion MBL levels within the first 6-12 months post-insertion.

In relation to OC use, Fraser et al. (1985) reported that women using OCs had lower overall MBL and had a significantly lower proportion of blood in their MF (17.3%) compared to those not using a form of HCs (35.5%). In contrast, participants using IUDs had a significantly higher proportion of blood in their MF (56.5%). Similar results have been reported in an earlier study, where Andrade et al. (1979) investigated the influence of OC use in women using non-hormonal IUDs. In their study, women using the combination of the OC and non-hormonal IUD had lower mean MBL at 3, 6 and 12 months after non-hormonal IUD insertion (59.7 mL, 62.6 mL and 66.6 mL, respectively) compared to the women who were not using the OC but had a non-hormonal IUD inserted (77.7 mL, 72.0 mL and 72.2 mL, respectively). While the women using the OC had lower MBL compared to those not using OCs, it was noted that all women in the study had higher MBL following the insertion of the non-hormonal IUD. These findings align with existing knowledge that the use of HCs typically reduces MFL (Bradley & Gueye, 2016).

In summary, substantial evidence indicates that MFL varies across contraceptive types, with HCs, such as OCs, generally associated with reduced MBL, whereas IUDs, particularly earlier plastic and copper models, are more frequently linked to increased MBL and a higher proportion of blood in MF. Notably, heavy MBL is often observed post-IUD insertion, with several studies demonstrating that these increases in MBL may persist for at least 6-12 months post-insertion. These findings are based on both objective and subjective methodologies, many of which were conducted between 1960 and the 1980s. The contraceptive devices used in this available research are now obsolete or infrequently used, such as the Dalkon Shield, Lippes Loop, Copper T 300, and Copper-7. These earlier devices have since been replaced with alternatives such as modern levonorgestrel IUDs (Mirena or Jaydess) or modern copper IUDs (e.g. TCu 380 Plus Normal, Choice TT380 Standard). Consequently, while historical research provides foundational insights into the association between MFL and contraception, further investigation using modern contraceptive methods is essential to provide updated information on MFL and MBL variations with hormonal and non-HC use. This will ensure relevant and applicable findings can be communicated to current populations and used within clinical practice.

2.6 Conclusion

This literature review investigated the understanding of, and methodologies used to quantify MFL over the last six decades. It has highlighted the multifaceted nature of MFL and its association with sociodemographic and lifestyle factors, body composition metrics, and menstrual health.

The review of methodologies used to quantify MFL highlights the strengths and limitations of various approaches, as well as the importance of accurate and reliable measurement techniques. Differences in reported MFL and MBL across studies may reflect variation in measurement approaches rather than true differences in MFL. After comparing methods used to determine MFL and MBL, it would seem that direct measurement techniques, such as the use of menstrual cups or gravimetric methods, are required to accurately determine MFL.

The discussion on sociodemographic and lifestyle factors suggests that age is significantly associated with MFL, while ethnicity and PA have been scarcely researched. Body composition, particularly BMI, has been shown to be positively associated with MFL. To the best of this author's knowledge, other body composition metrics such as body fat percentage, lean body mass, and fat mass and their association with MFL have not yet been investigated. Menstrual health factors, specifically parity and contraceptive use, play a significant role in influencing MFL, whereas age of menarche may not be strongly associated with MFL.

Overall, this literature review highlights the complexity of MFL and the numerous factors that contribute to the inter- and intra-individual variability. While existing research provides valuable insights, significant gaps remain in understanding the precise sociodemographic factors that are associated with variations in MFL. Confounding factors and inconsistencies in study methodologies highlight the need for more standardised and objective methods of measuring MFL, along with improved control of key confounders, to accurately assess these associations. Future studies should also aim to include more diverse populations to strengthen the reliability and generalisability of findings to more women. Addressing these gaps will support a more comprehensive understanding of menstrual health and its broader implications for women's health and well-being.

Chapter 3: Manuscript

3.1 Abstract

Background: Despite menstruation occurring regularly for reproductive-aged females, the understanding of menstrual fluid loss (MFL) volumes remains limited. This study aimed to determine the daily and total MFL in healthy menstruating women and explore associations with sociodemographic factors, body composition, and menstrual health.

Methods: Menstruating females (*n*=40; 18–45 years) from Auckland, New Zealand, participated in a prospective observational study measuring daily and total MFL using a menstrual cup or disc across one to three consecutive menstrual cycles (MC). At baseline, questionnaires captured sociodemographic and menstrual health data, and anthropometric and body composition measurements were taken. Participant characteristics were summarised descriptively, and MFL groups (light-medium and medium-heavy) were defined by the sample medium (43.5 g). Associations between MFL and participant characteristics were explored using independent samples *t*-tests, Mann-Whitney *U*, Fisher-Freeman-Halton exact tests, Friedman's *ANOVA*, and multivariate linear regression.

Results: Median MFL differed significantly across MCs and days one to eight of menstruation. The medium-heavy MFL group was found to have significantly more MFL and to subjectively rate their MFL as heavier compared to the light-medium MFL group. No other significant differences were found between the two MFL groups. No significant associations were observed between total MFL and sociodemographic ($p = 0.95$), body composition ($p = 0.98$) and menstrual health factors ($p = 0.61$).

Conclusion: Menstrual fluid loss exhibited substantial inter- and intra-individual variability. No significant predictors of MFL variability were identified. These results highlight the multifactorial nature of menstrual physiology and the need for larger, more diverse studies using objective measurement methods.

3.2 Introduction

Menstruation is a fundamental aspect of female health, yet the factors contributing to both inter- and intra-individual variability in MFL remain underexplored. This outcome is likely due to menstruation research focusing more on menstrual blood loss (MBL), a component of menstrual fluid (MF), rather than on total MFL in menstruating females. In this previous research, MBL has typically been quantified using the laboratory-based alkaline hematin method first described by Hallberg and Nilsson (1964). However, this research method for quantifying MBL is impractical for clinical or large-scale use due to its complex and time-consuming procedure, as well as the discomfort and invasiveness of storing and analysing used sanitary products (Donoso et al., 2019). Furthermore, the alkaline hematin method does not capture the full composition and total volume of MFL, which also contains cervicovaginal secretions and endometrial tissue (Yang et al., 2012).

Compared to the traditional alkaline hematin method used to estimate MBL, both the gravimetric and menstrual cup methods are simpler, faster, and less labour-intensive. These methods are relatively non-invasive and can be conducted in the privacy of the participant's home, allowing for real-time, self-directed data collection without the need for clinical oversight or handling of used menstrual products. However, gravimetric methods using single-use menstrual products (e.g. menstrual pads or tampons) carry a risk of fluid evaporation, which can compromise the accuracy and reliability of the reported MFL volumes. While this loss is considered negligible in the context of total MFL estimations (Fraser et al., 1985), it remains a limitation of this research methodology. In contrast, menstrual cups minimise the risk of fluid evaporation by directly collecting total MFL, offering a more ecologically valid measurement of total MFL (Donoso et al., 2019). Nonetheless, the use of menstrual cups only quantifies total MF and does not distinguish between the fluid's components, such as menstrual blood or cervicovaginal secretions. As a result, findings from MFL studies using menstrual cups cannot be directly compared to studies that have quantified MBL alone.

Despite the potential of menstrual cups to advance research on menstruation, the absence of established clinical thresholds or guidelines for interpreting MFL volumes highlights a significant gap in both the research literature and patient care. Notably, only one known study to date has used menstrual cups to objectively measure MFL (Donoso et al., 2019), further emphasising the need for continued investigation. To date, no studies have quantified MBL within complete samples of MFL, limiting our understanding of what constitutes normal or heavy MFL volumes and how these relate to heavy or non-heavy MBL volumes. This information could be valuable for establishing a practical measure of heavy MFL that could be used to screen for risk of iron deficiency in menstruating females.

During a female's reproductive years, MFL and MBL can vary significantly among and between individuals (Atsuko et al., 2024). Previous research has suggested that MFL may be influenced by a range of biopsychosocial factors, including lifestyle and physical characteristics such as body composition and health-related factors (Mena et al., 2021; Reavey et al., 2021). Sociodemographic factors such as advancing age may increase MFL (Dasharathy et al., 2012; Mena et al., 2021); however, factors such as physical activity (PA) present mixed findings of both positive and negative associations with MFL (Bruinvels et al., 2016; Mena et al., 2021). Research examining the association between body composition and anthropometry on MFL volumes remains mixed. While most research suggests statistically significant positive correlations between height, weight, body mass index (BMI), and MBL (Hahn et al., 2013; Mena et al., 2021; Reavey et al., 2021; Santos et al., 2011), others report no clear associations (Atsuko et al., 2024; Dasharathy et al., 2012; Donoso et al., 2019). However, there is a notable absence of studies examining the relationship between objectively measured MFL and more precise body composition metrics such as fat mass, lean body mass, or body fat percentage, highlighting an important gap in the literature. Finally, whilst parity has been reported to be positively associated with MFL (Atsuko et al., 2024; Donoso et al., 2019), age of menarche does not appear to significantly influence MFL (Ji et al., 1981; Napolitano et al., 2014). Due to the mixed findings and lack of research on MFL, further research examining biopsychosocial factors in relation to MFL volumes is required.

Therefore, the primary aim of the study was to determine the total and daily MFL in healthy menstruating women across one to three consecutive MCs using menstrual discs or cups. The secondary aim included exploring the associations between total

MFL and (a) sociodemographic and lifestyle variables, (b) body composition metrics, and (c) menstrual health factors.

3.3 Methodology

3.3.1 Study Design

This study is an observational, prospective cohort study design with data collected over one to three consecutive MCs.

3.3.2 Participants and Recruitment

Recruitment was conducted through convenience snowball sampling as part of the Female Health Research Programme at Massey University, Auckland, New Zealand. Advertisements were distributed via social media platforms (e.g. researchers' personal pages and community groups), email, flyers, and word of mouth. Potential participants completed an online screening questionnaire, and if eligible, were emailed an information sheet and invited to schedule their first laboratory visit (baseline) with the research officer on the heaviest day of their next menstrual bleed (days 2 or 3 of their MC). Eligible participants were menstruating women aged 18–45 years. All participants were assigned female sex at birth and were neither pregnant nor planning to become pregnant during the study period. The present study was approved by the Health and Disability Ethics Committee, reference number 2023 EXP 19295.

A prior power analysis using G*Power software (3.1.9.7) determined that a minimum of 36 participants was required for a repeated measures *ANOVA* with three measurements, assuming a small effect size (f = 0.25), a statistical power of 0.90, and a Type I error rate of 0.05. Accounting for 10% withdraw rate, we recruited 40 participants. Data collection was conducted between March 2024 and March 2025.

3.3.3 Data Collection

Prior to baseline data collection, each participant was sent either a Hello Period Ltd. menstrual cup or disc, with the choice of product selected based on their individual preference. At the baseline session, participants were again provided with information about the study to confirm their understanding before they provided their written informed consent for data collection. Participants then completed a baseline demographic and health questionnaire, which collected information on their age, ethnicity, parity, age of menarche, current and past contraceptive use, frequency and duration of menstrual bleeding, any diagnosed reproductive or bleeding disorders, and PA levels. Within the baseline questionnaire, the questions on reproductive status and menstrual bleeding were adapted from the Reproductive Status Questionnaire (Schmalenberger et al., 2021) and the Female Health Questionnaire (Bruinvels et al., 2016). Physical activity levels were assessed using the short form New Zealand Physical Activity Questionnaire (Moy et al., 2008) and converted into MET minutes per week (total MET) using the short form International Physical Activity Questionnaire (Craig et al., 2003). Following the completion of the questionnaire, participants had their resting blood pressure measured and body composition assessed via Bioelectrical Impedance Analysis (BIA) (InBody 230).

To conclude the baseline data collection session, participants were provided with a calibrated digital scale and an MF collection dish and were asked to estimate and report the anticipated start date of their next menstruation (e.g. their period). Prior to the onset of their next menstruation, participants were instructed to photograph the weight of the empty collection dish and their menstrual cup/disk to document the baseline weight of the dish and menstrual product. At the onset of menstruation, participants notified the research officer and began data collection. On each day where MFL was visible, and with each product change, which typically occurred twice daily (e.g. morning and evening), participants expressed the contents of the menstrual disc/cup into the collection dish. They recorded the weight of MFL using the digital scale. A photo of the weight reading was then sent to the research officer on the same day via an end-to-end encrypted messaging application. Participants were instructed to submit these photos as close to the time of measurement as possible. A research officer recorded the data daily upon receipt. Data collection for each menstruation continued until the weight of the collected MF sample returned to baseline (e.g. empty dish and product). This procedure was repeated for up to three consecutive MCs.

Each evening during data collection, participants received a short survey that asked about menstrual cup/disc leakage experienced that day. The questionnaire was adapted from the leakage questionnaire developed by Donoso et al. (2019) and was used to identify any potential MFL that could influence the accuracy of the recorded MFL volumes. Participants were asked to indicate whether leakage had occurred and, if so, to estimate the extent of MFL by selecting one of the three categories: minimal, moderate, or large. Any recorded leakage was documented accordingly during daily data collection. While MFL data was not adjusted when leakage was reported, instances of large leakage were noted in the dataset and considered in the interpretation of results.

3.3.4 Statistical Analysis

The dataset was reviewed and cleaned prior to being imported into IBM SPSS Statistics (version 30.0) for analysis. During data cleaning, responses from the MFL leakage survey were considered, and days in which participants reported substantial leakage, menstrual cup/disc drops, or malfunctions were excluded from the cleaned MFL dataset. Of the 455 total days recorded, only 21 responses (4.6%) indicated substantial leakage, suggesting that substantial leakage for participants in this study was low. The majority of responses (n=358; 78.7%) reported no leakage or minimal spotting, while the remaining responses (n=77; 17%) indicated moderate leakage, defined as the approximate volume contained in one tampon. Additionally, incomplete cycles were addressed; if a participant did not complete a full cycle, that cycle was excluded from the total cycle analyses. However, for daily MFL analyses, valid days within those incomplete cycles were retained and included in the daily MFL analysis.

Descriptive statistics, including measures of central tendency (mean and median), and variability (min, max, SD, and IQR), were calculated to summarise total MFL across valid MCs for each participant and each day of MFL. Data are presented as mean ± SD for normally distributed variables and median with IQR (25^{th}–75^{th} percentile) for non-normally distributed variables. The 95^{th} percentile, representing the upper normal limit for menstrual bleeding as proposed by Donoso et al. (2019), was calculated, and the proportion of participants with MFL exceeding this threshold was determined within the overall sample (i.e. across all recorded cycles).

A Friedman's *ANOVA* was used to examine differences in median MFL across cycles and days, which were treated as repeated, related, non-normally distributed categorical measures (median MFL per cycle/day). Post-hoc tests were not conducted for these analyses, as comparing the days and cycles would involve a large number of pairwise comparisons, increasing the risk of Type I errors. Daily differences were considered less clinically meaningful than the overall trend across the cycle.

Participants were categorised into two groups (light-medium and medium-heavy MFL) based on the sample's total median MFL. Associations between participant characteristics and MFL per group were examined. For this analysis, assumptions of normality and homogeneity of variances were assessed using the Shapiro-Wilk and Levene's tests, respectively, and parametric tests were applied only when these assumptions were met. Independent samples *t*-tests were used to assess associations between normally distributed continuous variables (age, age of menarche, gynaecological age, and body composition measures excluding BMI) and the two MFL groups. Mann-Whitney *U* tests were used to assess associations between non-normally distributed continuous variables (parity, median MFL per cycle, and BMI) and the two MFL groups. Fisher-Freeman-Halton exact tests were selected instead of the chi-square test due to low expected cell counts in some categories and were applied to categorical variables (ethnicity, PA group, HC use, and subjective MFL description) to assess associations with the two MFL groups.

Three multivariable linear regression models were developed using demographic and physiological variables collected at baseline to explore factors that contributed to variability in MFL. The independent variables included in the sociodemographic and lifestyle model (model one) were gynaecological age (current age–age of menarche), ethnicity (European and Other (including Māori, Pacific, and Asian)), and PA level, represented by Z-score total MET. Physical activity (total MET) was converted into a Z-score to improve the stability of the regression estimates, given the wide range of values within the sample. The independent variables in the body composition model (model two) included body fat percentage, while the independent variables in the menstrual health model (model three) included parity and current HC use (yes/no). Gynaecological age was selected as a primary variable over age and age of menarche because it reflects the duration of reproductive experience and physiological maturity, incorporating information from both age and menarche while avoiding multicollinearity in the regression models. To avoid multicollinearity, we chose one body composition metric from the BIA measures, body fat percentage, as it differentiates between adiposity and lean tissue. Commonly reported potential confounders in the literature, parity and gynaecological age, were included as covariates in all models to adjust for their possible influence on MFL. For each model, assumptions of linear regression (normality, linearity, homoscedasticity, and absence of multicollinearity) were assessed. For all data analyses, statistical significance was set at $p < 0.05$.

3.4 Results

3.4.1 Participant Characteristics

A total of 40 participants provided data for this study, of which four participants collected data over one MC, five participants over two MCs, and 31 participants over three MCs. Three participants withdrew from the study due to individual lifestyle reasons after completing baseline testing. Post data cleaning, a total of 455 daily MFL volumes were collected across one to three MCs. From which, 417 days and 96 cycles were analysed in the cycle analyses, and 455 days were analysed in the daily and linear regression models. Figure 3.1 provides an overview of participant data collection within the study and the MFL data processing from the initial collection through to the final dataset used in the statistical analysis.

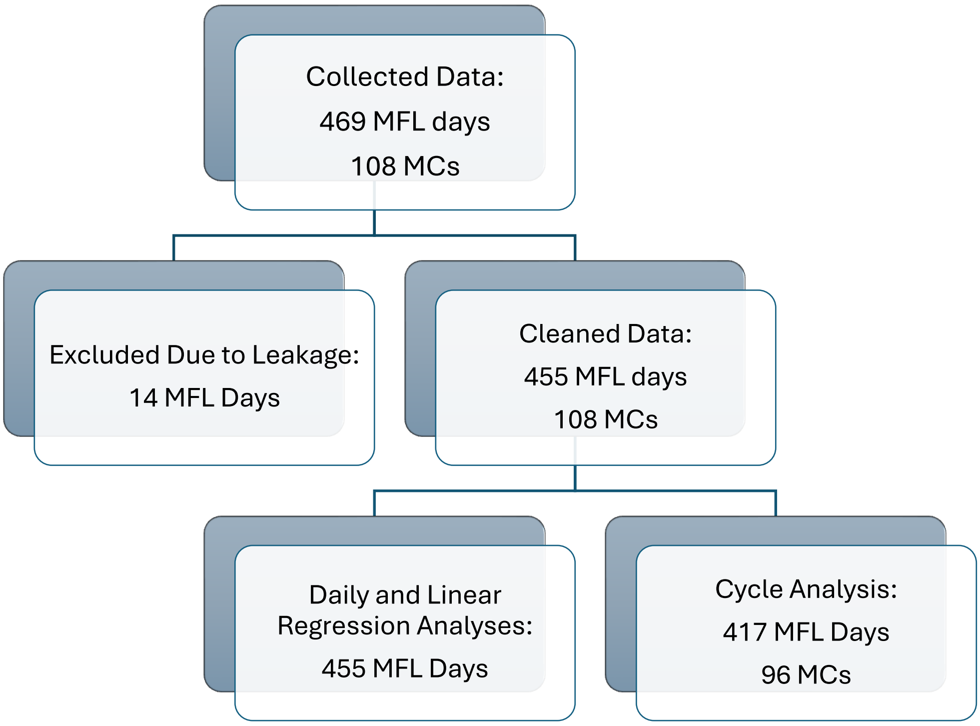

Figure 3.1 Flow Chart of Participant Data Collection and the Menstrual Fluid Loss Data Process

Abbreviations: MC, Menstrual Cycle; MFL, Menstrual Fluid Loss.

Of the 40 participants, 39 completed the baseline demographic and health questionnaire. The participant characteristics for light-medium and medium to heavy MFL groups are presented in Table 3.1. The majority of the participants identified as European and were aged between 23 and 41 years old, with a mean age of 32 ± 5.1 years. Twenty participants reported no previous pregnancies (52.6%), four were on HCs (10.3%), and over half reported high PA (61.5%). Menstrual fluid loss ranged from 0.0 g (in cases of spotting) to 122 g, with a median of 45.3 g. The 95th percentile of the MFL dataset was 167.0 g.

There was a statistically significant difference in subjective MFL description (p = 0.04) and median MFL per cycle (p < 0.001) between the two MFL groups, with more women in the medium-heavy group subjectively rating themselves as heavy, and those in the light-medium group more likely to rate themselves as light. There were no significant differences in gynaecological age (p = 0.8) or parity (p = 0.9) between the two MFL groups.

Table 3.1 Participant Characteristics by Menstrual Fluid Loss Group[1]

Variable	**Overall**	**Light-Medium**	**Medium-Heavy**	***p***
	n=40 (100%)	*n*=20 (50%)	*n*=20 (50%)	
Age *(yrs)*[a]	32.0 ± 5.1	31.4 ± 5.1	32.6 ± 5.3	0.5[d]
Min–Max	23.0 – 41.0	23 – 41	25 – 40	
Missing Data	8	4	4	
Ethnicity[b]				0.5[g]
European	27 (71.1)	14 (77.8)	13 (65.0)	
Māori	4 (10.5)	2 (11.1)	2 (10.0)	
Pacific	2 (5.3)	0 (0.0)	2 (10.0)	
Asian	5 (13.2)	2 (11.1)	3 (15.0)	
Missing Data	2	2	0	
PA Category[4b]				0.6[g]
Low	7 (17.9)	4 (21.1)	3 (15.0)	
Moderate	8 (20.5)	5 (26.3)	3 (15.0)	
High	24 (61.5)	10 (52.6)	14 (70.0)	
Missing Data	1	1	0	
Age Of Menarche[a]	12.8 ± 1.6	12.7 ± 1.5	12.8 ± 1.7	0.8[d]
Min–Max	9.0–17.0	9.0–15.0	10.0–17.0	
Missing Data	1	1	0	
Gynaecological Age[a]	19.2 ± 4.9	19.0 ± 4.9	19.5 ± 5.0	0.8[d]
Min–Max	11.0–27.5	11–27.5	13.0–27.0	
Pregnancies[c]	0.0 (0.0–3.0)	0.0 (0.0–3.0)	0.5 (0.0–2.8)	0.9[f]
Min–Max	0.0–4.0	0.0–4.0	0.0–4.0	
HC Use[b]				0.3[g]
Yes	4 (10.3)	3 (15.8)	1 (5.0)	
No	35 (89.7)	16 (84.2)	19 (95.0)	
Missing Data	1	1	0	
Median MFL Per Cycle (g)[2c]	45.3 (30.3–65.8)	30.7 (19.4–39.1)	65.5 (55.1–83.6)	< 0.001[f]
Min–Max	13.2–228.7	13.2–43.2	47.3–228.7	
Subjective MFL Description[b]				0.04[g]
Scant/Spotting	0 (0.0)	0 (0.0)	0 (0.0)	
Light	5 (12.8)	4 (21.1)	1 (5.0)	

Normal	26 (66.7)	14 (73.7)	12 (60.0)	
Heavy	8 (20.5)	1 (5.3)	7 (35.0)	
Missing Data	1	1	0	
BMI *(kg/m²)*[3c]	23.5 (21.2–27.4)	23.2 (21.2–26.2)	24.7 (21.3–29.9)	0.4[f]
Min–Max	16.9–41.1	16.9–30.3	18.9–41.1	
Body Weight *(kg)*[a]	67.2 ± 12.9	65.0 ± 10.8	69.3 ± 14.6	0.3[d]
Min–Max	45.5–102.7	45.5–83.1	52.2–102.7	
Height *(cm)*[a]	165.6 ± 5.9	166.0 ± 6.2	165.2 ± 5.7	0.7[d]
Min–Max	157.0–180.0	157.0–180.0	157.0–180.0	
BIA Measures				
Muscle Mass *(kg)*[a]	25.7 ± 3.5	25.2 ± 3.7	26.2 ± 3.3	0.4[d]
Min–Max	17.8–32.8	17.8–31.8	19.8–32.8	
Body fat percent[a]	29.3 ± 8.3	28.8 ± 6.5	29.8 ± 10.0	0.7[d]
Min–Max	14.0–50.2	19.2–44.1	14.0–50.2	

[a] Mean ± *SD*
[b] *n* (%)
[c] Median (IQR)
[d] Independent samples *t*-test
[f] Mann-Whitney *U* test
[g] Fisher-Freeman-Halton exact test

1 Based on medium total menstrual fluid loss from each cycle (45.3 g)
2 Median total menstrual fluid loss across all participants and cycles
3 BMI categories: underweight < 18.5 kg/m²; normal 18.5–24.9 kg/m²; overweight 25.0–29.9 kg/m²; obesity class I 30.0–39.9 kg/m²; obesity class II > 40.0 kg/m²
4 PA categories are defined by the IPAQ scoring method

Abbreviations: BIA, Bioelectrical Impedance Analysis; cm, centimetre; HC, hormonal contraceptive; kg, kilogram; kg/m2, kilogram per square metre; max, maximum; MC, Menstrual Cycle; min, minimum; mins, minutes; mL, millilitre; *n*, number of participants; *p*, predictive value; PA, Physical Activity; sd, standard deviation; yrs, years.

3.4.2 Menstrual Fluid Loss Overview

A Friedman's *ANOVA* (Table 3.2) showed a statistically significant difference in median MFL across MCs one, two, and three (χ^2 (1) = 768.0, p < 0.001), indicating that the median MFL differed significantly between at least two cycles.

Table 3.2 Median Menstrual Fluid Loss (g) for Menstrual Cycles One, Two, and Three[1]

Descriptive	Cycle 1 *n*=34	Cycle 2 *n*=32	Cycle 3 *n*=30	*p*[a]
Median (IQR)	46.5 (27.0–65.0)	42.5 (25.3–63.5)	47.0 (35.0–63.0)	< 0.001
Min–Max	10.0–225.1	6.0–208.1	11.0–253.0	

[a] Friedman's *ANOVA*

[1] Only complete valid cycles were used in this analysis

Abbreviations: IQR, Interquartile Range; Max, maximum; Min, minimum; *n*, number of participants; *p*, predictive value.

A second Friedman's *ANOVA* (Table 3.3) showed a statistically significant difference in median MFL across days one to eight (χ^2 (1) = 167.3, p < 0.001), indicating that the median MFL differed significantly between at least two days during menstruation. Menstrual fluid loss was consistently heavy on the second day of each MC (median: 17.7 g). The first three days of menstrual bleeding were notably heavier than the later days of menstruation, where MFL declines and typically ceases by day seven or eight.

Table 3.3 Median Daily Menstrual Fluid Loss (g)[1]

Descriptive	**Day 1** *n=40*	**Day 2** *n=40*	**Day 3** *n=39*	**Day 4** *n=35*	**Day 5** *n=25*	**Day 6** *n=11*	**Day 7** *n=5*	**Day 8** *n=1*	***p***[a]
Median (IQR)	9.0 (4.3–14.3)	17.7 (10.0–26.8)	11.0 (4.0–15.5)	5.7 (3.3–12.0)	3.0 (2.0–6.0)	2.7 (1.0–5.0)	4.0 (3.0–13.0)	2.0 (2.0–2.0)	< 0.001
Min–Max	0.0–40.7	1.0–95.6	1.7–60.7	1.0–22.7	0.0–19.2	1.0–17.0	2.0–13.0	2.0–2.0	
Missing Data	0	0	1	5	15	29	35	39	

[a] Friedman's *ANOVA*

[1] All cleaned days were used in this analysis. Calculated from the mean MFL per day per participant.

[2] The median of the mean MFL per day per participant.

Abbreviations: IQR, Interquartile Range; Max, maximum; Min, minimum; *n*, number of participants; *p,* predictive value.

3.4.3 Associations Between Menstrual Fluid Loss and Sociodemographic and Lifestyle Factors

The multivariate linear regression analysis presented in Table 3.4 determined the association between MFL and sociodemographic and lifestyle factors, including gynaecological age, ethnicity, physical activity level, and parity. The model was not statistically significant ($F(4,30) = 0.17$, $p = 0.95$) and explained only 3% of the variance in total MFL ($R^2 = 0.03$), indicating that none of the included variables contributed significantly to predicting MFL.

In this model, the model diagnostics were acceptable (residual plots, Durbin-Watson: 2.21, and variance inflation factor (VIF) mean: 1.47). There was a weak correlation between pregnancies and gynaecological age seen in the Pearson's correlation plot ($r = 0.62$, $p < 0.001$). However, the VIF indicated that the correlation was not sufficient to cause concern about multicollinearity.

Table 3.4 Multivariate Linear Regression Model of Menstrual Fluid Loss (g) and Sociodemographic and Lifestyle Factors

Predictor Variable	Coefficient (β)	SE (β)	95% CI β	Standardised β	*p*
Intercept	70.11	38.04	-8.07, 148.30		0.08
Gynaecological age	-0.60	1.89	-4.48, 3.28	-0.08	0.75
Ethnicity[1]	-3.48	15.87	-36.10, 29.15	-0.04	0.83
Z-score total MET[2]	-4.92	7.59	-20.53, 10.69	-0.14	0.52
Pregnancies	-0.57	5.83	-12.55, 11.40	-0.03	0.92

n = 31 R^2 = 0.03 Adjusted R^2 = -0.12

[1] Reference variable is European compared with other (category with Māori, Pacific and Asian).

[2] Physical activity total MET converted into a z-score.

Abbreviations: β, Beta; CI, Confidence Interval; R^2, multiple coefficients of determination; PA, Physical Activity; *p*, predictive value; SE, Standard Error.

3.4.4 Associations Between Menstrual Fluid Loss and Body Composition

The multivariate linear regression analysis presented in Table 3.5 determined the association between MFL and body composition, including gynaecological age, body fat percentage, and parity. The model was not statistically significant ($F(3,31) = 0.06$, $p = 0.98$) and explained only 1% of the variance in total MFL ($R^2 = 0.01$), indicating that none of the included variables contributed significantly to predicting MFL.

In this model, the model diagnostics were acceptable (residual plots, Durbin-Watson: 2.15, and VIF mean: 1.45).

Table 3.5 Multivariate Linear Regression Model of Menstrual Fluid Loss (g) and Body Composition

Predictor Variable	Coefficient (β)	SE (β)	95% CI β	Standardised β	*p*
Intercept	59.64	35.37	-12.81, 132.08		0.10
Body fat percent	-0.02	0.89	-1.84, 1.81	-0.01	0.99
Pregnancies	-1.24	5.34	-12.18, 9.70	-0.06	0.82
Gynaecological age	-0.22	1.69	-3.68, 3.23	-0.03	0.90

n = 32 R^2 = 0.01 Adjusted R^2 = -0.10

Abbreviations: β, Beta; CI, Confidence Interval; R^2, multiple coefficients of determination; PA, Physical Activity; *p*, predictive value; SE, Standard Error.

3.4.5 Associations Between Menstrual Fluid Loss and Menstrual Health

The multivariate linear regression analysis presented in Table 3.6 determined the association between MFL and menstrual health, including gynaecological age, parity, and HC use. The model was not statistically significant ($F(3,31) = 0.61$, $p = 0.61$) and explained only 6% of the variance in total MFL ($R^2 = 0.06$), indicating that none of the included variables contributed significantly to predicting MFL.

In this model, the model diagnostics were acceptable (residual plots, Durbin-Watson: 1.99, and VIF mean: 1.48). There was a weak correlation between pregnancies and gynaecological age across the three Pearson's correlation plots ($r= 0.60–0.62$, $p < 0.001$). However, the VIF values consistently indicated that this level of correlation was not sufficient to raise concerns about multicollinearity.

Table 3.6 Multivariate Linear Regression Model of Menstrual Fluid Loss (g) and Menstrual Health

Predictor Variable	Coefficient (β)	SE (β)	95% CI β	Standardised β	*p*
Intercept	21.11	41.04	-62.97, 105.18		0.61
Pregnancies	0.42	5.26	-10.35, 11.18	0.02	0.94
Gynaecological age	-0.85	1.70	-4.33, 2.62	-0.12	0.62
HC use[1]	25.52	19.88	-15.21, 66.24	0.25	0.21

n = 32 $R^2 = 0.06$ Adjusted $R^2 = -0.04$

[1] Reference variable is “yes” compared with “no”.

Abbreviations: β, Beta; CI, Confidence Interval; HC, hormonal contraceptive; R^2, multiple coefficients of determination; PA, Physical Activity; *p*, predictive value; SE, Standard Error.

3.5 Discussion

The present study aimed to determine daily and total MFL in menstruating women and whether variations in MFL volumes between women were associated with sociodemographic and lifestyle factors, body composition, and menstrual health. The key findings from the analyses include statistically significant differences in median MFL between consecutive MCs and across days of menstruation. Subjective MFL was significantly different between objectively classified light-medium and medium-heavy MFL groups, with more women in the medium-heavy group subjectively rating themselves as heavy, and those in the light-medium group more likely to rate themselves as light. No significant associations were observed between MFL and sociodemographic and lifestyle, body composition, and menstrual health factors.

3.5.1 Menstrual Fluid Loss Overview

The results from the present study indicated that median MFL may differ significantly between consecutive MCs and between menstruation days one to eight. Subsequently, it may be expected that in healthy and regularly menstruating women, median MFL can differ significantly between at least two consecutive MCs and between at least two menstruating days. The observed daily variation in MFL aligns with previous research that has also reported that the second day of menstruation is typically the heaviest (Atsuko et al., 2024). Cumulatively, the results from the current study and those of previous research reiterate that MFL is heaviest within the first couple of days of the MC. Following this, MFL will gradually decline thereafter, typically ceasing by day seven or eight.

Interestingly, despite daily and cycle MFL variations, the median total MFL in the present study (45.3 g) was observed to be within the normal range of MFL proposed by Atsuko et al. (2024) (36.5–96.8 g). As the current study sample comprised of healthy, regularly menstruating women, this finding of participants generally exhibiting physiologically normal menstrual bleeding, as described by previous research, is somewhat expected. Despite this, comparable studies that have used menstrual cups to measure MFL have reported higher MFL volumes than those reported in the present study. Specifically, Donoso et al. (2019) observed a median MFL of 81 mL among 28 women aged 24–49 years old. Moreover, assuming that 1 mL of MF is equivalent to 1 g of MF, studies that have used the gravimetric method to estimate MFL (Atsuko et al., 2024; Fraser et al., 1985) have also reported higher mean MFL values than those presented in the present study (74.4 mL and 77.6 g, respectively). Thus, the reported MFL volumes within previous research studies are consistently higher than those observed in the present study. However, it is worth noting that the differences observed in average MFL between studies may reflect variations in participant characteristics, such as parity or menstrual cup use experience, both of which have been shown to influence MFL (Atsuko et al., 2024; Donoso et al., 2019; Stewart et al., 2010). For example, in the present study, 48% of participants were multiparous compared with 79% in Donoso et al. (2019), who reported parity was found to be associated with greater MFL. Nonetheless, it is worth noting that there is limited research on total MFL in healthy, regularly menstruating women. Defining clinically meaningful thresholds of MFL (e.g. normal, heavy) remains an area in need of further investigation.

Despite differences in medium MFL values between the current study and previous research, heavy MFL values appear to be similar. Within the present study, the 95th percentile for MFL was 167.0 g. This result closely aligns with Donoso et al. (2019), who proposed the 95th percentile as the upper limit of normal MFL, which was 162.0 mL for their whole sample and 169.0 mL for multiparous women. These values of MFL also closely correspond to the threshold for abnormally heavy MFL as proposed by Atsuko et al. (2024) (≥ 166.5 g). Interestingly, Donoso et al. (2019) suggested that with approximately 50% of total MFL comprising of blood, the upper normal limit of MFL in their cohort (162.0 mL) would equate to around 80 mL of blood. This estimate was based on earlier work by Fraser et al. (2001), who reported that menstrual blood typically accounts for about 50% of total MFL. Furthermore, this estimated MBL value within the upper limit of MFL would then align with the traditional upper limit for normal MBL (80 mL), as proposed by Hallberg, Hôgdahl, et al. (1966). Subsequently, it may be suggested that HMB may be estimated from direct MFL. However, before this is considered to be a reliable recommendation, more research is required in larger samples of menstruating women to verify the value of the 95th percentile of MFL during menstruation. Additionally, future research needs to consider the direct quantification of MBL within whole MFL samples, which to date has not yet been completed, in research using ecologically valid methods, such as menstrual cups or discs.

A significant association was observed between participants' subjective descriptions of MFL and their objectively measured MFL group. Specifically, participants in this study who perceived their MFL as heavy generally had higher objectively measured MFL. However, the majority (66.7%) of participants described their MFL as "normal", including 60% of those in the medium-heavy MFL group. This aligns with previous research that has reported that participants may underestimate or normalise heavier MFL Hallberg, Hôgdahl, et al. (1966). Specifically, Hallberg, Hôgdahl, et al. (1966) found substantial misperception, with more than 40% of participants who exceeded the 80 mL HMB threshold describing their bleeding as "moderate" or "small". This underestimation of MFL may result from low menstrual health literacy and limited awareness of what constitutes HMB (Damian et al., 2019; Fletcher, 2023). Recent studies have reported persistent gaps in menstrual health knowledge, particularly in key clinical areas such as the threshold for HMB, of which 51.7% of women were unaware of (Fletcher, 2023). In addition, 52.5% of women misreported on the typical duration and volume of menstruation (Damian et al., 2019). Collectively, these findings may suggest that gaps or low awareness in regard to menstrual health literacy may contribute to a poor understanding or awareness of HMB. This low menstrual health literacy may perpetuate poor health-seeking behaviours when experiencing HMB, as previous research has reported that menstruating women tend to endure HMB for an average of five years before receiving effective treatment (Warner et al., 2021). However, the association between subjective MFL, objective MFL, menstrual health literacy and health-seeking behaviours requires further research to verify this proposition.

3.5.2 Associations Between Menstrual Fluid Loss and Sociodemographic and Lifestyle Factors

Previous research has demonstrated that sociodemographic and lifestyle factors such as age (Atsuko et al., 2024) and PA level (Mena et al., 2021) may be associated with total MFL. Within the present study, factors such as ethnicity and PA level explained little of the variation in MFL (total or daily) among the participants.

Specifically, within our cohort of healthy menstruating women, no significant difference in age was observed between the light-medium (31.4 years) and medium-heavy (32.6 years) MFL groups, suggesting that age was not associated with total MFL. This finding is consistent with much of the existing literature, which reports no significant age-related changes in MBL or MFL (Atsuko et al., 2024; Donoso et al., 2019). However, it is worth noting that these studies, including the present one, included participants of similar age (32–36 years old), so age-related differences may not be expected. Similarly, no significant association was found between ethnicity and MFL, which is consistent with findings from the BioCycle study (Dasharathy et al., 2012). However, the large proportion of European participants (71.1%) in the present study is a significant sampling bias that currently limits the generalisability of these results to women from diverse ethnic groups. Future MFL research should incorporate more ethnically diverse samples to thoroughly explore potential ethnic differences in MFL. Of note, within the current study, PA level also showed no significant association with total MFL. This finding is consistent with previous research, which reported no significant association between the amount of vigorous PA or sedentary time and MBL or HMB (Dasharathy et al., 2012; Hahn et al., 2013). However, these findings contrast with other studies, which have reported that the prevalence of HMB varies according to PA level (Mena et al., 2021) and that HMB is common among exercisers and elite athletes (Bruinvels et al., 2016). Interestingly, within the present study, participants who were classified as highly active (61.5%) accounted for 70% of the medium-heavy MFL group, suggesting a potential trend consistent with previous research indicating that HMB is common among regular and highly active exercisers (Bruinvels et al., 2016). Given that much of the existing literature relies on self-reported bleeding rather than objectively measured MFL, as assessed in the current study, future research may consider quantifying both PA and MFL objectively to determine the association between PA and the risk of HMB in menstruating females.

3.5.3 Associations Between Menstrual Fluid Loss and Body Composition

Analysis of body composition, including body fat percentage, revealed no significant associations with MFL. In the current study, participants' mean body fat percentage was 29.3%, with no significant difference observed between the light-medium and medium-heavy MFL groups. Moreover, the median BMI of 23.5 kg/m² also showed no significant differences between groups. However, a higher proportion of participants in the medium-heavy MFL group were classified as obese (> 30.0 kg/m^2; 25.0% vs 5.0%). This may suggest a possible trend toward higher MFL among participants with higher BMI or adiposity, although this did not reach statistical significance. Within the available literature, most existing research examining the association between body composition and MFL has focused solely on MBL and HMB, often relying on anthropometric measures alone, which do not distinguish between fat and lean mass (Duren et al., 2008). Subsequently, findings have been inconsistent, with some studies reporting a positive association between BMI categories and MBL (Mena et al., 2021; Tang et al., 2020), while others have found no clear associations (Atsuko et al., 2024; Donoso et al., 2019). The trends observed in the present study, in which a greater proportion of medium-heavy MFL participants were classified as obese, are broadly consistent with previous findings, although, as in most previous research, the association was not statistically significant. In contrast to previous literature, the present study, using body fat percentage, provides a more precise approach to examining body composition and ecologically valid MFL variations. Future research should continue to use objective measures of MFL and body composition measures (e.g. BIA or DEXA) to better understand the potential influence of adiposity and body composition on MFL across the reproductive lifespan.

3.5.4 Associations Between Menstrual Fluid Loss and Menstrual Health

Menstrual health characteristics, including parity (Donoso et al., 2019), age of menarche (Dasharathy et al., 2012), gynaecological age (Napolitano et al., 2014), and contraceptive use (Fraser et al., 1985), have been frequently associated with variations in MBL and MFL. However, findings from the present study indicate that these variables did not significantly predict MFL. Within the current study, no significant associations were observed between the number of pregnancies and total MFL or MFL group. However, findings from the present study indicate that these variables did not significantly predict MFL. Among the three regression models, menstrual health variables collectively explained the greatest proportion of variance in total MFL (6%), compared with sociodemographic and lifestyle (3%) and body composition (1%) factors. Despite this, no significant associations were observed between the number of pregnancies and total MFL or MFL group. However, the median MFL was slightly higher among parous participants (52.0 g) than among nulliparous participants (45.3 g), though substantial individual variability was observed. These findings contradict previous research that has generally indicated that parity is significantly and positively associated with MFL (Atsuko et al., 2024; Dasharathy et al., 2012). Previously, Donoso et al. (2019) reported significantly higher MFL among parous women (93.0 mL) than among nulliparous women (45.9 mL). However, it should be noted that the participant cohort in Donoso et al. (2019) included a higher proportion of multiparous participants (79%) than the present study (48%), which may partly explain the higher MFL values and the association between MFL and parity observed. The increase in MFL with parity and in multiparous women has been proposed to be due to physiological adaptations following pregnancy, such as increased uterine volume and endometrial surface area (Atsuko et al., 2024). The absence of a significant association in the present study may therefore reflect differing participant characteristics, such as the relatively large proportion of nulliparous participants in the current sample. Future MFL research should continue to consider parity when examining variations between women, to provide a more comprehensive understanding of how MFL may differ according to parity status in menstruating women.

The mean age of menarche among participants in the present study was 12.8 years and did not differ significantly between MFL groups. However, within the medium-heavy MFL group, a higher age range of menarche (10.0–17.0 years) was reported compared with the light-medium MFL group (9.0–15.0 years). Whilst not a significant finding of the current study, Dasharathy et al. (2012) and Hefnawi et al. (1979) observed a trend toward higher MBL among individuals with earlier menarche, although these associations were also not statistically significant. Overall, most existing research findings are inconsistent, leaving the association between age of menarche and MFL uncertain. Future research should further explore the potential association between age of menarche and MFL to clarify its influence on total MFL. Additionally, no significant difference was observed between gynaecological age and the MFL groups. Previously, Dasharathy et al. (2012) and Napolitano et al. (2014) reported that women with normal or light menstrual bleeding had a younger gynaecological age (12.7 and 17.7 years, respectively) compared with those experiencing HMB (16.6 and 21.7 years, respectively). These results would suggest a positive relationship between gynaecological age and MFL, particularly when women are perimenopausal and have a higher gynaecological age (Dasharathy et al., 2012; Mena et al., 2021). However, previous research has also suggested that gynaecological age influences MC regularity, with older gynaecological age associated with more regular cycles and potentially more consistent MFL (Harley et al., 2024). This would align with the results of the current study, where participants had a mean gynaecological age of 19.2 years, with similar values observed across MFL groups (light-medium: 19.0 years, medium-heavy: 19.5 years). The participants within this study may be considered to be at a relatively mature stage of reproductive life, during which menstrual patterns and MFL may be consistent. In addition, despite having a mature gynaecological age, the majority of participants were in their early thirties, and thus are not likely to have entered into perimenopause. Cumulatively, this may potentially explain the absence of a significant association between gynaecological age and MFL within this sample.

Previous research consistently indicates that HCs influence menstrual patterns, often reducing or even suppressing MFL (Bradley & Gueye, 2016), which aligns with the present study's findings of a higher proportion of HC users in the light-medium MFL group (15.8%) compared with the medium-heavy group (5.0%). In this study, four participants (10.3%) reported using HCs at the time of data collection. However, the small proportion of HC users resulted in an inability to determine a significant association between HC use and MFL variations. Substantial previous evidence also indicates that MFL varies across contraceptive types, with hormonal methods such as OCs generally associated with reduced MBL and non-hormonal IUDs more frequently associated with increased MBL and a higher proportion of blood in MF (Fraser et al., 1985; Hefnawi et al., 1974). However, the majority of the available research examining MFL in relation to contraceptive use is dated, with most studies conducted between 1971 and 1985 and only a few published in the 2000s (Cole et al., 1971; Fraser et al., 1985; Mena et al., 2021). Consequently, while historical research provides foundational insights into the association between MFL and contraception, further investigations using modern contraceptive methods and larger cohorts of participants are needed to clarify how modern hormonal and non-HCs influence MFL.

3.5.5 Conclusion

The present study is among the first to quantify total MFL using menstrual cups and discs, and to explore its associations with sociodemographic and lifestyle, body composition, and menstrual health factors. Findings revealed significant variation in MFL between and within MCs, with the heaviest bleeding consistently occurring on the second day and declining thereafter. The median total MFL observed fell within the proposed physiologically normal range, and the 95th percentile value closely aligned with previously reported upper limits of normal menstrual bleeding.

No significant associations were found between total MFL and sociodemographic and lifestyle, body composition, or menstrual health variables. While previous research suggests that factors such as age, parity, BMI, and HC use influence MBL and MFL, these associations were not evident in the present sample. These results may be due to small stratified sample sizes in each subgroup, a relatively homogenous participant cohort, a limited number of contraceptive users, and a predominance of multiparous women.

In summary, the present study contributes to the current understanding of menstrual fluid dynamics and supports the use of the menstrual cup as a valid tool for quantifying MFL. The findings underscore the substantial inter- and intra-individual variability in MFL, reflecting the complex, multifactorial nature of menstrual physiology, even though the independent variables were not significant predictors within this sample. Future research should include larger, more diverse samples (e.g. ethnic groups and ages), detailed contraceptive profiling, and consistent methodological approaches to establish reference ranges and clarify the determinants of MFL variation. Such efforts could help to improve menstrual health literacy and contribute to more individualised, evidence-based approaches to menstrual care.

Chapter 4: Conclusion and Recommendations

4.1 Overview and Achievement of Study Aims and Objectives

The present study aimed to determine the daily and total menstrual fluid loss (MFL) in menstruating New Zealand women and to examine whether MFL was associated with sociodemographic and lifestyle, body composition, and menstrual health factors.

The first objective was to quantify daily and total MFL and to assess variability during menstruation. It was hypothesised that there would be significant differences in daily MFL among menstruating women. The findings of this study support this hypothesis, revealing significant differences in median MFL across MCs and days one to eight of menstruation. Within this study, MFL peaked on day two and gradually declined until cessation by day seven or eight. A significant association was also observed between subjective MFL descriptions and light-medium and medium-heavy MFL groups, indicating that those who perceived their MFL as heavy generally had higher measured MFL.

The second objective was to explore associations between MFL and sociodemographic and lifestyle factors, including age, ethnicity, and physical activity (PA) level. It was hypothesised that total MFL would be positively associated with age, and that women with higher levels of PA would experience lower MFL than those with lower PA levels. However, the findings did not support these hypotheses, as no significant associations were observed between total MFL or MFL groups and sociodemographic and lifestyle factors, specifically age, ethnicity, and PA level.

The third objective was to explore associations between MFL and body composition using bioimpedance and anthropometric measures, including body fat percentage. It was hypothesised that total MFL would be positively associated with body fat percentage. However, the findings did not support this hypothesis, as no significant association was observed between total MFL or MFL groups and body composition, specifically body fat percentage.

The fourth objective was to examine menstrual health-related factors, including parity, gynaecological age, and hormonal contraceptives (HC). It was hypothesised that parous women would experience higher MFL than nulliparous women, and that HC users would experience significantly lower MFL than non-HC users. These hypotheses were also not supported, as no significant association was observed between total MFL or MFL groups and menstrual health factors, specifically parity, gynaecological age, and HCs.

Overall, while the present study confirmed the hypothesis that daily MFL differs significantly across menstruation, the findings did not support the proposed associations between total or group MFL and sociodemographic and lifestyle, body composition, or menstrual health factors.

4.2 Strengths and Limitations

To the best of the author's knowledge, the present study is the second study to quantify total MFL using menstrual cups/discs. Unlike most previous research, which has predominantly examined menstrual blood loss (MBL), the present study measured both the total volume of MFL and its variation across MCs, days of menstruation, and between participants. This approach enhances ecological validity by capturing menstrual fluid (MF) dynamics under natural conditions. Specifically, reusable products such as menstrual cups and discs offer a direct, reliable, and less labour-intensive alternative to traditional MFL and MBL quantification methods. They enable at-home data collection, in real-time, that is non-invasive, eliminating the need to store used sanitary products. Subsequently, this reduces the risk of fluid evaporation, which was a common limitation of gravimetric methods (Fraser et al., 1985). Using photographic evidence of scale weights, rather than MFL itself, not only improves the accuracy of reporting and data collection but also preserves participant privacy, minimises discomfort, and likely contributed to the study's low attrition rate (7.0%), reflecting strong participant retention.

Leakage of MFL is another common limitation in MFL and MBL research (Donoso et al., 2019; Wyatt et al., 2001). To address this, the present study incorporated daily MFL leakage surveys, similar to Donoso et al. (2019), providing contextual information for the interpretation of MFL results. While Wyatt et al. (2001) reported that quantifying and accounting for extraneous blood loss increased median MBL estimates by up to 60% among women presenting with heavy menstrual bleeding (HMB), MFL leakage was not quantified in the present study, as participants reported minimal leakage. Only 4.6% (*n*=21) of total responses reported substantial leakage, while the majority (*n*=358; 78.7%) reported no leakage or minimal spotting. Consequently, reported leakage was not used to adjust MFL volumes. Instead, days in which participants reported substantial leakage, device drops, or malfunctions were excluded during data cleaning to preserve data integrity. This approach prioritised data integrity by excluding compromised observations rather than subjectively estimating fluid leakage.

Although menstrual cups provide a promising advancement for measuring MFL, their wider use in research and clinical practice is constrained by the lack of standardised guidelines and reference ranges. To the best of the author's knowledge, only one other study has quantified total MFL using menstrual cups (Donoso et al., 2019). Therefore, clinical guidance for interpreting MFL measurements remains limited, as research has not yet established how MFL volumes correspond to the thresholds for HMB or MBL as quantified using traditional research methods. This lack of standardisation constrains understanding of total MFL and may contribute to low awareness among women regarding their own menstrual volumes, as subjective perception often serves as the primary reference point. This was evident in the present study, where 60% of women in the medium-heavy MFL group described their MFL as "normal". Without objective data or clinical guidelines, these perceptions may be inaccurate, potentially leading to undiagnosed abnormal uterine bleeding (AUB), including HMB and amenorrhea (Munro et al., 2018).

4.3 Recommendations

The following recommendations are informed by the study findings and the limitations acknowledged within this research.

4.3.1 Recommendations for Healthcare Professionals

- *Promote Awareness of Individual Variability in Menstrual Fluid Loss:*

The observed inter- and intra-individual MFL variability highlights the importance of recognising that MFL differs between and within individuals. Clinicians should normalise this variation when discussing menstrual health to help distinguish physiological variability from AUB, such as HMB and amenorrhea. Recognising this variability also supports the need for an individualised and holistic approach when assessing menstrual symptoms or suspected AUB, and reinforces the urgent need for improved menstrual education and support as proposed by Musulin et al. (2025).

- *Encourage Subjective Awareness of Menstrual Fluid Loss:*
  Given that the majority of participants with medium-heavy MFL in the present study perceived their MFL as "normal", clinicians should encourage women to monitor their menstrual patterns. This can be achieved through visual assessments or tracking apps, which have been shown to enhance women's health literacy, reduce stigma, and support self-management (Eschler et al., 2019; Musulin et al., 2025). Such practices can facilitate more informed discussions about MC regularity, HMB, and changes in MFL over time. This may be particularly important for women who are prone to iron deficiency. Promoting awareness and self-monitoring will not only empower women to better understand their own menstrual health but also help normalise conversations around menstruation and women's health (Musulin et al., 2025). Furthermore, research indicates that self-advocacy in healthcare leads to improved health outcomes (Ruggiano et al., 2016). Therefore, encouraging women to actively track and understand their menstruation is an important step toward improving menstrual health literacy and awareness of HMB, which is a risk factor for iron deficiency (Napolitano et al., 2014).

4.3.2 Recommendations for Future Research

- *Validation of Measurement Methods:*
  Future research is needed to validate menstrual cup measurements against traditional MFL and MBL quantification methods and to establish clinically meaningful MFL thresholds that align with existing definitions of MBL and HMB. Establishing these reference values will provide a foundation for clinical guidelines and improve diagnostic accuracy for conditions such as HMB.

- *Contraceptive Profiling:*
  Future research should quantify MFL using ecologically valid methods, such as menstrual cups or discs, among women using modern contraceptives. As most contraceptive methods assessed in the existing literature are dated, including a wider range of modern contraceptive methods would enable a more comprehensive understanding of their associations with MFL.
- *Establishment of Reference Values:*
  Large-scale, longitudinal studies on diverse populations are required to determine MFL variations across the reproductive lifespan. For example, by tracking participants' MFL in adolescent and perimenopausal women. This research would enhance the generalisability of findings and support the development of MFL reference values in relation to age.

References:

Allen, A. M., McRae-Clark, A. L., Carlson, S., Saladin, M. E., Gray, K. M., Wetherington, C. L., McKee, S. A., & Allen, S. S. (2016). Determining menstrual phase in human biobehavioral research: A review with recommendations. *Experimental and Clinical Psychopharmacology*, *24*(1), 1-11. https://doi.org/10.1037/pha0000057

American Academy of Pediatrics, Committee on Adolescence, American College of Obstetricians and Gynecologists, & Committee on Adolescent Health Care. (2006). Menstruation in girls and adolescents: Using the menstrual cycle as a vital sign. *Pediatrics*, *118*(5), 2245-2250. https://doi.org/10.1542/peds.2006-2481

Andrade, A. T. L., de Souza, O. P., Rowe, P. J., & Shaw, S. T. (1979). Effect of prior pregnancy and combined oral contraceptives on baseline menstrual blood loss and bleeding response to intrauterine devices. *Contraception*, *20*(1), 19-27. https://doi.org/10.1016/0010-7824(79)90041-6

Atsuko, S., Hiromi, T., & Hiroki, I. (2024). Normal menstrual flow volume range and its characteristics measured from sanitary napkins in Japanese women: Discrepancy between measured and subjective menstrual flow volume. *Journal of Obstetrics and Gynaecology Research*, *50*(10), 1924-1934. https://doi.org/10.1111/jog.16064

Bradley, L. D., & Gueye, N.-A. (2016). The medical management of abnormal uterine bleeding in reproductive-aged women. *American Journal of Obstetrics and Gynecology*, *214*(1), 31-44. https://doi.org/https://doi.org/10.1016/j.ajog.2015.07.044

Bruinvels, G., Burden, R., Brown, N., Richards, T., & Pedlar, C. (2016). The prevalence and impact of heavy menstrual bleeding (menorrhagia) in elite and non-elite athletes. *PloS One*, *11*(2), 1-8. https://doi.org/10.1371/journal.pone.0149881

Chimbira, T. H., Anderson, A. B., & Turnbull, A. C. (1980). Relation between measured menstrual blood loss and patient's subjective assessment of loss, duration of bleeding, number of sanitary towels used, uterine weight and endometrial surface area. *British Journal of Obstetrics and Gynaecology*, *87*(7), 603-609. https://doi.org/10.1111/j.1471-0528.1980.tb05013.x

Cole, S. K., Billewicz, W. Z., & Thomson, A. M. (1971). Sources of variation in menstrual blood loss. *BJOG: An International Journal of Obstetrics and Gynaecology*, *78*(10), 933-939. https://doi.org/10.1111/j.1471-0528.1971.tb00208.x

Craig, C. L., Marshall, A. L., SjÖStrÖM, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U. L. F., Yngve, A., Sallis, J. F., & Oja, P. (2003). International Physical Activity Questionnaire: 12-country reliability and validity. In *Medicine and Science in Sports and Exercise* (Vol. 35, pp. 1381-1395).

Damian, W., Iwona, S., Bronislawa, P., Katarzyna, K.-K., Janusz, S., Nicole, S.-W., & Miroslaw, W. (2019). Sex education in Poland – A cross-sectional study evaluating over twenty thousand polish women’s knowledge of reproductive health issues and contraceptive methods. *BMC Public Health*, *19*(1), 1-8. https://doi.org/10.1186/s12889-019-7046-0

Dasharathy, S. S., Mumford, S. L., Pollack, A. Z., Perkins, N. J., Mattison, D. R., Wactawski-Wende, J., & Schisterman, E. F. (2012). Menstrual bleeding patterns among regularly menstruating women. *American Journal of Epidemiology*, *175*(6), 536-545. https://doi.org/10.1093/aje/kwr356

De Souza, M. J., & Williams, N. I. (2004). Physiological aspects and clinical sequelae of energy deficiency and hypoestrogenism in exercising women. *Human Reproduction Update*, *10*(5), 433-448.

Diorio, J. A., & Munro, J. A. (2000). Doing harm in the name of protection: Menstruation as a topic for sex education. *Gender and Education*, *12*(3), 347-365.

Donoso, M. B., Serra, R., Rice, G. E., Gana, M. T., Rojas, C., Khoury, M., Arraztoa, J. A., Monteiro, L. J., Acuña, S., & Illanes, S. E. (2019). Normality ranges of menstrual fluid volume during reproductive life using direct quantification of menses with vaginal cups. *Gynecologic and Obstetric Investigation*, *84*(4), 390-395. https://doi.org/10.1159/000496608

Duren, D. L., Sherwood, R. J., Czerwinski, S. A., Lee, M., Choh, A. C., Siervogel, R. M., & Chumlea, W. C. (2008). Body composition methods: Comparisons and interpretation. *Journal of Diabetes Science and Technology*, *2*(6), 1139-1146. https://doi.org/10.1177/193229680800200623

Egan, E., Reilly, T., Whyte, G., Giacomoni, M., & Cable, N. T. (2003). Disorders of the menstrual cycle in elite female ice hockey players and figure skaters. *Biological Rhythm Research*, *34*(3), 251-264. https://doi.org/10.1076/brhm.34.3.251.18806

Eschler, J., Menking, A., Fox, S., & Backonja, U. (2019). Defining menstrual literacy with the aim of evaluating mobile menstrual tracking applications. *CIN - Computers Informatics Nursing*, *37*(12), 638-646. https://doi.org/10.1097/CIN.0000000000000559

Fletcher, D. (2023). *The development of a questionnaire for the assessment of menstrual health literacy amongst active females [Unpublished doctoral dissertation]* Massey University].

Fraser, I. S., Mansour, D., Breymann, C., Hoffman, C., Mezzacasa, A., & Petraglia, F. (2015). Prevalence of heavy menstrual bleeding and experiences of affected women in a European patient survey. *International Journal of Gynecology and Obstetrics*, *128*(3), 196-200. https://doi.org/https://doi.org/10.1016/j.ijgo.2014.09.027

Fraser, I. S., McCarron, G., Markham, R., & Resta, T. (1985). Blood and total fluid content of menstrual discharge. *Obstetrics and Gynecology*, *65*(2), 194-198.

Fraser, I. S., Warner, P., & Marantos, P. A. (2001). Estimating menstrual blood loss in women with normal and excessive menstrual fluid volume. *Obstetrics and Gynecology*, *98*(5), 806-814. https://doi.org/10.1016/S0029-7844(01)01581-2

Gannon, M. J., Day, P., Hammadieh, N., & Johnson, N. (1996). A new method for measuring menstrual blood loss and its use in screening women before endometrial ablation. *BJOG: An International Journal of Obstetrics and Gynaecology*, *103*(10), 1029-1033. https://doi.org/10.1111/j.1471-0528.1996.tb09556.x

Gellersen, B., & Brosens, J. J. (2014). Cyclic decidualization of the human endometrium in reproductive health and failure. *Endocrine Reviews*, *35*(6), 851-905. https://doi.org/10.1210/er.2014-1045

Gleeson, N., Devitt, M., Buggy, F., & Bonnar, J. (1993). Menstrual blood loss measurement with gynaeseal. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, *33*(1), 79-80. https://doi.org/10.1111/j.1479-828X.1993.tb02061.x

Gudmundsdottir, B. R., Hjaltalin, E. F., Bragadottir, G., Hauksson, A., Geirsson, R. T., & Onundarson, P. T. (2009). Quantification of menstrual flow by weighing protective pads in women with normal, decreased or increased menstruation. *Acta Obstetricia et Gynecologica Scandinavica*, *88*(3), 275-279. https://doi.org/10.1080/00016340802673162

Guttorm, E. (1971). Menstrual bleeding with intrauterine contraceptive devices. *Acta Obstetricia et Gynecologica Scandinavica*, *50*(1), 9-16. https://doi.org/10.3109/00016347109157279

Hahn, K. A., Wise, L. A., Riis, A. H., Mikkelsen, E. M., Rothman, K. J., Banholzer, K., & Hatch, E. E. (2013). Correlates of menstrual cycle characteristics among nulliparous Danish women. *Clinical Epidemiology*, *5*(1), 311-319. https://doi.org/10.2147/CLEP.S46712

Hallberg, L., Hôgdahl, A. M., Nilsson, L., & Rybo, G. (1966). Menstrual blood loss–A population study: Variation at different ages and attempts to define normality. *Acta Obstetricia et Gynecologica Scandinavica*, *45*(3), 320-351. https://doi.org/10.3109/00016346609158455

Hallberg, L., Högdahl, A. M., Nilsson, L., & Rybo, G. (1966). Menstrual blood loss and iron deficiency. *Acta Medica Scandinavica*, *180*(5), 639-650. https://doi.org/10.1111/j.0954-6820.1966.tb02880.x

Hallberg, L., & Nilsson, L. (1964). Determination of menstrual blood loss. *Scandinavian Journal of Clinical and Laboratory Investigation*, *16*(2), 244-248. https://doi.org/10.1080/00365516409060511

Harley, K. G., Watson, A., Robertson, S., Vitzthum, V. J., & Shea, A. (2024). Menstrual cycle characteristics of U. S. adolescents according to gynecologic age and age at menarche. *Journal of Pediatric and Adolescent Gynecology*, *37*(4), 419-425. https://doi.org/https://doi.org/10.1016/j.jpag.2024.03.005

Hartz, A. J., Barboriak, P. N., Wong, A., Katayama, K. P., & Rimm, A. A. (1979). The association of obesity with infertility and related menstrual abnormalities in women. *International Journal of Obesity*, *3*(1), 57-73.

Hefnawi, F., Askalani, H., & Zaki, K. (1974). Menstrual blood loss with copper intrauterine devices. *Contraception*, *9*(2), 133-139. https://doi.org/10.1016/0010-7824(74)90026-2

Hefnawi, F., El Zayat, A. F., & Yacout, M. M. (1979). Physiologic studies of menstrual blood loss. II. Physiologic variables affecting the magnitude of menstrual blood loss. *International Journal of Gynecology and Obstetrics*, *17*(4), 348-352.

Higham, J. M., & Shaw, R. W. (1999). Clinical associations with objective menstrual blood volume. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *82*(1), 73-76. https://doi.org/10.1016/S0301-2115(98)00224-3

Hurskainen, R., Teperi, J., Turpeinen, U., Grenman, S., Kivelä, A., Kujansuu, E., Vihko, K., Yliskoski, M., & Paavonen, J. (1998). Combined laboratory and diary method for objective assessment of menstrual blood loss. *Acta Obstetricia et Gynecologica Scandinavica*, *77*(2), 201-204.

Israel, R., Shaw Jr, S. T., & Martin, M. A. (1974). Comparative quantitation of menstrual blood loss with the Lippes Loop, Dalkon Shield, and copper T intrauterine devices. *Contraception*, *10*(1), 63-71. https://doi.org/10.1016/0010-7824(74)90133-4

Jahanfar, S., Mortazavi, J., Lapidow, A., Cu, C., Al Abosy, J., Ciana, H., Morris, K., Steinfeldt, M., Maurer, O., Bohang, J., Anjali Oberoi, R., & Ali, M. (2024). Assessing the impact of hormonal contraceptive use on menstrual health among women of reproductive age – A systematic review. *The European Journal of Contraception & Reproductive Health Care*, 1-31. https://doi.org/10.1080/13625187.2024.2373143

Janssen, C. A. H., Scholten, P. C., & Heintz, A. P. M. (1997). Menorrhagia—A search for epidemiological risk markers. *Maturitas*, *28*(1), 19-25. https://doi.org/10.1016/S0378-5122(97)00065-0

Järvelaid, M. (2005). The effect of gynecologic age, body mass index and psychosocial environment on menstrual regularity among teenaged females. *Acta Obstetricia et Gynecologica Scandinavica*, *84*(7), 645-649. https://doi.org/10.1111/j.0001-6349.2005.00372.x

Ji, G., Li-yuan, M., Su, Z., Hui-min, F., & Li-hui, H. (1981). Menstrual blood loss in healthy Chinese women. *Contraception*, *23*(6), 591-601. https://doi.org/10.1016/S0010-7824(81)80002-9

Ji, G., Su, Z., Bo-ling, S., Hui-min, F., & Li-hui, H. (1987). Menstrual blood loss and hematologic indices in healthy Chinese women. *Journal of Reproductive Medicine for the Obstetrician and Gynecologist*, *32*(11), 822-826.

Levin, R. J., & Wagner, G. (1986). Absorption of menstrual discharge by tampons inserted during menstruation: Quantitative assessment of blood and total fluid content. *British Journal of Obstetrics and Gynaecology*, *93*(7), 765-772.

Liedholm, P., Rybo, G., Sjöberg, N. O., & Sölvell, L. (1975). Copper IUD - Influence on menstrual blood loss and iron deficiency. *Contraception*, *12*(3), 317-325. https://doi.org/10.1016/0010-7824(75)90091-8

Lobo, R. A. (2022). Menopause and care of the mature woman: Endocrinology, consequences of estrogen deficiency, effects of hormone therapy, and other treatment options. In D. M. Gershenson, G. M. Lentz, F. A. Valea, & R. A. Lobo (Eds.), *Comprehensive Gynecology (Eighth Edition)* (pp. 255-288). Elsevier. https://doi.org/10.1016/B978-0-323-65399-2.00023-1

Mansfield, P. K., Voda, A., & Allison, G. (2004). Validating a pencil-and-paper measure of perimenopausal menstrual blood loss. *Women's Health Issues*, *14*(6), 242-247. https://doi.org/10.1016/j.whi.2004.07.005

Mena, G. P., Mielke, G. I., & Brown, W. J. (2021). Prospective associations between physical activity and BMI with irregular periods and heavy menstrual bleeding in a large cohort of Australian women. *Human Reproduction*, *36*(6), 1481-1491. https://doi.org/10.1093/humrep/deab055

Moy, K. L., Scragg, R. K., McLean, G., & Carr, H. (2008). The New Zealand physical activity questionnaires: Validation by heart-rate monitoring in a multiethnic population. *Journal of Physical Activity & Health*, *5*(Suppl1), S45-S61.

Munro, M. G., Critchley, H. O. D., Fraser, I. S., & Figo Menstrual Disorders, C. (2018). The two FIGO systems for normal and abnormal uterine bleeding symptoms and classification of causes of abnormal uterine bleeding in the reproductive years: 2018 revisions. *International Journal of Gynecology and Obstetrics*, *143*(3), 393-408. https://doi.org/10.1002/ijgo.12666

Musulin, C., Yeshitila, N., & Harper, J. (2025). Periods and well-being: A focus group study to discuss how menstruation affects the well-being of women aged 18-40. *Womens Health (Lond)*, *21*, 1-20. https://doi.org/10.1177/17455057251362992

Napolitano, M., Dolce, A., Celenza, G., Grandone, E., Perilli, M. G., Siragusa, S., Carta, G., Orecchioni, A., & Mariani, G. (2014). Iron-dependent erythropoiesis in women with excessive menstrual blood losses and women with normal menses. *Annals of Hematology*, *93*(4), 557-563. https://doi.org/10.1007/s00277-013-1901-3

Nattiv, A., Loucks, A. B., Manore, M. M., Sanborn, C. F., Sundgot-Borgen, J., Warren, M. P., & American College of Sports, M. (2007). American College of Sports Medicine position stand. The female athlete triad. *Medicine and Science in Sports and Exercise*, *39*(10), 1867-1882. https://doi.org/10.1249/mss.0b013e318149f111

Nazem, T. G., & Ackerman, K. E. (2012). The female athlete triad. *Sports Health*, *4*(4), 302-311. https://doi.org/10.1177/1941738112439685

Newton, J., Barnard, G., & Collins, W. (1977). A rapid method for measuring menstrual blood loss using automatic extraction. *Contraception*, *16*(3), 269-282. https://doi.org/10.1016/0010-7824(77)90026-9

Poitras, M., Shearzad, F., Qureshi, A. F., Blackburn, C., & Plamondon, H. (2024). Bloody stressed! A systematic review of the associations between adulthood psychological stress and menstrual cycle irregularity. *Neuroscience and Biobehavioral Reviews*, *163*. https://doi.org/https://doi.org/10.1016/j.neubiorev.2024.105784

Querevalú-Pancorbo, I., Rojas-Cama, L. F., Soncco-Llulluy, F., Li, J., & Rosales-Rimache, J. (2024). Abnormal uterine bleeding and associated factors: A cross-sectional study in high-performance Peruvian athletes. *BMJ Open Sport and Exercise Medicine*, *10*(2). https://doi.org/10.1136/bmjsem-2023-001820

Reavey, J. J., Walker, C., Murray, A. A., Brito-Mutunayagam, S., Sweeney, S., Nicol, M., Cambursano, A., Critchley, H. O. D., & Maybin, J. A. (2021). Obesity is associated with heavy menstruation that may be due to delayed endometrial repair. *Journal of Endocrinology*, *249*(2), 71-82. https://doi.org/10.1530/JOE-20-0446

Rees, M. C. (1991). Role of menstrual blood loss measurements in management of complaints of excessive menstrual bleeding. *British Journal of Obstetrics and Gynaecology*, *98*(3), 327-328. https://doi.org/10.1111/j.1471-0528.1991.tb13406.x

Reid, P. (2006). Assessment of menorrhagia by total menstrual fluid loss. *Journal of Obstetrics and Gynaecology*, *26*(5), 438-441. https://doi.org/10.1080/01443610600747215

Reid, P. C., Coker, A., & Coltart, R. (2000). Assessment of menstrual blood loss using a pictorial chart: a validation study. *BJOG: An International Journal of Obstetrics and Gynaecology*, *107*(3), 320-322. https://doi.org/10.1111/j.1471-0528.2000.tb13225.x

Rohatgi, A., & Dash, S. (2023). Period poverty and mental health of menstruators during COVID-19 pandemic: Lessons and implications for the future. *Frontiers in Global Women's Health*, *4*. https://doi.org/10.3389/fgwh.2023.1128169

Ruggiano, N., Whiteman, K., & Shtompel, N. (2016). "If I don't like the way I feel with a certain drug, I'll tell them.": Older adults' experiences with self-determination and health self-advocacy. *Journal of Applied Gerontology*, *35*(4), 401-420. https://doi.org/10.1177/0733464814527513

Rybo, G. (1966a). Clinical and experimental studies on menstrual blood loss. *Acta Obstetricia et Gynecologica Scandinavica*, *45*(7 S), 1-23. https://doi.org/10.3109/00016346609158476

Rybo, G. (1966b). Menstrual blood loss in relation to parity and menstrual pattern. *Acta Obstetricia et Gynecologica Scandinavica*, *45*(7 S), 25-45. https://doi.org/10.3109/00016346609158477

Rybo, G. (1983). Population studies of menorrhagia. *Research and Clinical Forums*, *5*, 77-81.

Salamonsen, L. A., Henry, P., Kovacs, G. T., Findlay, J. K., & Henry'S, P. (1999). Current concepts of the mechanisms of menstruation. *Bailliere's Best Practice and Research in Clinical Obstetrics and Gynaecology*, *13*(2), 161-179. https://doi.org/10.1053/beog.1999.0015

Santos, I. S., Minten, G. C., Valle, N. C. J., Tuerlinckx, G. C., Silva, A. B., Pereira, G. A. R., & Carriconde, J. F. (2011). Menstrual bleeding patterns: A community-based cross-sectional study among women aged 18-45 years in Southern Brazil. *BMC Women's Health*, *11*, Article 26. https://doi.org/10.1186/1472-6874-11-26

Schmalenberger, K. M., Tauseef, H. A., Barone, J. C., Owens, S. A., Lieberman, L., Jarczok, M. N., Girdler, S. S., Kiesner, J., Ditzen, B., & Eisenlohr-Moul, T. A. (2021). How to study the menstrual cycle: Practical tools and recommendations. *Psychoneuroendocrinology*, *123*, 1-30. https://doi.org/10.1016/j.psyneuen.2020.104895

Seif, M. W., Diamond, K., & Nickkho-Amiry, M. (2015). Obesity and menstrual disorders. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *29*(4), 516-527. https://doi.org/10.1016/j.bpobgyn.2014.10.010

Shaw Jr, S. T., Aaronson, D. E., & Moyer, D. L. (1972). Quantitation of menstrual blood loss - Further evaluation of the alkaline hematin method. *Contraception*, *5*(6), 497-513. https://doi.org/10.1016/0010-7824(72)90015-7

Stewart, K., Greer, R., & Powell, M. (2010). Women's experience of using the Mooncup. *Journal of Obstetrics and Gynaecology*, *30*(3), 285-287. https://doi.org/10.3109/01443610903572117

Stoegererhecher, E., Kirchengast, S., Huber, J. C., & Hartmann, B. (2012). Amenorrhea and BMI as independent determinants of patient satisfaction in LNG-IUD users: Cross-sectional study in a Central European district. *Gynecological Endocrinology*, *28*(2), 119-124. https://doi.org/10.3109/09513590.2011.588751

Sukhija, V., & Katiyar, K. (2024). Metabolic dynamics throughout the menstrual cycle: Body temperature, blood sugar, and weight. *2024 IEEE International Conference on Interdisciplinary Approaches in Technology and Management for Social Innovation (IATMSI), Interdisciplinary Approaches in Technology and Management for Social Innovation (IATMSI), 2024 IEEE International Conference on*, *2*, 1-4. https://doi.org/10.1109/IATMSI60426.2024.10502831

Tang, Y., Chen, Y., Feng, H., Zhu, C., Tong, M., & Chen, Q. (2020). Is body mass index associated with irregular menstruation: A questionnaire study? *BMC Women's Health*, *20*(1), 226. https://doi.org/10.1186/s12905-020-01085-4

UNICEF. (n.d.). *Menstrual hygiene: Gender inequality, cultural taboos and poverty can cause menstrual health needs to go unmet.* https://www.unicef.org/wash/menstrual-hygiene

Van Eijkeren, M. A., Christiaens, G., Haspels, A. A., & Sixma, J. J. (1991). Measured menstrual blood loss in women with a bleeding disorder or using oral anticoagulant therapy. *International Journal of Gynecology and Obstetrics*, *34*(2), 1261-1263. https://doi.org/10.1016/0020-7292(91)90284-C

Van Hooff, M. H. A., Kaptein, M. B. M., Schoemaker, J., Voorhorst, F. J., Hirasing, R. A., & Koppenaal, C. (1998). Relationship of the menstrual cycle pattern in 14-17 year old adolescents with gynaecological age, body mass index and historical parameters. *Human Reproduction*, *13*(8), 2252-2260. https://doi.org/10.1093/humrep/13.8.2252

Van Voorhis, B. J., Santoro, N., Harlow, S., Crawford, S. L., & Randolph, J. (2008). The relationship of bleeding patterns to daily reproductive hormones in women approaching menopause. *Obstetrics and Gynecology*, *112*(1), 101-108. https://doi.org/10.1097/AOG.0b013e31817d452b

Warner, P., Whitaker, L. H. R., Parker, R. A., Weir, C. J., Douglas, A., Hansen, C. H., Madhra, M., Hillier, S. G., Saunders, P. T. K., Iredale, J. P., Semple, S., Slayden, O. D., Walker, B. R., & Critchley, H. O. D. (2021). Low dose dexamethasone as treatment for women with heavy menstrual bleeding: A response-adaptive randomised placebo-controlled dose-finding parallel group trial (DexFEM). *eBioMedicine*, *69*. https://doi.org/10.1016/j.ebiom.2021.103434

Wyatt, K. M., Dimmock, P. W., Walker, T. J., & O'Brien, P. M. S. (2001). Determination of total menstrual blood loss. *Fertility and Sterility*, *76*(1), 125-131. https://doi.org/10.1016/S0015-0282(01)01847-7

Yang, H., Zhou, B., Prinz, M., & Siegel, D. (2012). Proteomic analysis of menstrual blood. *Molecular & Cellular Proteomics*, *11*(10), 1024-1035. https://doi.org/10.1074/mcp.M112.018390

Yi, Y., Zhang, Q., Li, J., Xie, S., Fu, J., Li, Y., & Zhao, J. (2023). The association between SARS-CoV-2 infection with menstrual characteristics changes in China: a cross-sectional study. *Journal of Psychosomatic Obstetrics & Gynecology*, *44*(1), 1-7. https://doi.org/10.1080/0167482X.2023.2238243