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Assessing Menstrual Fluid as a Diagnostic Tool for Diabetes and Cardiovascular Disease Risk in Healthy Menstruating Women

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

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Abstract

Background: Women make up roughly half the global population (49.6%), yet women's health continues to be vastly underrepresented in medical research. Prevalence of chronic conditions including type 2 diabetes (T2D) and cardiovascular disease (CVD) is increasing, particularly among reproductive aged women. Menstrual fluid (MF), a routinely available biological sample has been growing in interest regarding its use as an alternative health screening tool.

Objectives: To assess the agreement between MF and venous blood (VB) for key biomarkers associated with T2D and CVD risk in healthy menstruating women, and to explore the influence of demographic factors (age, body mass index (BMI) and ethnicity) on these biomarkers.

Methods: A cross-sectional laboratory study was conducted in 102 premenopausal participants who provided paired MF and VB samples. Biomarkers related to T2D, and CVD were measured using point-of-care testing (POCT) using the COBAS b® 101 analyser. Relative agreement between sample types was assessed using Spearman's correlation coefficients, and Bland-Altman analysis evaluated absolute agreement, bias and limits of agreement (LoA). Additionally, linear regression models were used to explore whether MF could predict the values of VB biomarkers with addition of covariates (age, BMI, and ethnicity).

Results: Findings demonstrated that several biomarkers, including glycated haemoglobin (HbA1c), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), Triglycerides (TG) and TC:HDL ratio, showed moderate to strong association between MF and VB. However, weak absolute agreement was observed between MF and VB

biomarkers measurements, and equations using MF results demonstrated limited capacity to predict VB biomarker concentrations, even after adjustment for demographic factors (R^2 = 0.09 – 0.64).

Conclusions: This research provides evidence that MF may be associated with VB across several key health biomarkers used in T2D and CVD PCOT. However, these findings do not support MF as an alternative to VB diagnostics for T2D and CVD risk screening in healthy premenopausal women. Further refinement and validation are needed across a more diverse population to fully understand MF's clinical potential.

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List of Abbreviations

Abbreviation	Meaning
CVD	Cardiovascular Disease
CRP	C-Reactive Protein
DBS	Dried Blood Spot
E2	Oestradiol
FP	Follicular Phase
FSH	Follicle-Stimulating Hormone
HbA1c	Glycated Haemoglobin
HDL	High Density Lipoprotein
LP	Luteal Phase
LH	Luteinizing Hormone
LDL	Low Density Lipoprotein
LoA	Limits of Agreement
MC	Menstrual Cycle
MCIDs	Minimal Clinical Important Differences
MF	Menstrual Fluid
MP	Menstrual Phase
OC	Oral Contraception
POCT	Point-of-care Testing
P4	Progesterone
TC	Total Cholesterol
TC:HDL ratio	Total Cholesterol to High Density Lipoprotein Ratio
T2D	Type 2 Diabetes
TG	Triglycerides

VB	Venous Blood

Terminology disclaimer:

This research acknowledges that menstruation is not experienced only by cisgender women. It includes and recognises all individuals who may experience menstruation over their lifetime, including cisgender women, transgender women, gender-diverse, and gender fluid individuals who identify as women. Sex and gender are complex and flexible terms, and menstrual health is shaped by a mix of biological, cultural and social factors. Throughout this thesis, the language used will be reflective of the terminology found in the original research being cited. For instance, if a participant in a cited study is being referred to as a woman, that terminology will be maintained when discussing the research. When other terms or language are used such as *menstruating people* or *menstruators,* those original terms will also be preserved. To help with clarity across the thesis, *females* will be used to refer to the biological capacity to menstruate, while *women* will refer to individuals who identify as women, regardless of the sex they were assigned at birth. Despite this research only being inclusive of healthy menstruating women, it considers that menstrual health is relevant to a much wider group within society, counting some transgender men and non-binary individuals. It aims to reflect a respectful and inclusive approach, even when not all experiences are included within the study.

Additionally, throughout this thesis *menstrual fluid (MF)* will be the primary term to describe the sample collected by the participants, due to accurately reflecting the full composition (blood, endometrial tissue, vaginal secretions, and cervical mucus). Previous literature has used the terms *menstrual blood* or *menstrual effluent*, however the use of these terms is inconsistent, with some studies referring specifically to the blood component and other times to the whole fluid. This study will use the term MF for clarity, where *menstrual blood* will be retained when directly citing studies that use that term.

Chapter 1: Introduction

Cardiovascular disease (CVD) and type 2 diabetes (T2D) continue to rank as the top causes of adverse health outcomes including death among women globally, yet remain underrecognised in their sex-specific presentation, screening and outcomes (Vervoort et al., 2024; Worrall-Carter et al., 2011). Despite these significant health burdens, women have historically been underrepresented in health research and clinical trials (Bierer et al., 2022). For many decades, universal differences in sex and gender have had a pronounced impact on reproductive health, morbidity and mortality. Despite this, funding for women's health research has been disproportionately low (Mirin, 2021). Subsequently, due to underrepresentation in health research, clinical healthcare decisions for women are often based solely on male-dominant health research, with limited evidence suggesting the use of data from men is appropriate for women (Tobb et al., 2022).

Male-dominated data is not appropriate for women as it inadequately considers biological sex differences, including sex specific disease progression and fluctuations in sex steroid hormones. For example, oestrogen (E2) and progesterone (P4), which can significantly influence how chronic diseases develop and progress in women, particularly in the context of lifestyle conditions like CVD and T2D. For instance, younger women (< 65 years) often experience more subtle or atypical symptoms of heart disease compared to men, such as fatigue, shortness of breath, nausea and jaw pain. Conversely, research has suggested that women over 65 years tend to present with symptoms of heart disease which are more similar to men (Keteepe-Arachi & Sharma, 2017). This highlights the importance of age specific considerations in understanding sex differences and disease presentation. Research by

Thompson (2019) found that differing symptoms for heart disease in women contribute to misdiagnosis, delays in diagnosis and treatment. These delays may sometimes last months or even years, depending on the condition and population.

Unsurprisingly, existing research indicates that women have a higher risk of morbidity from chronic lifestyle diseases like T2D and CVD (Harreiter et al., 2020). However, when these diseases progress to advanced stages, the lack of timely and early diagnosis and interventions contributes to women facing a 50% higher risk of mortality from CVD than their male counterparts (Adam et al., 2023). Furthermore, these diagnostic delays reportedly contribute to worse health outcomes in women, including increased rates of CVD, greater complications from diabetes, higher prevalence of hypertension and elevated risks of stroke and heart failure (Broni et al., 2022). To reduce mortality and morbidity risks associated with CVD and diabetes, early detection through regular health screening and monitoring is essential (Leonards & Aguilar, 2024; Weir, 2024). Previous research and healthcare professionals have emphasised the importance of early detection in improving health outcomes for individuals at risk of chronic diseases, with many healthcare systems incorporating regular screenings as part of their standard care practices to manage these diseases effectively (Weir, 2024). Across several developed countries, healthcare systems have included regular CVD and diabetes screenings into standard care, however the extent of implementation varies, including England's NHS Health Check and Denmark's population based risk score to Finland's long standing use of Finnish Diabetes Risk Score (FINDRISC) and guideline led screening in the United states, with evidence reporting improved T2D detection, CVD risk and reduced CVD events in these screened populations (Livet et al., 2024; Nieto-Martínez et al., 2023; Palladino et al., 2020; Simmons et al., 2017; Tuomilehto et al., 2023). However, sex-specific health screening for

chronic lifestyle diseases remains an area of ongoing research and development (Kautzky-Willer et al., 2023; Le Bonniec et al., 2022).

For conditions such as CVD and T2D, recommended health screening includes regular monitoring of lipid profiles (Brett & MacDougall, 2023), blood pressure and blood glucose levels (Cabalar et al., 2024). However, sex-specific health research has shown that fluctuations in sex steroid hormones, such as E2 and P4, throughout the menstrual cycle (MC) can significantly influence biomarkers commonly used in routine health screening (Gaskins et al., 2012; Vashishta et al., 2017). Key metabolic processes such as glucose metabolism, lipid profiles, inflammation and insulin sensitivity have all been reported to change over the MC (Alemany, 2021; MacGregor et al., 2021). However, current screening guidelines generally do not consider whether hormonal variations potentially undermine the reliability and interpretation of health biomarker results in women.

Preliminary research suggests that menstrual fluid (MF) could be used as a regular and alternative sample for biomarker testing, potentially aiding in the early detection of conditions like T2D and CVD (Naseri et al., 2024; Naseri et al., 2022; Tindal et al., 2024). Recent studies have shown that key biomarkers, including glycated haemoglobin (HbA1c), glucose, total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), and C-reactive protein (CRP), can be detected in MF (Naseri et al., 2023). Furthermore, Naseri et al. (2023) research found a strong agreement between MF and venous blood (VB) for HbA1c. However, for other health biomarkers such as lipid parameters, inflammatory cytokines and CRP, there is limited research suggesting agreement between MF and paired VB samples (Naseri et al., 2023). It is worth noting that much of the current research has focused on dried blood spot (DBS) collection methods (Naseri et al., 2024; Naseri et al., 2022). While

convenient for researchers, DBS analysis presents several limitations, such as inconsistent sample volumes and reduced accuracy for specific biomarkers due to drying effects (Malsagova et al., 2020; McDade et al., 2007). Therefore, more comprehensive studies investigating health biomarkers using whole, non-dried samples of MF are needed. In addition, research comparing the clinical agreement of VB and MF is needed in whole samples, in order to comprehensively understand the practical usability of MF as a health screening resource.

In summary, significant gaps remain in women's health screening, mainly due to limited sex-specific research and historical underrepresentation of female physiology in clinical studies. These gaps have contributed to inequities in the early detection and management of chronic lifestyle diseases such as T2D and CVD. However, in recent research, MF has emerged as a promising, regular and self-collected sample that may be a viable resource for health screening.

1.1 Aims

This research aims to assess the agreement between MF and VB in the measurement of biomarkers relevant to T2D and CVD in healthy menstruating women.

1.1.1 Objectives

To assess the level of agreement between MF and VB for blood biomarkers, namely HbA1c, lipid parameters (LDL, HDL, TG and TC), and CRP, using point of care (POC) analytical methods.

1.1.2 Hypotheses

It is hypothesised that:

1) HbA1c values measured from MF will show strong agreement with VB values.
2) Lipid profile and CRP values will demonstrate lower levels of agreement between MF and VB.

3) Women with higher BMI and those who are older are likely to have increased lipid levels and elevated CRP across both MF and VB samples.

1.2 Structure of Thesis

This thesis begins by discussing the significant gap in women’s health research, particularly regarding CVD and T2D, and introduces the potential of MF as a novel diagnostic medium. Chapter Two reviews the existing literature on prevalence and diagnosis of CVD and T2D in premenopausal women, an overview of the menstrual cycle (MC) and variations in specific biomarkers across the MC, as well as previous research using MF. Chapter Three describes the methods used in this study and presents the key findings of the data. The final chapter considers the implications of these findings for health professionals, researchers, and menstruating individuals, discussing the study's limitations, strengths, and suggesting directions for future research in women’s health.

1.3 Researchers Contributions

Table 1.1: Summary of Researcher's Contributions to the Study

Author	Contribution to Thesis
Mimi Bond Masters of Science Student (Nutrition and Dietetics)	Primary author of thesis Statistical Analysis Interpretation of results
Dr Kaio Vitzel Primary Supervisor Senior Lecturer, Physiology School of Health Sciences	Assisted in research design Data collection Assisted in statistical analysis Revised and approved thesis
Associate Professor Claire Badenhorst Co – Supervisor Associate Professor School of Sport, Exercise and Nutrition	Designed research Applied for ethics Participant recruitment Data collection Assisted in statistical analysis Revised and approved thesis
Karen Mumme Statistician	Assisted in data analysis Data cleaning Reviewed thesis

Chapter 2: Literature review

2.1 Introduction

Women make up nearly half of the global population (49.6%), with the female population expected to outnumber males by 2025 (Spaander, 2024). However, women also remain consistently underrepresented in the data that guides decisions in various fields, including health, politics, technology and business (Brooks & Saad, 2020; Kalaitzi et al., 2017; Smith & Sinkford, 2022). This lack of representation affects the design and development of pharmacological treatments and diagnostic tools as well as the ergonomics of transportation and fitness equipment (Havan, 2023; Younes, 2024). Traditionally, scientists have considered the female body as too complex and variable for inclusion in medical research due to fluctuating sex steroid hormonal concentrations (Nunamaker & Turner, 2023). Subsequently, women's health remains vastly under-researched, particularly in regards to sex-specific guidelines, diagnostics, treatment, and preventive care (Parry et al., 2022).

This literature review considers whether menstrual fluid (MF) could be used as a diagnostic tool for menstruating women. It discusses the biological composition of MF, its potential source of clinically relevant biomarkers, and how MF based testing compares with standard venous blood (VB) analysis. By exploring existing evidence and identifying current barriers, this review aims to clarify MF's potential role in supporting and improving women's health diagnostics.

2.2 Prevalence and Incidence of CVD and Diabetes in Women

Cardiovascular disease (CVD) continues to rank as the top cause of death for women globally, contributing to 35% of all female deaths each year, surpassing all forms of cancer combined (Kalibala et al., 2020; Woodward, 2019). Cardiovascular diseases include a range of conditions

that impact the heart and blood vessels, such as coronary heart disease, also referred to as ischemic heart disease, strokes, peripheral arterial disease and aortic disease (Al Hamid et al., 2024). In New Zealand, around 65,000 women are living with CVD, with ischemic heart disease and stroke being the most common forms (Vogel et al., 2022; WHO, 2021).

Prevalence of CVD in premenopausal women varies quite a bit depending on the population and study design, reflecting differences in study populations, disease definitions and methodology. In a cross-sectional survey of 1,622 premenopausal Canadian women aged 19-49 years, Szakun et al. (2024) found that 47% were at risk of developing CVD. Higher prevalence rates were also observed in a study of premenopausal women who were referred for coronary angiography, with 66.7% of participants diagnosed with coronary artery disease (CAD) (Sahu et al., 2023). Interestingly, a systematic review investigating CVD risk in premenopausal women on oral contraceptives (OC) showed a significant increase in traditional CVD risk factors among these individuals (Fabunmi et al., 2023). Specifically, they reported that OC users (49.5%) demonstrated a significantly higher prevalence of CVD risk factors (hypertension, dyslipidemia, obesity, T2D) compared to non-OC users (50.5%), with a pooled standardised mean difference of 0.73

Incidence studies have reported development of new CVD events over time. For instance, in a population based study in Korea with over 1.2 million premenopausal women (20-49 years), Jeong et al. (2022) reported an incidence rate for major CVD events, specifically myocardial infarction and ischemic stroke, of 1.0 per 1,000 people. Over the follow-up time of 8.3 years, a total of 10,876 incidents were reported, indicating an important but generally low risk of CVD events in the Korean population. Another study by Zhu et al. (2019), examined 177,131

premenopausal women across nine international cohort studies. Findings resulted in 0.9% of women experienced a CVD event with a mean onset age of 41.3 years

Even among the differences, the overall findings across studies remain consistent. Incidence of CVD events within the premenopausal women population tend to be low. Nonetheless, sex specific awareness of CVD risk factors is inadequate, with only 36% of premenopausal women stating they can identity risk factors, as well as 70.4% unaware CVD is the leading cause of death for women (Szakun et al., 2024). This indicates a critical gap in public health knowledge further contributing to underdiagnosis and delayed interventions in this specific population.

Globally, an estimated 199 million women are living with diabetes, with T2D ranking as the 9th leading cause of death among women worldwide (Kapur & Seshiah, 2017). Type 2 diabetes is also a well-established risk factor for CVD (Matheus et al., 2013). Prevalence of T2D among women in developed countries has risen substantially over the past three decades (Islam et al., 2023; Goodall et al., 2021). For example, between 1990 and 2019, age standardised prevelance rates of T2D in women in the United Kingdom increased by 114.6%. Even larger increases were observed in other countries, including Luxembourg (+219.2%) and Ireland (+165.7%) (Goodall et al., 2021). In Australia, prevalence rose from 2,000 to 3,400 per 100,000 people over the same time period, resulting in doubling the overall burden, despite men having greater absolute rates (Islam et al., 2023). In the United States, incidence rates of T2D per 100,000 rose from 101 to 205, whereby the largest increase was observed in reproductive age women aged 15-39 years (Luo et al., 2025). Geographically, incidence rates for T2D showed the greatest increase in high income regions of North America. However, the highest overall burden was observed in low to middle sociodemographic regions, with a prevalence of 104.44 per 100,000 people (Luo et al., 2025).

These findings suggest that among premenopausal women in developed areas, prevalence and incidences of T2D are rising. Comparing findings across studies is complex due to differences in screening methods, demographic factors, and diagnostic criteria. Several studies have not separated their data by reproductive stage. This reduces understanding of trends particularly in premenopausal women, a gap this study aims to address by focusing on healthy menstruating women in a developed country.

2.3 Diagnosis of CVD and Diabetes in New Zealand

Initial CVD diagnosis often involves an extensive clinical assessment, based on medical history and physical findings. This is typically followed by diagnostic tests such as electrocardiograms, cardiac biomarkers (lipids and C-reactive protein - CRP), and imaging techniques, for example, echocardiography and coronary angiography. However, evidence suggests that these standardised tools may be less effective in women, particularly when symptoms are due to microvascular dysfunction or non-obstructive coronary artery disease, conditions that are more prevalent in women than men (Ji & Liu, 2024).

In New Zealand, cardiovascular risk assessment is commonly conducted in primary care using the PREDICT tool, a sex and ethnicity-specific algorithm that estimates five-year CVD risk based on factors such as age, blood pressure, cholesterol levels, smoking, diabetes and family history (Ministry of Health NZ, 2020). According to the New Zealand CVD risk assessment and management guidelines, women without risk factors should begin screening at the age of 55 years. Additionally, for high-risk groups, such as Māori, Pacific, and Asian women, and those who experience serious mental health illnesses (schizophrenia, major depressive disorder, bipolar disorder), screening should begin sooner at the age of 40 years and 25 years, respectively (Ministry of Health, 2018).

Within New Zealand, diabetes is diagnosed based on fasting plasma glucose (FPG ≥ 7.0 mmol/l), glycated haemoglobin (HbA1c ≥ 50 mmol/mol), or a 2-hour post-load glucose level of ≥ 11.1 mmol/l during an oral glucose tolerance test (OGTT) (NZSSD, 2021). However, the most commonly used diagnostic tool is the HbA1c measurement, which reflects the individual's average blood glucose levels over the past two to three months. An HbA1c level of 50 mmol/mol or higher on two separate tests indicates diabetes (New Zealand Diabetes, 2024). Screening for diabetes in asymptomatic women is recommended for all individuals over 55 years. Within New Zealand, women considered to be at risk of T2D include those who are of non-European ethnicity, have a history of prediabetes or T2D, who are a first-degree relative of an individual with T2D diagnosed < 40 years, who have associated health conditions such as polycystic ovary syndrome (PCOS), hypertension, or dyslipidemia, or a history of CVD, post-transplant, smoking and long-term corticosteroid or antipsychotic therapy. Similar to CVD screening guidelines, T2D screening for these at-risk women is recommended from 40 years of age (NZSSD, 2021). Additionally, women with a BMI ≥ 25 kg/m^2 (or ≥ 23kg/m^2 for Asian ethnicity) and who present with at least one additional risk factor should take part in T2D risk-based screening every 3 years.

Lipid thresholds are relatively identical between the sexes, with the exception of high-density lipoprotein (HDL). Previous research has noted physiological differences between men and women that influence lipid metabolism and CVD risk, however current guidelines still apply the same targets for both sexes. For lipids, premenopausal women generally present with higher HDL compared with men (Ahmed et al., 2016; Haeckel et al., 2023). Table 2.1 shows that these sex-specific lipid targets are only applied to HDL, whereas the rest of the lipid parameters remain uniform between the sexes.

Table 2.1: Fasting Lipid Targets by Sex

Lipid Marker	Target for Women	Target for Men
TC	< 5.0 mmol/l	< 5.0 mmol/l
LDL	< 3.0 mmol/l	< 3.0 mmol/l
HDL	>1.3 mmol/l	> 1.0 mmol/l
TG	< 1.7 mmol/l	< 1.7 mmol/l
TC:HDL Ratio	< 5.0	< 5.0

Values from Sundjaja et al., 2020; Ahmed et al., 2016. TC = Total Cholesterol; LDL = Low-Density Lipoprotein; HDL = High-Density Lipoprotein; TC = Triglycerides

In contrast, HbA1c thresholds remain the same between sexes. Recent evidence indicates that current diagnostic thresholds for T2D presented in Table 2.2 may not adequately reflect sex-specific differences, particularly in premenopausal women. A sizeable study based in United Kingdom (n = 1.1 million) explored sex differences in HbA1c distribution across two cohorts. Results found that participants younger than 50-years, lower HbA1c levels were consistently observed in women compared to men of the same age, with a mean difference of 1.6 mmol/mol (Stedman et al., 2025). Interestingly, another recent study estimated that approximately 35,000 additional women under 50 years of age in England and Wales could be recategorised as having diabetes if sex-specific thresholds were applied to diagnostic criteria (46 mmol/mol for women and 48 mmol/mol for men) (Holland et al., 2024). Reasons may include menstruation, whereby the red blood cells overall lifespan limits how long haemoglobin is exposed to glucose. Less exposure to glucose may reflect the differing values between menstruating people and those who do not menstruate (Holland et al., 2024). The findings highlight a critical gap in current diagnostic practice, specifically the identical HbA1c threshold may not adequately capture early T2D onset in premenopausal women.

Table 2.2: HbA1c thresholds by sex

Glycemic status	HbA1c target for women	HbA1c target for men
Non-diabetes	< 41 mmol/mol	< 41 mmol/mol
Prediabetes	41- 49 mmol/mol	41- 49 mmol/mol
Diabetes	≥ 50 mmol/mol	≥ 50 mmol/mol

Values from Florkowski et al., 2014. HbA1c = glycated haemoglobin

This is similar to CRP which continues to be interpreted using uniform thresholds across both sexes, presented in Table 2.3. C-reactive protein (CRP) levels may vary between men and women. Greater CRP levels have been reported in premenopausal women compared to men (3.3 vs. 1.8 mg/l; $p < 0.001$), with CRP levels differing across ethnic groups (Khera et al., 2005). For instance, higher CRP levels have been reported in African American women compared to Caucasian ethnicity, suggesting inflammatory profiles may be influenced by demographic factors (Kelley-Hedgepeth et al., 2008; Nagar et al., 2021). More current findings from Ireri (2022) report similar patterns, whereby females present with meaningfully higher median CRP concentrations than males (11.7 mg/L vs. 4.4 mg/L, $p < 0.01$). Combined, these findings acknowledge the importance of sex and demographic consideration when assessing inflammatory markers.

Table 2.3: CRP thresholds by sex

CRP levels	CRP target for women	CRP target for men
Healthy baseline	< 0.3 mg/dL	< 0.3 mg/dL
Normal – Minor Inflammation	0.3 to 1.0 mg/dL	0.3 to 1.0 mg/dL
Moderate inflammation	1.0 to 10.0 mg/dL	1.0 to 10.0 mg/dL
Severe inflammation	≥ 50.0 mg/dL	≥ 50.00 mg/dL

Values from Singh et al., 2025. CRP = C-Reactive Protein

2.4 Barriers to health screening for premenopausal women

Various barriers to healthcare and regular screening for women have been reported (Le Bonniec et al., 2022). These barriers can be roughly grouped into structural, social, cultural, personal and psychological factors. Structural barriers include economic and geographical constraints that hinder women's access to regular healthcare. Although New Zealand has a publicly funded healthcare system, out of pocket healthcare expenses can still create notable challenges for many individuals. Examples include the financial burden of regular doctor visits and the high costs of diagnostic tests, which often prevent women from accessing routine healthcare (Tobb et al., 2022). Previous research has shown that being female, particularly a Māori woman, was

a significant predictor for an individual to experience cost related challenges to primary healthcare access (Jeffreys et al., 2024). Women living in rural and remote areas may face geographical limitations, where access to specialised healthcare services is restricted and long travel time may create further barriers to regular healthcare access (Lilley et al., 2019). Systemic issues in healthcare, such as poor accessibility for women with disabilities and the lack of inclusive care for ethnic and sexual minorities, are additional barriers to regular healthcare access and screening for those population groups (Le Bonniec et al., 2022; Worrall-Carter et al., 2011). Women with disabilities may struggle with inaccessible clinics or untrained providers, while ethnic minorities face language barriers, cultural insensitivity, and medical bias. LGBTQ+ individuals often encounter discrimination and limited gender affirming care, which cumulatively will discourage preventive healthcare behaviors (Nowaskie & Menez, 2024; Soled et al., 2022).

Women's health care engagement is highly influenced by social and cultural disparities. In some cultures, traditional gender roles prioritise family responsibilities over personal health. As a result, women often delay seeking medical care, focusing on childcare responsibilities and the needs of others before their own (Craig & Mullan, 2011). Cultural expectations and social stigma surrounding certain health issues can lead to embarrassment and discourage participation in healthcare, particularly in communities where discussing topics like menstruation is considered taboo (Olson et al., 2022). Additionally, Western healthcare systems may conflict with cultural beliefs around health and illness. For example, some women may have preferences for female healthcare providers, rely on traditional medicine, or feel stigmatized when discussing reproductive and metabolic health (Kropi et al., 2024; Riaz et al., 2021). Language barriers can also restrict an understanding of medical advice and navigation

of health services, especially for immigrant and refugee populations (O'Lawrence et al., 2023; Pandey et al., 2021).

Personal and psychological factors further heighten these challenges. Many women have a low perceived risk of illness and may lack awareness about the importance of health screenings (Kling et al., 2013). This may be worsened by low health literacy and high levels of fear and misconception about screening that can both contribute to lower rates of health screening (Poon et al., 2023). Additionally, fear of diagnosis can discourage some women from seeking primary healthcare (Le Bonniec et al., 2022). The invasive nature of some screening procedures, along with limited privacy and feelings of vulnerability further hinder women's participation in health screenings (Butler et al., 2022; Nagendiram et al., 2020). For instance, gynecological exams like pap smears and pelvic exams cause discomfort, embarrassment, or anxiety (Deffieux et al., 2023), particularly for trauma survivors (Gorfinkel et al., 2021; Iraola et al., 2024). Additionally, breast screenings, such as mammograms, can be exposing or physically uncomfortable for many patients (Montoro et al., 2023). Women continue to face systemic barriers to accessing healthcare, which can reduce engagement in routine screening. Addressing these barriers is therefore an important step towards improving equity in preventive healthcare.

2.5 Overview of Menstruation

The menstrual cycle (MC) refers to the regular, cyclical changes in a woman's reproductive system, characterised by the fluctuations in sex steroid hormones, estrogen (E2) and progesterone (P4). Commonly described as a biphasic cycle, it broadly consists of two phases, the follicular (proliferative) phase (FP) dominated by E2 and the luteal (secretory) phase (LP), dominated by P4, set apart by ovulation (Draper et al., 2018). These hormonal changes are regulated by the hypothalamus and driven by gonadotrophic hormones, follicle-stimulating hormone (FSH) and luteinising hormone (LH), produced by the anterior pituitary gland. Follicle-stimulating hormone stimulates the growth and maturation of ovarian follicles, which promotes E2 production. A LH surge triggers ovulation and supports the formation of the corpus luteum, which secretes P4 and E2. Collectively, these two hormones regulate key reproductive processes, including the growth and maturation of the endometrium for implantation. The down regulation of both these hormones at the end of the MC is a key factor leading to the breakdown and shedding of the secretory phase endometrial lining during menstruation (Thiyagarajan et al., 2024). The MC begins at menarche (first menstrual bleed), which typically occurs between the ages of 10 and 16 years, with the median age of menarche being 12.25 years in developed countries (Lacroix et al., 2017).

A regular MC can range from 21 to 35 days (Cabre et al., 2024), with the start of each cycle marked by the shedding of the endometrial lining and commencement of menstruation. During menstruation, inflammatory signals including cytokines, prostaglandins and immune cells, induce constriction of spiral arteries, which supply the upper (functional) layer of the endometrium (Maybin & Critchley, 2015). The reduced blood flow to the endometrium contributes to local cell death, and eventually, the spiral arteries rupture, filling the endometrial tissue with peripheral blood (Chaudhry & Chaudhry, 2018). This bleeding plays an active role

in dislodging the functional endometrial layer from the basal layer (Tindal et al., 2024). As shedding of the functional endometrial layer progresses, continued bleeding occurs from the exposed blood vessels left behind, which is ultimately released during menstruation as MF (Cousins et al., 2022). Throughout this process of menstruation, the basal layer of the endometrium remains intact. This layer of the endometrium contains stem/progenitor cells that drive regeneration of the functional layer during the subsequent MC (Cousins et al., 2022).

Most women who experience a natural cycle typically find menstruation lasts 3-7 days (Najmabadi et al., 2020). A study by Bull et al. (2019) with over 600,000 MCs reported an average bleed length of 4 ± 1.5 days. The heaviest bleeding usually occurs around day two of the cycle, with an average blood loss of around 33 ml. The total menstrual blood loss across the entire cycle varies widely, commonly ranging from 10 to 84 ml (Itriyeva, 2022; Mihm et al., 2011). Dasharathy et al. (2012) similarly reported that women experience heavier MF loss during their first three days of menstruation. In a separate study, median blood loss during menstruation was 59.3 ml (± 25.1) with individual losses ranging from 25 ml to 110 ml (Li et al., 2011). Over a lifetime, the average woman experiences approximately 400 menstrual cycles, producing MF each time they menstruate (Tyson et al., 2024). Notably, MF patterns tend to change with age. For example, after 35 years, the bleeding duration typically shortens by half a day, while women around 50 years lose 6 ml more blood on average than younger women (Harlow, 2000). Research suggests that differences in MC characteristics, such as FP and LP length, bleeding duration, and blood volume, may be influenced by regional, ethnic, or socioeconomic factors (Harlow, 2000). However, more research is needed to confirm these findings.

Despite inter-individual differences in MF volumes, MF may be considered a regularly obtainable biological sample that remains largely unexplored for medical diagnostics and is accessible throughout much of a woman's reproductive life (Naseri et al., 2022). Given MF is regular and available to women for around four decades, the biological sample had potential to enhance health screening practices by offering an alternative to traditional VB sampling.

2.6 Health biomarker measurement during menstruation

Currently, there is no recommended time point for VB biomarker collection for women within the natural MC. Samples for the assessment of health biomarkers are typically obtained at any phase of the MC, with no consideration of how hormonal variations, ovulation or menstruation may influence results (Ramsey et al., 2016). Evidence states certain biomarker concentrations can vary throughout the MC, such as lipids and CRP (Barnett et al., 2024; Wilson et al., 2025), however literature exploring health biomarker measurements from VB specifically during menstruation remains limited. Additionally, understanding on biomarker levels collected from VB during menstruation are particularly of interest for this research, due to being the only point at which MF can be collected. Together, the existing literature suggests that the menstrual phase (MP) may influence VB biomarker levels, yet studies specifically measuring HbA1c, lipids, or CRP during menstruation are scarce, representing a critical gap in research.

In addition, women on hormonal contraceptives who experience a withdrawal bleed may show increases in certain biomarkers. This is because synthetic hormones alter endogenous endocrine function and hepatic metabolism. For example, oestrogen-containing contraceptives can stimulate liver protein synthesis, leading to elevated levels of some circulating biomarkers compared to naturally cycling individuals (Bandenhorst et al., 2023). Research suggests that OCs consistently raise CRP in both healthy women and those with PCOS, as well as increasing

certain lipid parameters such as triglycerides (TG) and HDL (Bieglmayer et al., 1995; Collomp et al., 2025; Larsen et al., 2020; Wang et al., 2016). Other changes that women may encounter using OCs include reduced menstrual blood loss and alterations in the hormonal and biochemical environment, leading to changes in MF composition (Lethaby et al., 2019). Specifically, in OC using individuals, it has been observed that menstrual blood loss is reduced, and the endometrial lining tends to be thinner, leading to less tissue and blood being shed (Zaheer et al., 2024) . For example, Larsen et al. (2020) reported that menstrual blood loss decreased significantly after starting OCs, from 60.2 + 5.6 ml (range 22-116 ml) before use to 36.5 + 5.2 ml (range 7-80 ml) and 33.7 + 4.1 ml (range 5-70 ml) after 3 to 6 months, respectively. Additionally, this study also noted that the reduction in MF was most apparent during the first two days of menstruation. Understanding the differences in MF composition between naturally menstruating women and those using OCs is essential, as variations in volume and content can directly affect whole blood analysis and influence how samples are collected and tested. Furthermore, these differences also reflect physiological variability, highlighting the importance of being aware of intra-individual variation, especially when interpreting results for clinical application.

2.6.1 HbA1c measured during menstruation

Glycated haemoglobin (HbA1c) is a key biomarker utilised in clinical practice, providing an important measure to diagnose diabetes (Sherwani et al., 2016). It reflects the glycemic control over the past 2-3 months and captures glucose levels across approximately three MCs (Naseri et al., 2022; Sherwani et al., 2016). Several studies have explored short-term glycemic biomarkers across the MC in the FP and LPs. However, few studies have collected HbA1c in the early follicular also known as the menstruation phase of the cycle, while menstrual bleeding

is present. For example, MacGregor et al. (2023) conducted a cross-sectional observation study examining metabolic biomarkers in a large cohort of 8,694 premenopausal women. In this study, minimal blood glucose variations were reported between women and HbA1c measurements were not reported. However, VB sampling was standardised to a three specific points in the MC (menstruation, FP and LP) based on participants' self-reported onset of last menstruation. Conversely, in a prospective observation study, Lin et al. (2023) monitored 49 healthy premenopausal women using continuous glucose monitoring over 79 days, capturing a total of 149 cycles. They observed biphasic patterns in daily median blood glucose levels, with the lowest levels reported during the late FP and the highest during the LP. However, within this study, blood glucose during the early FP was not reported. Similarly, in a comparative observational study, Rani et al. (2013) investigated variations in blood glucose across the MC in 50 healthy women aged 18-22 years in India. Blood glucose levels were found to be lower during the MP compared with the week preceding menstruation. Although in a similar manner to previous research, this study primarily measured blood glucose and did not measure HbA1c. Despite differences in study design and biomarker measurements, these studies demonstrate short-term glycemic fluctuations across the cycle, with lower blood glucose levels reported during menstruation. However, there is a lack of evidence on HbA1c measurements in VB, specifically during menstruation. Given that HbA1c reflects long-term glycemic exposure over the lifespan of red blood cells, it is unlikely to vary substantially across the MC.

2.6.2 Lipid parameters measured during menstruation

Lipid parameters such as TC, LDL, HDL, and TG are commonly assessed for CVD risk as dyslipidemia is a major modifiable risk factor for CVD events (Islam et al., 2023).

Methods and results from prior research that have measured VB lipid levels during menstruation are presented in Table 2.4. A large study conducted by MacGregor et al. (2023),

with 8,694 menstruating women, observed across the MC that TC:HDL ratio varied non-linearly, there was also evidence of elevated lipid levels observed in VB samples taken while participants were experiencing menstruation. Similarly, Barnett et al. (2024) reported that in 48 women aged 18-36 years, LDL and TC were higher during menstruation. Furthermore, HDL remained stable, and the LDL:HDL ratio declined. These results also align with Mumford et al. (2010), who found that among 259 women aged 18-44 years, more participants exceeded recommended LDL (> 130 mg/dL) or TC (> 200 mg/dL) thresholds during menstruation compared to other time points in the MC. In addition, these researchers observed that TC, TG, and LDL were all higher during menstruation compared to the LP, whilst HDL remained unchanged regardless of whether it was measured during menstruation or the LP. Even in studies that have collected blood samples in the later stage of the MP (8th day of the cycle) in 20 women, increases in LDL were observed (Hasan and Khalifa, 2023). At this later time point in the MP, increases in TC and TG were noted, but they were not statistically significant, and again, no change in HDL during menstruation was observed (Hasan and Khalifa, 2023). Finally, in a smaller sample of eight healthy women, Jensen et al. (2017) reported LDL and TC levels to be relatively similar between the phases, without mention of the MP specifically.

Collectively, these studies suggest that in VB samples collected while a woman may be experiencing menstruation, lipids, specifically, TC and LDL, may be higher than at other timepoints in the cycle (MacGregor et al., 2023; Barnett et al., 2004; Mumford et al., 2010; Hansan & Khalifa, 2023; Jensen et al., 2017). The magnitude of this increase in lipid levels may vary depending on the exact day of collection during menstruation. For example, VB lipid levels may be higher in the earlier days of the cycle versus one week after menstruation has commenced. In VB samples, HDL levels appear largely unaffected by the MP, and variations in TG levels are not consistent across studies (Barnett et al. 2024; Mumford et al., 2010; Hasan

and Khalifa, 2023). Both TC and LDL may show a predictable rise during menstruation, which is important to consider when using these biomarkers for diabetes and CVD risk assessments. Results from this research may suggest that having a standardised time point in the MC for VB lipid biomarker assessment could allow for improved accuracy in lipid results and reporting in premenopausal women.

Table 2.4: Lipid parameters in venous blood during menstruation

Author	Sample size	Age (Mean ± SD, range)	Timing of sample collection	Key results
Barnett et al., 2004	44	26.8 ± 4.1 (18-36)	- FP (days 3-9) - LP (days 10-12 after OvuStick colour change)	- LDL and TC ↑ start of cycle (menstruation) - LDL: ↓ 6.2% in LP ($P = 0.015$) - Total: HDL cholesterol ratio: ↓ 5.1% in LP ($P = 0.0006$) - LDL:HDL cholesterol ratio: ↓ 8.4% in LP ($P = 0.002$) - TC: slight decrease in LP (not always significant) - HDL: minimal change across cycle (variation smaller than LDL)
Mumford et al., 2010	259	27.3 ± 4.9 (18-44)	- 8 clinic visits per cycle across two MCs - Menses - Mid follicular - Late follicular - Ovulation - Early luteal - Mid luteal - Late luteal	- TC: ↓ during luteal phase vs follicular phase ($P < 0.001$) - LDL:↓ during luteal phase vs follicular phase ($P < 0.001$) - HDL: Highest around ovulation ($P < 0.001$) - More women exceeded LDL > 130mg/dl or TC > 200 mg/dl during menstruation; TC, TG, LDL ↑ compared to LP, HDL stable
Gupta et al., 2015	120	22 ± 2.5 (17-27)	- Menstruation (days 1-3 of cycle)	- HDL moderate, not as high as LP - LDL and TC slightly higher during MP - TG relatively lower during MP

				- TC:HDL, TG/HDL and LDL/HDL ratios remained within normal ranges but less favorable during menstruation - All changes significant ($p < 0.001$) -
Jensen et al., 2017	8	Mean ± SD not reported (21-40)	- 20 blood samples collected from each participant during 4 different physiological states - Natural MC (n = 7 samples) - Mid follicular (days 7-9) - Late follicular (1-3 days after high fertility) - Ovulation (0-2 days post LH surge) - Early luteal (3-6 days post LH surge) - Mid-luteal (7-9 days) - Late luteal (10-14 days) - Menes (days 1-3) - OC cycle (n = 3 samples) - Menopause (n = 3 samples)	TC very similar between phases - FP - 134.1 mg/dl - LP - 134.0 mg/dl LDL very similar between phases - FP - 80.3 mg/dl - LP - 80.2 mg/dl HDL lower during FP - FP: 44.9 mg/d - LP - 44.9 mg/dl TC higher in FP - FP - 45.3 mg/dl - LP - 41.3 mg/dl

VaShiShta, Gahlot, and Goyal. 2017	111	Mean ± SD not reported (15-45)	- FP (10th day) - LP (22nd day)	- TC and LDL ↑ during FP - HDL moderately lower in FP - TG and VLDL slightly ↑ in FP - Significant decreases in luteal vs. follicular phase: - TC ↓, $p = 0.006$ - LDL ↓, $p = 0.004$ - TC:HDL-C ratio ↓, $p = 0.006$ - LDL/HDL ratio ↓, $p = 0.01$ - TG/HDL ratio ↓, $p = 0.02$ - Non-significant changes: - TG ↓, $p = 0.18$ - HDL↑, $p = 0.16$ - VLDL↓, $p = 0.17$
Hasan & Khalifa, 2023	30 (15 group 1, 15 group 2)	Group 1: 22.5 ± 1.5 (20-25) Group 2: 42.5 ± 1.5 (40-45)	- 8th day (FP) - 22nd day (LP)	- LDL significantly ↑ on 8th day ($p \leq 0.05$) - TC and TG not significantly ↑ - HDL stable
MacGregor et al., 2023	8,694	44.9 ± 2.8	Self-reported cycle days: - Menstruation (0.00-0.18; day 1-5) - Follicular (0.18-0.54; to ~day 15) - Luteal (0.54-1.00; ~day 16 to cycle end)	- TC (phase 0.25) and LDL (0.21) peak in early follicular/menstruation - TC ranged from 5.32 mmol/l in the LP to 5.44 mmol/l in the FP (EDF 3.4, $p < 0.001$) - HDL moderately elevated in menstruation, peaks at ovulation (0.46)

				- FP (1.59 mmol/l) compared with the LP (1.53 mmol/l, EDF 2.9, $p < 0.001$) - TG: 1.18 mmol/l in the LP to 1.24 mmol/l in the FP, variation not statistically significant (EDF 4.0, $p = 0.066$)

TC = Total Cholesterol; LDL = Low-Density Lipoprotein; HDL = High-Density Lipoprotein; TG = Triglycerides; VLDL = Very Low-Density Lipoprotein; FP = Follicular Phase; LP = Luteal Phase; MP = Menstrual Phase; MC = Menstrual Cycle; EDF = Estimated Degrees of Freedom; OC = Oral Contraceptive

2.6.3 CRP measured during menstruation

C-reactive protein (CRP) is a well-established acute phase protein produced by the liver and is widely recognized as a key biomarker of inflammation and infection. Circulating CRP concentrations reflect levels of systemic inflammation and commonly measured in clinical and research settings. Elevated CRP levels have been consistently associated with increased CVD risk, making CRP a common measurement utilised in CVD risk assessment (Banait et al., 2022). The methods and results from previous research that have measured CRP during menstruation are presented in Table 2.5.

The current evidence would suggest that CRP levels may be higher during menstruation. For example, Wilson et al. (2025) conducted an observational study measuring urinary CRP in 98 women (76 Polish, 22 Polish American) and found that CRP measured in urine was elevated during the first three days of menstruation before declining throughout the rest of the MC. Within this study, population differences were observed, with higher levels of CRP observed in Polish American participants. Such results suggest that there may be population differences in MP CRP levels, however, this remains to be explored. Similarly, Gaskins et al. (2012) reported a significant increase in serum CRP during early menstruation (days 1-3) in 259 women aged 18-44 years, with 12.3% of women classified as having CRP > 3mg/l, although there was considerable intra-individual variation. Vashisthta et al. (2017) observed a comparable pattern in women aged 15-45 years with CRP levels found to be significantly higher during menstruation (mean 1.88 ± 1.49 mg/l) compared to LP (1.54 ± 1.30 mg/l). Finally, in a smaller study, Wander et al. (2008) measured CRP in eight healthy women across 11 MCs and noted a non-significant 17% increase in CRP levels during menstruation.

Prior research exploring CRP fluctuations across the MC have used varied biological samples and study designs. For instance, Gaskins et al. (2012) completed a prospective cohort study on 259 women with an age range between 18-44 years measuring serum CRP, compared to Wilson et al. (2025) whereby urinary CRP was measured in 98 women (76 Polish, 22 Polish American). Even with differences in study methods such as sample size, type of biological sample (urine and serum), and timing of collection, similar findings were reported with elevated CRP during the early MP (days 1-3), with some variations between groups. The majority of studies collected data over a single cycle, however in the longitudinal Bio Cycle Study (Gaskins et al., 2012), elevations in CRP during menstruation were still present, indicating a consistent physiological pattern seen during the MP where CRP increases.

Altogether, these findings suggest that collection of CRP in VB samples during menstruation may potentially be elevated compared to other time points in the MC. This is notably evident in the early phase (days 1-3) of the cycle. Therefore, it’s important to consider these fluctuations when interpreting biomarkers for diabetes and CVD risk in premenopausal women.

Table 2.5: CRP in urine and venous blood during menstruation

Author	Sample size	Age in years (Mean ± SD, range)	Timing of sample collection	Key results
Blum et al., 2005	17 (8 normal weight, 9 overweight)	Normal weight: 29.8 ± 3.7 Overweight: 32.3 ± 2.6	- 15 measurements across the MC - Early FP corresponds to menstruation	- CRP significantly ↑ in the early follicular phase ($p < 0.001$)
Wander et al., 2008	8 (11 MCs)	Mean ± SD not reported (21-47)	- Blood and urine samples collected on 12 days across the MC - Menes self-reported	- Menstruation associated with 17% rise in CRP - 10-fold increase in P4 = 23% increase in CRP
Gaskins et al., 2012	259	27.3 ± 8.2 (18-44)	- 8 serum samples per cycle, timed by fertility monitors, for 1-2 MCs	- CRP ↑ during menses (median 0.74 mg/l) - ↓ in the FP - lowest at ovulation (0.45 mg/l) - ↑ again in the LP - 50% ↑ variance during menses (1.23 vs. 0.82 $(mg/l)^2$)
Vashishta et al., 2017	111	28 ± 8 (15-45)	- Menstruation (days 1-3) - FP (10th day) - LP (22nd day)	- Higher in FP (Mean 1.88 mg/l, SD 1.49) - LP (Mean 1.54 mg/l, SD 1.30) - $p < 0.001$, significant.
Wilson et al., 2025	98 (76 Polish, 22 Polish American)	31.8 ± 7 (18-46)	- Daily urine samples from day 1 of menstruation until start of next period	Polish population: - CRP ↑ during the first 3 days of menses at 0.365. - No significant differences were reported between the phases. Polish American population: - CRP ↑ during the first 3 days of menses at 0.466 - CRP lowest during the LP at 0.277.

				- For both populations, CRP ↑ in the first 3 days of menses. - The Polish American population had ↑ CRP levels than the Polish population during this phase (0.466 vs 0.365).

CRP = C-reactive protein; FP = Follicular Phase; LP = luteal Phase; MC = Menstrual Cycle; P4 = Progesterone

2.7 Research using Menstrual Fluid

During menstruation, an individual can lose between 86-148 ml of MF (Schwalie et al., 2024). Three components comprise MF: peripheral blood, cervicovaginal mucous, and the cells and fluid of the secretory endometrial lining and the cervix, which is shed during menstruation (Naseri et al., 2019; Tindal et al., 2024). Research has reported that MF contains 385 extra proteins that are not present in VB. It also contains higher levels of inflammatory molecules, such as matrix metalloproteinases and cytokines like interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α). These are present in only small amounts in VB. These components play key roles in tissue remodelling, immune response and angiogenesis (Naseri et al., 2023; Yang et al., 2012).

Literature exploring the use of MF for clinical applications remains limited (Table 2.6). However, it is a gradually growing research area with some promising evidence. For instance, Naseri et al. (2019) measured clinically relevant biomarkers in 84 women, including lipids (HDL, LDL, TC), HbA1c, CRP, and glucose in VB and participant-matched dried MF samples. The study showed strong correlation for several biomarkers, TC ($P = 0.89$, $R^2 = 0.89$), LDL ($P = 0.21$, $R^2 = 0.84$), TG ($P = 0.45$, $R^2 = 0.89$), HDL ($P = 0.33$, $R^2 = 0.77$), HbA1c ($P = 0.54$, $R^2 = 0.80$), and CRP ($P = 0.89$, $R^2 = 0.99$). However even despite the high R^2 values, none of these correlations reached statistical significance. A more recent cross-sectional study by Naseri et al. (2024) including 152 women reported a strong correlation between HbA1c levels in dried MF and VB ($r = 0.96$), however the specific p-value was not reported. Similar studies completed by the same research group have reported statistical significance, typically within the $p < 0.001$ especially in studies with larger sample sizes (Naseri et al., 2024; Naseri et al., 2022; Naseri et al., 2019). Similarly, in a study by Srinivasan et al. (2019) where self-collected menstrual tampons were used to measure HbA1c in 53 participants, results showed that

menstrual HbA1c was comparable to venous HbA1c for prediabetes detection at the standard 39 mmol/mol (5.7%) threshold. Additionally, within this research, the participants expressed a strong preference for quarterly MF testing over venous phlebotomy ($p = 0.015$), indicating that there may be good acceptability of MF testing for regular health monitoring.

Cumulatively, these initial findings suggest that MF could be used as an alternative fluid for HbA1c monitoring. In addition, most of the studies have collected MF on day 2-3 of menstruation, typically the heavier flow days and most representative of the endometrium environment (Tindal et al., 2024; van der Molen et al., 2013). However, it is worth noting that these studies have used dried blood spots (DBS) and tampons to collect MF samples. The use of DBS is a technique that collects a small amount of fluid and dries it onto specialised filter paper for analysis. This sample method has been suggested as an ethical and cost-effective method for MF collection, delivery and storage of biomaterial (Malsagova et al., 2020). However, this method comes with its limitations. Firstly, one limitation is that DBS only collects small volumes of blood (50-100 µl). This limits the detection of analytes that may require larger sample volumes (Malsagova et al., 2020; McDade et al., 2007). Secondly, MF and VB are different fluids with different physical properties. Variations in blood viscosity can affect how the fluid spreads on filter paper, presenting challenges for DBS analysis (McClendon-Weary et al., 2020). With this in mind, clotting and significant intra-individual variation in MF (Tindal et al., 2024) may impact the consistency and reliability of biomarker measurements using DBS. Another important challenge to note is the recovery of analytes from the filter paper, as there is limited information on the stability of molecules and the proportion of each analyte that can actually be retrieved. Recovery and stability of analytes from DBS depends on filter paper, extraction methods and storage conditions, with some analytes like urea or benzodiazepines recovering well, while others degrade quickly or can be lost by up to

41.6% if dried or stored improperly, highlighting the need for optimal conditions such as low temperature and desiccation (Alfazil & Anderson, 2008; Choi et al., 2014; Moat et al., 2025; Quraishi et al., 2013). No research to date, to the best of the author's knowledge has measured whole samples of MF and VB and determined the agreement between the two samples for acute point of care testing (POCT).

Collectively, the literature that has analysed MF has several recurring limitations. Firstly, many studies have small sample sizes that are often insufficient for biomarker validation. Within the available studies, many lack standardised collection and processing protocols or comparison to participant-matched VB samples. This limits our confidence in using MF as a reliable clinical tool for diagnosis or health monitoring. Without comparison to VB, the diagnostic gold standard, the clinical application of MF remains uncertain. In addition, there is minimal reporting of participant characteristics and limited or no evaluation of MF biomarker values to previously defined diagnostic cut-offs or clinical thresholds. For MF to be considered as a potential screening tool for T2D or CVD risk, research studies are needed with matched VB comparisons, larger samples, standardised protocols, and validation of biomarkers in whole MF, instead of DBS, to ensure accuracy and reliability, particularly for acute POCT.

Table 2.6: Research Using MF to Assess Biomarkers Compared with VB

Author	Sample size	Age group	MF collection method	Biomarkers measured	Comparison with VB	Key results
Naseri et al., 2019	84 (35 menstrual and serum sample, 20 sample adequate for analysis)	28 ± 8.7 (18-45)	DBS	Lipids (HDL, LDL, TC), HbA1c, CRP, and glucose	Yes	- TC: $R^2 = 0.89$, $p = 0.89$ - LDL: $R^2 = 0.84$, $p = 0.21$ - TG: $R^2 = 0.89$, $p = 0.45$ - HbA1c: $R^2 = 0.80$, $p = 0.54$ - HDL: $R^2 = 0.77$, $p = 0.33$ - CRP: $R^2 = 0.99$, $p = 0.89$
Srinivasan et al., 2019	53	Non-applicable	Tampons (Subjects collected 2-3 consecutive tampons starting on day 2 of their period)	HbA1c	Yes	- MF HbA1c comparable to venous HbA1c for prediabetes detection at 39 mmol/mol - Participants preferred quarterly menstrual testing over venous phlebotomy ($p = 0.015$)
Naseri et al., 2024	152	33.0 ± 7.9 (19-50)	Modified menstrual pad with DBS	HbA1c		- Menstrual HbA1c levels were highly correlated with venipuncture samples ($r = 0.96$) - Menstrual HbA1c values were stabilized in the DBS strips through 53 days -

MF = Menstrual Fluid; VB = Venous Blood; DBS = Dried Blood Spot; HbA1c = Haemoglobin A1c; TC = Total Cholesterol; LDL = Low-Density Lipoprotein; HDL = High-Density Lipoprotein; TG = Triglycerides; CRP = C-reactive protein

2.8 Conclusion

Lifestyle related diseases such as CVD and T2D are rising among the female population, yet research indicates that these conditions tend to be underdiagnosed in premenopausal populations. Several barriers such as lack of sex specific resources, low awareness and social stigma all hinder timely detection and treatment for these lifestyles diseases. As a biological sample, MF offers a unique opportunity in this context, as it is regularly available throughout a woman's reproductive life and may provide a non-invasive alternative (e.g. no needles) for biomarker assessment.

Lipid parameters and CRP are key markers used to screen for CVD and T2D risk, however research has shown these biomarkers fluctuate throughout the MC. Lipid parameters specifically LDL, TC and CRP have been shown to peak around menstruation. This suggest that measuring these biomarkers at a standardised point in the cycle could improve accuracy and reliability of results. Although limited research has been conducted using MF, the available evidence suggests potential agreement with VB for some biomarkers such as HbA1c, lipids, and CRP. Venous blood (VB) remains the gold standard, but no study has yet compared whole MF with paired VB samples, leaving an important gap to explore. Collectively, these findings support the idea that MF could offer a non-invasive, regularly available sample for monitoring key biomarkers. With potential for helping improve early detection of lifestyle related diseases like CVD and T2D in premenopausal women, a population where underdiagnosis remains a real challenge.

Chapter 3: Manuscript

3.1 Abstract

Background: Cardiovascular disease (CVD) and diabetes are prevalent among women of premenopausal age yet are often underdiagnosed. Traditional diagnostics rely on venous blood (VB), however, menstrual fluid (MF) may provide a naturally available, sex-specific, non-invasive alternative for assessing biomarkers for diabetes and CVD.

Objectives: To assess the agreement between MF and VB for key biomarkers associated with CVD and diabetes risk, and explore the influence of demographic factors (age, BMI and ethnicity) on these biomarkers.

Methods: Premenopausal women (n =102) completed a laboratory visit, providing paired MF and VB samples. Paired MF and VB samples were evaluated using a biochemistry analyser (COBAS® 101) to measure biomarkers of glucose regulation and lipids. Relative agreement between the paired MF and VB samples was assessed using Spearman's correlation coefficient (*r*). Bland-Altman analysis was performed to examine the absolute agreement and identify any systematic bias between each biomarker assessed in VB and MF. Linear regression models were conducted to identify whether MF could predict VB, the second model had addition of demographic variables (age, BMI and ethnicity).

Results: Ninety paired MF and VB samples were analysed. Bland-Altman plots showed no absolute agreement across biomarkers MF and VB, indicating that MF is not reliable enough to be used as a substitute to VB for individual level assessment for diabetes and CVD risk. Moderate to strong correlations were observed between MF and VB for several biomarkers including HbA1c (r = 0.68, $p < 0.001$), LDL (r = 0.57, $p < 0.001$), HDL (r = 0.57, $p < 0.001$)

TC:HDL ratio ($r = 0.73$, $p = 0.368$) and TC ($r = 0.58$, $p < 0.001$). Weaker correlations were observed in TG ($r = 0.36$, $p < 0.001$). These associations indicate relative agreement between MF and VB but do not support clinical replacement. Linear regression analysis found MF biomarkers were associated with VB biomarkers for HbA1c, HDL, LDL, TG, TC and TC:HDL ratio (all $p < 0.05$). However, the obtained predictive equations had limited ability to predict VB biomarker concentrations based on MF values, even after being adjusted for demographic factors (age, ethnicity and BMI) ($R^2 = 0.09 – 0.64$).

Conclusion: Menstrual fluid may be associated with VB across several key health biomarkers used in diabetes and CVD acute point-of-care testing. However, findings show limited absolute agreement between the two samples and small predictable ability even with the addition of demographic factors. These findings do not support MF as an alternative to VB diagnostics for screening of diabetes and CVD risk in premenopausal women. Future research is needed to address the potential of MF for clinical utility.

3.2 Introduction

Cardiovascular disease (CVD) and type 2 diabetes (T2D) prevalence is rising among menstruating women worldwide (Vervoort et al., 2024; Worrall-Carter et al., 2011; Yoshida et al., 2023). In contrast, improved screening has contributed to declining trends in T2D and CVD in men and post-menopausal women, suggesting lower awareness and inadequate testing among premenopausal women (Fang et al., 2022). Lower awareness and testing within premenopausal women may be due to clinicians underestimating their risk, less reliable diagnostic tools tailored to younger women, atypical presentations, limited education, gender roles, cultural norms and financial barriers (Babeaux, 2025; Sinha et al., 2024; Terefe et al., 2025).

Risk factors such as obesity, dyslipidemia, and hypertension, alongside female-specific conditions including gestational diabetes and polycystic ovary syndrome (PCOS), have previously been reported to contribute to the increase in both CVD and T2D in premenopausal women (Dubey et al., 2024). Additionally, demographic factors such as high BMI (overweight/obesity) and increasing age may further compound CVD and T2D risk in premenopausal women (Goldney et al., 2025; Yu et al., 2020). Notably, women may develop CVD at lower glucose levels than men and experience a greater risk of CVD associated with prediabetes (Levitzky et al., 2008). They are also more likely to have undiagnosed diabetes, reflecting disparities in healthcare access, screening frequency, and awareness of disease risk (Levitzky et al., 2008). These disparities have been reported in research to be more pronounced in women of other ethnicities compared with White European women. Prior research has reported non-Hispanic black women have a higher prevalence of diabetes (15.0%) and prediabetes (38.5%) compared with white European women (4.8% and 16.6%, respectively) (Britton et al., 2018). Similarly, Asian women are two to four times more likely to develop

T2D than white European women (Britton et al., 2018; Yeo et al., 2021). In New Zealand, Pacific (16.3%) and Māori (10%) women experience higher diabetes prevalence and disproportionately greater CVD-related hospitalisations and mortality compared with European women (Ke et al., 2023; Selak et al., 2020; Yu et al., 2021). These findings highlight ethnicity as a key independent risk factor for both T2D and CVD in premenopausal women.

Currently, within healthcare settings, biomarkers such as lipids (high density lipoprotein - HDL, low density lipoprotein - LDL, triglycerides - TG, total cholesterol - TC, total cholesterol to high density lipoprotein ratio - TC:HDL ratio), glycated haemoglobin (HbA1c) and C-Reactive protein (CRP) are most commonly measured in venous blood (VB) that has been collected through venipuncture (Garzarelli et al., 2022). Timing of VB collection is well recognised as an important factor to consider in biomarker assessment, as circadian variations in biomarkers and feeding status can influence results of blood or urine specimens (İhtiyar et al., 2022; Ruskovska et al., 2021). However, physiological fluctuations across the menstrual cycle (MC) may further impact biomarker measurements in women (Schisterman et al., 2014). Research suggests variability in biomarker concentrations could vary based on the MC phase, indicating that biomarker measurement timing relative to the MC in premenopausal women may be critical for accurate assessment in this population. Interestingly, emerging research suggests that menstrual fluid (MF) lost during the first week of the cycle (early follicular phase - FP) could be a useful diagnostic sample (Naseri et al., 2019; Tindal et al., 2024; Zaheer et al., 2024). Existing studies have investigated MF as a resource for the assessment of endometriosis, human papillomavirus, pregnancy outcomes and infertility, and, more recently, systemic biomarkers such as lipids and HbA1c assessments. Specifically, Naseri et al. (2022) demonstrated that MF biomarkers can reflect systemic levels of HbA1c. However, the ability

of MF to capture both diabetes and CVD-related biomarkers in healthy premenopausal women remains unknown as no studies to date have validated MF biomarkers for CVD risk.

Another limitation in this field is that, although MF has been used in prior research, there is a lack of studies that have used whole MF while directly comparing it with VB to assess biomarker agreement. Much of the existing research have separated cells, specific analytes or have utilised dried blood spot (DBS) for analysis (Harzif et al.; Naseri et al., 2023). This creates a methodological gap which makes it hard to determine how well whole MF fluid reflects VB measurements. Menstrual fluid is a complex biological mixture of endometrial tissue, immune cells, and vaginal secretions, giving it a distinct composition compared with VB (Tindal et al., 2024). Existing studies have primarily aimed to identify unique MF biomarkers or distinguish MF from other biological fluids, rather than to evaluate systemic biomarker comparability (Quinn & Elkins, 2017; Zhang et al., 2024). Without robust comparisons to VB, the clinical utility of MF biomarkers for monitoring systemic disease remains uncertain. Including point-of-care testing (POCT) into MF analysis may offer a pathway forward by allowing efficient, low-volume, and accessible testing (Cheng, 2020). Given that MF is non-invasive and can be self-collected, similar to saliva or urine, POCT could support at-home screening, improving compliance and accessibility, particularly for women in low-resource or remote settings.

Therefore, this study aimed to assess the level of agreement, both relative (correlation) and absolute (Bland Altman), between MF and VB for biomarkers including HbA1c, lipid parameters (HDL, LDL TC, TG, TC:HDL ratio), and CRP using POCT. Secondly, the study aimed to explore the influence of demographic factors (age, body mass index (BMI) and ethnicity) on these biomarkers.

3.3 Methodology

3.3.1 Study Design

Cross-sectional study conducted in healthy premenopausal women in Auckland, New Zealand. Data collection commenced in March 2024 and was completed in March 2025.

3.3.2 Participants and recruitment

Convenience recruitment was carried out through social media posts, community group pages, and advertisements placed across Massey University campus, including noticeboards and email lists. Additional interest came through word of mouth. An online screening questionnaire was completed by all interested and potential participants to assess eligibility for inclusion in the study. Figure 3.1 illustrates the recruitment and screening process. To be included in the study, participants were required to be between the ages of 18 and 45 years, either have a natural and regularly occurring MC or be users of a hormonal contraceptive but still experience a menstrual bleed, and be able to attend a lab visit at Massey University, Auckland. Access to a smartphone and a willingness to use a menstrual cup or disc during the study were also necessary criteria. If eligible, participants received study information prior to booking their data collection session with the research team. Ethics approval for this study was granted by Health and Disability Committee New Zealand [HDEC 2023 EXP 19295]. The sample size for this study was based on previous work by Naseri et al. (2019), this calculation used a 5% significance level (Type I error), 80% power, (Type II error = 0.20), and an expected mean difference of 0.08% with a standard deviation of 0.09% for HbA1c. This indicated a required sample size of 102 participants, which was achieved in the present study (Figure 3.1).

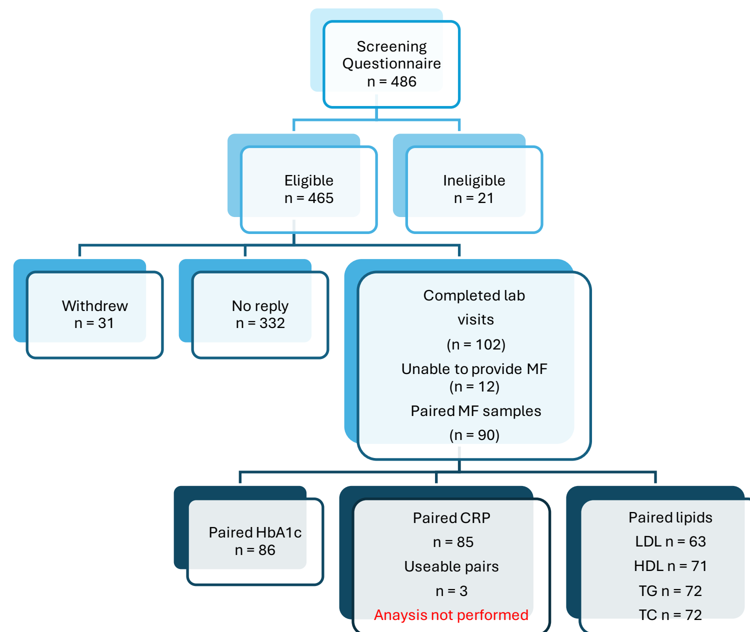

Figure 3.1: Flow chart of total number of women screened to the final number of paired blood samples of each biomarker. CRP = C-Reactive Protein; HbA1c = Glycated Haemoglobin; LDL = Low Density Lipoprotein; HDL = High Density Lipoprotein; TG = Triglycerides; TC = Total Cholesterol.

3.3.3 Study Protocols

A data collection session was conducted at Massey University in Auckland, New Zealand. This session was scheduled to coincide with each participant's predicted menstrual bleed and days of heaviest flow (usually on days 2-3 following the start of menstruation). When menstrual bleeding commenced, participants notified the researchers, and if needed, the testing session was adjusted accordingly to ensure data collection on the day of the heaviest flow. All participants were provided with a personal menstrual cup or disc (Hello Period Ltd, Hawke's Bay, New Zealand). The reusable menstrual product provided to each participant was dependent on the individual's preference and comfort. On the day of data collection, participants were instructed to insert their reusable menstrual product at least three hours prior to attending their laboratory session. Following the provision of informed consent, all participants completed an online demographics survey. This survey included several components: (1) a demographic questionnaire designed to gather general health information, such as age, ethnicity, diet, medical history, reproductive health; (2) menstrual history and

menstrual bleeding questionaries, with questions selected from the Reproductive Status Questionnaire (Schmalenberger et al., 2021) and the Female Health Questionnaire (Bruinvels et al., 2016). Having completed the online survey in a rested seated position, the researchers then completed a resting blood pressure measurement using a calibrated professional-grade digital automatic blood pressure monitor (Omron HEM series, Omron Healthcare, Kyoto, Japan). Following the resting blood pressure measurement, the participant's height and weight were measured using standard procedures. Body composition, including lean and fat mass, was assessed using a bioelectrical impedance analyser (InBody 230, Seoul, South Korea). A rested VB sample was then collected (see section 3.3.4).

Participants were provided a sample collection dish and a sample collection container, and were directed to a private bathroom to self-collect their sample of MF. All participants, prior to MF self-collection, were provided with clear instructions on how to collect their MF into the collection dish with the change of their reusable menstrual product. Following the completion of MF collection, participants reported back to the researchers if there was sufficient volume and their data collection process was completed.

3.3.4 Venous Blood and Menstrual Fluid Analysis

Venous blood (VB) as the clinical gold standard was included to provide a baseline comparison and to assess whether any observed MF variation reflected broader physiological changes or was specific to MF matrix. Venous blood samples were collected by a trained phlebotomist. Participants were seated and rested for 5 minutes prior to sample collection. Venous blood samples (approximately 5 ml) were collected from the antecubital vein using 23-G butterfly needles into EDTA vacutainers. Menstrual fluid samples were pipetted from the collection container to similar EDTA vacutainers. All MF and VB samples were analysed for HbA1c, lipid panel and CRP using the COBAS b® 101 analyser (Roche Diagnostics, Switzerland)

immediately following collection and in accordance with manufacturer's instructions. Previous research results, for HbA1c and lipids have reported this system to be highly comparable to standard laboratory measurements, supporting its application for POCT (Yu et al., 2017). The single-use COBAS b® 101 reagent discs require approximately 2 µL for HbA1c, 19 µL for lipids and 12 µL for CRP. For this analysis, HbA1c was measured directly from whole MF and VB samples. The VB and MF samples were then centrifuged at 2000 x g for 20 minutes at 4°C. The plasma that was separated from both MF and VB was then used to measure CRP and lipids using the appropriate discs.

3.3.5 Statistical Analysis

Data were initially collected and processed in Microsoft Excel before being transferred to IBM SPSS Statistics (version 29, Armonk, NY, USA) for Mac for statistical analyses. Normally distributed variables are presented as means ± standard deviation (SD), while categorical variables, such as ethnicity and menstrual status are reported as frequency and percentage.

Paired sample sizes differed across biomarkers due to varying levels of missing data resulting from insufficient sample volume or assay errors. Figure 3.1 shows the total number of women included in the analysis for each paired MF and VB biomarker sample.

For some biomarkers, values fell outside the detection limits of the assays and were substituted with predefined values to allow inclusion in analyses. Triglyceride (TG) values below 0.5 mmol/l were assigned a value of 0.4 mmol/l (n = 2 venous), and TG values above 7.35 mmol/l were assigned 7.36 mmol/l (n = 1 menstrual). High density lipoprotein (HDL) values below 0.38 mmol/l were assigned 0.37 mmol/l (n = 1 venous; n = 2 menstrual), and values above 2.6

mmol/l were assigned 2.7 mmol/l (n = 2 menstrual; n = 1 venous). Total cholesterol (TC) values below 1.28 mmol/l were assigned 1.27 mmol/l (n = 1 menstrual).

C- Reactive Protein (CRP) was excluded from the study due to insufficient datapoints. The majority of the CRP values (n = 82) were below the lower limit of detection of the COBAS b® 101 device (< 3.00 mg/l), reflecting the limited analytical sensitivity of the platform at low CRP concentrations. As a result, only three paired measurements exceeded the detection threshold, which was insufficient to continue meaningful agreement analysis. Clinically, CRP values below 3.0 mg/l are within normal range, with concentrations above this threshold being considered at high cardiovascular risk.

Ethnicity was categorised using the prioritised ethnicity approach according to HISO 10001:2017 Ethnicity Data Protocols. Participants reporting multiple ethnicities were assigned to a single group following that standard priority hierarchy: Māori, Pacific Peoples, Asian, MELAA (Middle Eastern, Latin American, and African), Other, and European (Ministry of Health, 2017).

In context of POCT, analytical feasibility alone is insufficient, meaning it is important to understand how well novel sampling methods agree with established reference standards. In measurement studies, agreement is described in both absolute and relative terms, and differentiation between the two terms is important when assessing whether one method can purposely substitute for another. Absolute agreement refers to how closely measurements align in their original units or within clinically acceptable limits, reflecting the degree of measure error (Vanbelle et al., 2025). This was assessed using a typical tool Bland-Altman analysis (Ranganathan et al., 2017). In contrast, relative agreement often known as correlation,

describes how well two methods track or rank individuals in relation to one another, rather than if they produce equivalent values (De Vet et al., 2013). As the primary aim of this study was to assess whether MF could serve as an alternative to VB diagnostics for diabetes and CVD risk, absolute agreement was prioritised. While relative agreement provides useful supporting information on the relationship between MF and VB, only absolute agreement can determine whether MF measurements are sufficiently comparable for potential clinical or research use.

Relative agreement was assessed using correlation analyses. Normality of the data was checked using the Shapiro-Wilk test. Relative agreement between MF and VB biomarker concentrations were assessed using Spearman's rank correlation coefficient (r) for population coefficient. Spearman's correlation was deemed more appropriate than Pearsons correlation, due to the shapes of the scatter plots for each biomarker.

Bland-Altman plots were used to assess absolute agreement between MF and VB measurements for each biomarker. For each Bland-Altman plot, the x-axis represents the mean of the MF and VB measurements for a participant. The y-axis represents the difference between MF and VB (MF-VB). The central blue dotted line represents the mean difference between MF and VB values. The outer blue dotted lines represent the 95% limits of agreement (LoA), calculated as the mean difference ± 1.96 x standard deviation of the differences. The LoA represent the range within which most individual differences between MF and VB are expected to fall. The shaded blue area represents differences considered clinically insignificant, based on the minimal clinically important differences (MCIDs) for each parameter or other equivalent criteria further explained below. The pink line (bias) represents the linear fit for the data point spread. Ideal agreement is indicated by a mean difference close to zero, a flat pink line (the pattern of MF-VB values is similar across different concentrations of the biomarker), and

narrow LoA that fall within predefined clinically acceptable boundaries. The clinical relevance of agreement was therefore interpreted by evaluating whether the observed LoA were sufficiently small to support the use of MF as an alternative to VB measurement rather than relying on correlation alone.

The minimally clinical important differences (MCID) thresholds were used to interpret Bland-Altman limits of agreement and to assess whether differences between MF and VB values were likely to be clinically meaningful. For HbA1c we considered a MCID of 5.5 mmol/mol, reflecting the smallest change likely to produce meaningful physiological or clinical effects (Lenters-Westra et al., 2014). There is no MCID consensus for the other parameters, but conservative threshold values were adopted. For LDL we considered a MCID of 0.4 mmol/l (Mach et al., 2020), though an alternative threshold of 1.0 mmol/l has also been reported (Baigent, 2005). For HDL we considered a MCID of 0.3 mmol/l (Pedersen, 2005), while a value of 0.5 mmol/l has also been reported (Zhou et al., 2021). For TG we used a threshold of 0.3 mmol/l, which accounts for a minor variation related to food intake (Pancholia et al., 2024). Alternative approaches have considered a 30% reduction (Bradley et al., 2009), although higher thresholds (for example, 2.0 mmol/l) are generally only relevant in hyperlipidemia (Zhou et al., 2021). For TC, a MCID of 0.75 mmol/l was applied (Jeong et al., 2018). Lastly, for TC:HDL ratio a difference of 1 unit was considered clinically important, which was applied, reflecting a value that has already been described to produce physiological effects rather than a formal MCID (Millán et al., 2009).

To assess whether MF measures could be used to predict VB measures, we conducted linear regression. Separate regression analyses were run for each biomarker. Model 1 included the VB measure as the outcome and the MF measure as the predictor, whilst Model 2 included

covariates, specifically age (years), ethnicity (European, Other), and BMI to determine whether theses covariates contributed to the predictive relationship between MF and VB.

Model assumptions were assessed visually. Residuals versus fitted plots were used to assess linearity. For all VB measures except HbA1c, the residuals versus fitted plots showed curvature, indicating that a simple linear term (e.g. MF) did not adequately describe the relationship between MF and VB. To address this, a quadratic term for the MF measure was added to the model. This flattened the curvature and resulted in residuals that were more evenly scattered around zero, indicating an improved model fit. The quadratic model was therefore retained for these biomarkers. Preferred model was decided by comparing Model 1 and Model 2 using ANOVA. Where the ANOVA was similar between models ($p > 0.05$), Model 1 (being the simpler) was used. If ANOVA showed the models to be different ($p < 0.05$), the model with the lowest residual sum of squares (least amount of error) was used. Cook's distance identified a few influential values for TG and TC. When explored, sensitivity analyses appeared to reflect real biological variation rather that error within the data, whereby removing them did not change interpretation of results. They were retained, and this indicates greater sample variability for these lipid measures. Other biomarkers (HbA1c, LDL, HDL, and TC:HDL ratio) measures had no influential values, indicating more stable results.

In a secondary analysis, to explore whether MF and VB biomarker concentrations varied between early (days 1-2) and later (days 3-5) collection timepoints, independent t-tests were conducted between the 'day' groups for the mean difference (MF-VB) for each participant. Statistical significance was set at $p < 0.05$.

3.4 Results

3.4.1 Participant Characteristics

The majority of participants had a natural MC (88.5%), while 11.5% were users of oral contraceptives (OCs). Among OC users, the most common method of hormonal contraception was the OC pill (n = 3), followed by the hormonal IUD/Mirena (n = 2), and hormonal implant/ rods (n = 2). Participant characteristics are presented in Table 3.1.

Table 3.1: Summary of participant characteristics

Participants (n)	**90**
Characteristic	Mean ± SD
Age (years)	32.0 ± 5.8
Height (cm)	167.4 ± 6.5
Weight (kg)	70.6 ± 13.4
BMI (kg/m²)	25.2 ± 4.7
Systolic Blood Pressure (mmHg)	116.1 ± 11.4
Diastolic Blood Pressure (mmHg)	73.8 ± 8.0
Priority Ethnicity	**n (%)**
European	62 (72.9)
Māori	8 (9.4)
Pacific Islander	2 (2.4)
Asian	9 (10.6)
Middle Eastern, Latin American, and African	4 (4.7)
Other	0 (0)
Menstrual status	**n (%)**
Natural Menstrual Cycle	80 (92)
Oral Contraception	**7** (8)

Age unknown n = 10; Height unknown n = 1; Weight unknown n = 1; Blood Pressure unknown n = 2; Ethnicity unknown n = 5; Contraceptive unknown n = 3.

3.4.2 Absolute and Relative Agreement between MF and VB biomarkers

Out of the 102 women who completed a lab visit at Massey University, 12 women were unable to provide an MF sample, therefore results reflect 90 individuals. Menstrual fluid samples were lost for several reasons including insufficient volume (n = 11), technical or collection issues (n = 11) and sample composition (n = 6).

Overall, the highest paired sample availability was for HbA1c (n = 86, 96%), while lipids had lower amounts of paired samples (HDL: n = 71, 79%, LDL: n = 63, 70%, TG: n = 72, 80%, TC: n = 72, 80%, TC:HDL ratio: n = 64, 71%). Table 3.2 displays the paired comparison of biomarker concentrations in MF and VB samples. All biomarkers in MF were significantly lower than VB, except TG which tended to be higher in MF (1.9 ± 1.0 mmol/l) compared with VB (1.1 ± 0.6 mmol/l) ($p = 0.002$).

Relative agreement between MF and VB measurements was assessed using Spearman's correlation. Positive correlations were observed for HbA1c, LDL, HDL, TC, and the TC:HDL ratio (all $p < 0.01$), with correlation coefficients ranging from r = 0.36 to 0.74 (Table 3.2).

Table 3.2: Paired Comparison of Biomarker Concentrations in MF and VB: Means, Differences, and Correlations

Biomarker	Paired sample	MF Mean ± SD	VB Mean ± SD	Mean Difference (95% CI) (VB-MF)	p (paired t test)	95% Limits of Agreement	Bias	Spearman's Correlation (*r*)	p (corr)
HbA1c (mmol/mol)	86	30.7 ± 3.1	32.4 ± 3.1	-1.73 (-2.27, 1.19)	< 0.001	-6.73 to 3.27	No	0.68	< 0.001
LDL (mmol/l)	63	1.8 ± 0.7	2.6 ± 0.8	-0.76 (-0.93, -0.58)	< 0.001	-2.12 to 0.60	Yes	0.63	< 0.001
HDL (mmol/l)	71	1.4 ± 0.5	1.6 ± 0.4	-0.23 (-0.33, -0.14)	< 0.001	-0.98 to 0.52	Yes	0.60	< 0.001
TG (mmol/l)	72	1.9 ± 1.0	1.1 ± 0.6	0.73 (0.47, 0.99)	< 0.001	-1.43 to 2.89	Yes	0.36	0.002
TC (mmol/l)	72	3.9 ± 1.0	4.7 ± 0.9	-0.75 (-0.95, -0.56)	< 0.001	-2.36 to 0.86	Yes	0.61	< 0.001
TC:HDL ratio	71	3.0 ± 0.9	3.0 ± 0.8	0.5 (-0.11, 0.21)	0.52	-1.24 to 1.34	No	0.74	< 0.001

HbA1c = Glycated Haemoglobin; LDL = Low Density Lipoprotein; HDL = High Density Lipoprotein; TG = Triglycerides; TC = Total Cholesterol; TC:HDL ratio = Total Cholesterol/ High Density Lipoprotein Ratio. 95% Limits of agreement (LoA) were calculated using Bland-Altman analysis and represent the range within which 95% of the differences between VB and MF measurements are expected to lie (mean difference ± 1.96 SD).

The Bland–Altman plot for HbA1c (Figure 3.2A) showed a significant negative mean difference (MF–VB) of −1.73 mmol/mol ($p < 0.001$; paired t-test), with LoA from −6.73 to 3.27 mmol/mol (Table 3.2). These limits fall within the MCID of 5.5 mmol/mol for HbA1c.

The Bland–Altman plot for HDL (Figure 3.2B) demonstrated a significant mean difference of −0.23 mmol/l ($p < 0.001$; paired t-test), with LoA between −0.33 and −0.14 mmol/L (Table 3.2). These limits do not fall within the MCID of 0.3 mmol/l for HDL.

For LDL, the Bland–Altman plot (Figure 3.2C) showed a significant mean difference of −0.76 mmol/l ($p < 0.001$; paired t-test), with LoA from −0.58 to 0.93 mmol/l (Table 3.2), exceeding the MCID of 0.4 mmol/l.

The Bland–Altman plot for TG (Figure 3.2D) showed a significant positive mean difference of 0.73 mmol/l ($p = 0.002$; paired t-test), with LoA between 0.47 and 0.99 mmol/l (Table 3.2). These limits fall outside the MCID of 0.3 mmol/l.

For TC, the Bland–Altman plot (Figure 3.2E) showed a significant mean difference of −0.75 mmol/L ($p < 0.001$; paired t-test), with LoA from −2.46 to 0.86 mmol/l (Table 3.2). Compared with the MCID of 0.75 mmol/l, the LoA exceed the clinically acceptable range.

The Bland–Altman plot for the TC:HDL ratio (Figure 3.2F) showed a significant mean difference of 0.50 ($p < 0.001$; paired t-test), with LoA between −1.24 and 1.34 (Table 3.2). These limits extend beyond the clinically important difference of ±1 unit.

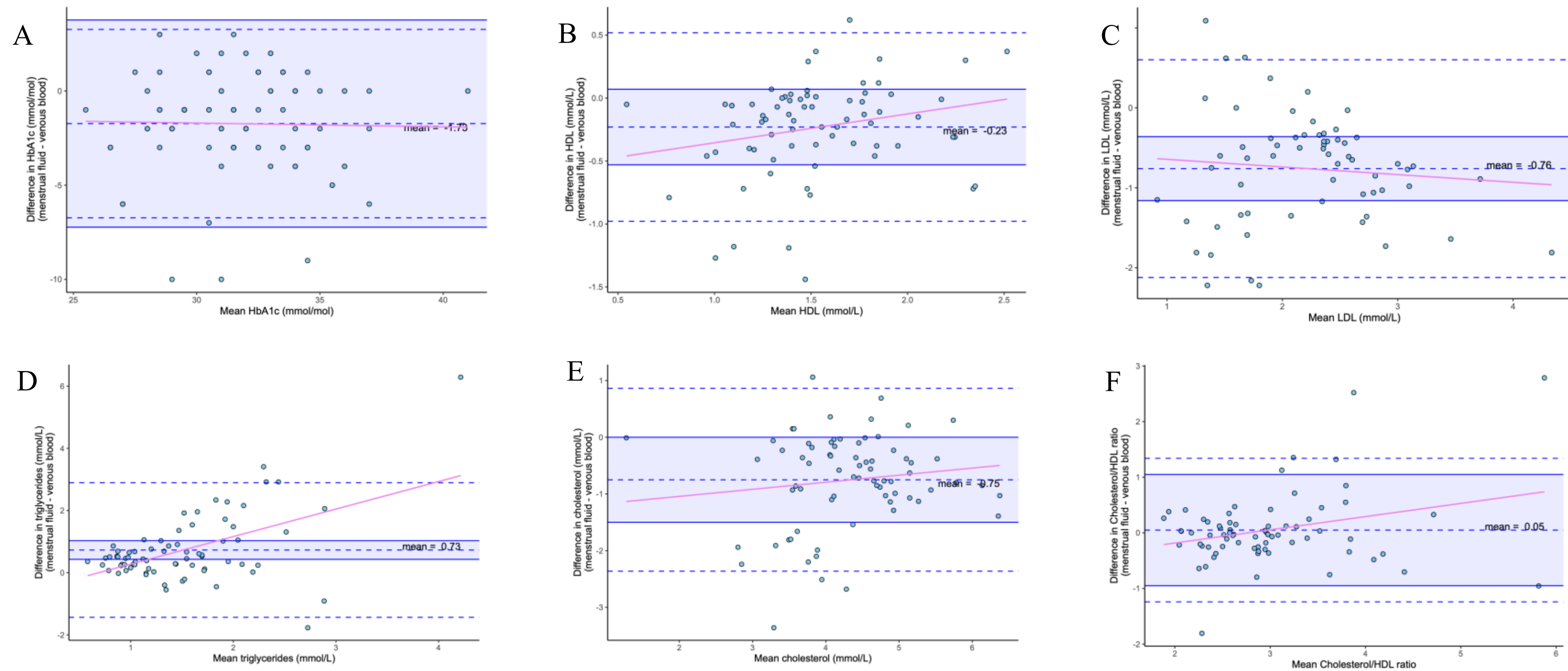

Figure 3.2: Bland-Altman plots assessing agreement between menstrual fluid (MF) and venous blood (VB) for (A) HbA1c, (B) HDL, (C) LDL, (D) TG, (E) TC and (F) TC:HDL ratio. HbA1c = Glycated Haemoglobin; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; TG = Triglycerides; TC = Total Cholesterol. The pink line represents the linear fit for data point spread. Blue dotted line represents that average mean difference between MF-VB concentrations. The blue shaded area signifies the predefined clinically acceptable range against which agreement was assessed

3.4.3 Menstrual Fluid as a Predictor of Venous Blood Values

Table 3.3-3.8 shows the linear regression outputs for the relationship between VB and MF and a possible equation to predict VB from MF. Model 1 (MF values predicting VB values) and Model 2 (addition of other covariates alongside MF to predict VB values) were statistically similar for HbA1c, HDL, LDL, TG and TC. Therefore Model 1 was considered the best model. Model 1 explained between 9% and 54% of the relationship between VB and MF. For TC:HDL ratio Model 2 was a better fit for this data (p = 0.02) and explained 64% of the variation between MF and VB.

Table 3.3: Linear Regression showing whether MF can predict VB HbA1c

Characteristic	**Model 1**				**Model 2**			
	N	**Beta**	**95% CI**	**p-value**	**N**	**Beta**	**95% CI**	**p-value**
(Intercept)	86	11.74	6.60, 17.00	<0.001	74	11.00	4.5, 18	0.001
Menstrual fluid (mmol/mol)	86	0.67	0.51, 0.84	<0.001	74	0.64	0.44, 0.85	<0.001
Age (years)					74	-0.02	-0.12, 0.08	0.6
Ethnicity								
European					56	—	—	
Other					18	0.92	-0.49, 2.3	0.2
BMI					74	0.08	-0.06, 0.22	0.3
R^2		0.44				0.45		
Adjusted R^2		0.43				0.41		
p-value		<0.001				<0.001		
Compare models with ANOVA (likelihood ratio test nested linear models): Model 1: RSS = 427, Df = 72; Model 2: RSS = 411, Df = 69; p-value = 0.46								
Possible predictive equation: Venous blood (HbA1c mmol/mol) = 0.67 * menstrual fluid (HbA1c mmol/mol) + 11.74								

BMI = Body Mass Index. CI = Confidence Interval. Df = degrees of freedom. RSS = residual sum of squares

Table 3.4: Linear Regression showing whether MF can predict VB HDL

Characteristic	**Model 1**				**Model 2**			
	N	**Beta**	**95% CI**	**p-value**	**N**	**Beta**	**95% CI**	**p-value**
(Intercept)	71	1.37	0.90, 1.80	<0.001	61	1.80	1.0, 2.5	<0.001
Menstrual fluid (mmol/l)	71	-0.19	-0.86, 0.48	0.60	61	-0.24	-1.0, 0.52	0.50
Menstrual fluid2 (mmol/l)	71	0.25	0.02, 0.48	0.03	61	0.29	0.02, 0.56	0.04
Age (years)					61	-0.01	-0.02, 0.01	0.40
Ethnicity								
European					47	—	—	
Other					14	-0.13	-0.30, 0.04	0.12
BMI					61	-0.01	-0.03, 0.01	0.40
R^2		0.42				0.50		
Adjusted R^2		0.41				0.45		
p-value		<0.001				<0.001		

Compare models with ANOVA (likelihood ratio test nested linear models):
Model 1: RSS = 4.46, Df = 58;
Model 2: RSS = 4.14, Df = 55;
p-value = 0.23

Possible predictive equation: Venous blood (HDL mmol/l) = -0.19 * menstrual fluid (HDL mmol/l) + 0.25 * menstrual fluid2 (HDL mmol/l) + 1.37

BMI = Body Mass Index. CI = Confidence Interval. Df = degrees of freedom. RSS = residual sum of squares

Table 3.5: Linear Regression showing whether MF can predict VB LDL

Characteristic	Model 1				Model 2			
	N	Beta	95% CI	p-value	N	Beta	95% CI	p-value
(Intercept)	63	2.70	2.10, 3.30	<0.001	55	2.90	1.30, 4.50	<0.001
Menstrual fluid (mmol/l)	63	-1.20	-2.00, -0.50	0.001	55	-1.40	-2.40, -0.28	0.02
Menstrual fluid (mmol/l) (squared)	63	0.56	0.34, 0.77	<0.001	55	0.58	0.26, 0.90	<0.001
Age (years)					55	0.00	-0.03, 0.03	0.90
Ethnicity								
European					44	—	—	
Other					11	0.04	-0.36, 0.43	0.90
BMI					55	0.00	-0.04, 0.05	> 0.9
R^2		0.54				0.41		
Adjusted R^2		0.52				0.35		
p-value		<0.001				< 0.001		

Compare models with ANOVA (likelihood ratio test nested linear models):
Model 1: RSS = 16.26, Df = 52;
Model 2: RSS = 16.23, Df = 49;
p-value = 0.99

Possible Predictive equation: Venous blood (LDL mol/l) = -1.2 * menstrual fluid (LDL mol/l) + 0.56 * menstrual fluid2 (LDL mol/l) + 2.7

BMI = Body Mass Index. CI = Confidence Interval. Df = degrees of freedom. RSS = residual sum of squares

Table 3.6: Linear Regression showing whether MF can predict VB TG

Characteristic	Model 1				Model 2			
	N	Beta	95% CI	p-value	N	Beta	95% CI	p-value
(Intercept)	72	0.51	0.02, 1.00	0.04	62	-0.54	-1.80, 0.68	0.40
Menstrual fluid (mmol/l)	72	0.49	0.12, 0.87	0.01	62	0.67	0.24, 1.10	0.003
Menstrual fluid (mmol/l) (squared)	72	-0.06	-0.12, -0.01	0.03	62	-0.08	-0.14, -0.02	0.02
Age (years)					62	0.00	-0.03, 0.02	> 0.90
Ethnicity								
European					47	—	—	
Other					15	-0.03	-0.38, 0.31	0.90
BMI					62	0.04	0.00, 0.08	0.08
R^2		0.09				0.24		
Adjusted R^2		0.07				0.17		
p-value		0.03				0.01		

Compare models with ANOVA (likelihood ratio test nested linear models):
Model 1: RSS = 18.77, Df = 59;
Model 2: RSS =17.72, Df =56
p-value = 0.36

Possible predictive equation: Venous blood (TG mmol/l) = 0.67 * menstrual (TG mmol/l) - 0.08* menstrual 2 (TG mmol/l) – 0.00 Age (years) – 0.03 Ethnicity – 0.04 BMI + - 0.54

BMI = Body Mass Index. CI = Confidence Interval. Df = degrees of freedom. RSS = residual sum of squares

Table 3.7: Linear Regression showing whether MF can predict VB TC

Characteristic	**Model 1**				**Model 2**			
	N	**Beta**	**95% CI**	**p-value**	**N**	**Beta**	**95% CI**	**p-value**
(Intercept)	72	4.10	2.50, 5.80	<0.001	62	3.50	1.10, 6.0	0.01
Menstrual fluid (mmol/l)	72	-0.41	-1.30, 0.49	0.4	62	-0.24	-1.30, 0.79	0.60
Menstrual fluid (mmol/l) (squared)	72	0.13	0.01, 0.25	0.03	62	0.11	-0.03, 0.24	0.12
Age (years)					62	-0.01	-0.04, 0.02	0.60
Ethnicity								
European					47	—	—	
Other					15	-0.22	-0.65, 0.21	0.30
BMI					62	0.03	-0.02, 0.08	0.30
R^2		0.44				0.43		
Adjusted R^2		0.42				0.38		
p-value		<0.001				< 0.001		

Compare models with ANOVA (likelihood ratio test nested linear models):
Model 1: RSS = 29.60, Df = 59;
Model 2: RSS = 28.21, Df = 56;
p-value = 0.44

Possible predictive equation: Venous blood (TC mmol/l) = -0.41 *menstrual fluid (TC mmol/l) + 0.13 * menstrual fluid2 (TC mmol/l) + 4.1

BMI = Body Mass Index. CI = Confidence Interval. Df = degrees of freedom. RSS = residual sum of squares

Table 3.8: Linear Regression showing whether MF can predict VB TC:HDL ratio

Characteristic	Model 1				Model 2			
	N	**Beta**	**95% CI**	**p-value**	**N**	**Beta**	**95% CI**	**p-value**
(Intercept)	71	0.49	-0.58, 1.50	0.40	61	-0.67	-1.90, 0.57	0.03
Menstrual fluid	71	1.00	0.42, 1.60	0.001	61	1.10	0.57, 1.50	< 0.001
Menstrual fluid (squared)	71	-0.05	-0.13, 0.02	0.20	61	-0.07	-0.13, -0.01	0.03
Age (years)					61	0.00	-0.02, 0.02	0.70
Ethnicity								
European					47	—	—	
Other					14	0.06	-0.19, 0.32	0.60
BMI					61	0.04	0.01, 0.07	0.01
R^2		0.53				0.64		
Adjusted R^2		0.52				0.61		
p-value		<0.001				< 0.001		
Compare models with ANOVA (likelihood ratio test nested linear models): Model 1: RSS = 9.97, Df = 56; Model 2: RSS = 9.12, Df =55; p-value = 0.02								
Possible predicative equation: Venous blood (TC:HDL ratio) = - 0.67 + 1.1 * menstrual (TC:HDL ratio) + -0.07 *menstrual2 (TC:HDL ratio) – 0.00 Age (years) – 0.06 Ethnicity - 0.04 BMI								

BMI = Body Mass Index. CI = Confidence Interval. Df = degrees of freedom. RSS = residual sum of squares

3.4.4 Influence on sample collection timing on MF and VB biomarkers

The majority of participants collected MF during days 1-2 (n = 77), with day 2 being the most common day of data collection (n = 66). Fewer participants collected MF during days 3-5 (n = 28).

The mean differences between MF and VB did not differ between samples collected on days 1-2 and days 3-5 for any of the biomarkers (HbA1c, HDL, LDL, TC, TG, or TC:HDL ratio; all p > 0.2, independent t-tests; Table 3.9). This suggests that timing of sample collection within the study did not affect the MF-VB differences.

Table 3.9: Comparison of MF and VB concentrations between days 1-2 and 3-5.

Biomarker	MF-VB Day 1-2 (Mean ± SD)	MF-VB Day 3-5 (Mean ± SD)	Mean difference between days	95% CI	p-value
HbA1c (mmol/mol)	-1.73 ± 2.60	-1.80 ± 2.36	0.06	-1.13 to 1.26	0.22
HDL (mmol/l)	-0.22 ± 0.33	-0.17 ± 0.39	-0.04	-0.22 to 0.14	0.41
LDL (mmol/l)	-0.72 ± 0.67	-0.80 ± 0.74	0.07	-0.28 to 0.44	0.60
TC (mmol/l)	-0.77 ± 0.80	-0.73 ± 0.88	-0.04	-0.45 to 0.37	0.50
TG (mmol/l)	0.59 ± 0.95	0.74 ± 0.82	-0.15	-0.61 to 0.29	0.65
TC:HDL ratio	-0.01 ± 0.42	-0.03 ± 0.77	0.02	-0.27 to 0.32	0.20

HbA1c = Glycated Haemoglobin; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; TC = Total Cholesterol; TG = Triglycerides.

3.5 Discussion

The aim of this study was to determine the absolute agreement between MF and VB for potential to use as a diagnostic tool for T2D and CVD risk in menstruating women. Overall, moderate to strong relative agreement was observed between MF and VB for all biomarkers at the population level. In particular, menstrual fluid (MF) HbA1c showed a strong relative agreement with VB values. Absolute agreement was poor for all biomarkers, with some improvement seen in TC:HDL ratio. Overall, the findings indicate that while MF measurements reflect VB values at a population level (relative agreement), differences in absolute values limit their interchangeability at the individual level.

Although Bland-Altman analysis demonstrated a consistent negative mean difference between MF and VB HbA1c (-1.73 mmol/l), the magnitude of the differences was small and remained stable across concentrations. The LoA fell within the MCID (5.5 mmol/mol), these differences between values are not large enough to reflect a meaningful physiological or clinical effect. However, MF values were consistently lower than VB HbA1c values, indicating that measures are not directly interchangeable. The reduction present in HbA1c values measured from MF may reflect shorter erythrocyte exposure to circulating glucose compared with VB samples (Kokumai et al., 2025). As MF contains endometrial blood and shed red blood cells of varying age, the biomarker may be influenced by altered red cell lifespan rather than true glycemic status (Tindal et al., 2024). Additionally, the presence of non-blood components within MF may result in dilution of red blood cells, further contributing to the observed lower HbA1c (Tindal et al., 2024). Although the present study did not directly compare sexes, similar reductions in HbA1c have been consistently observed in women relative to age-matched men in population studies with a mean difference of 1.6 mmol/mol (Stedman et al., 2025). Menstrual red blood cell turnover may be able to partially explain this mean difference observed (Holland

et al., 2024). Supporting the clinical relevance of this mechanism, application of sex specific diagnostic thresholds has been estimated to reclassify approximately 35,000 women under 50 years in England and Wales as having diabetes (Holland et al., 2024). The lower values observed in MF in this study may reflect differences in sample composition rather than systemic physiological processes affecting HbA1c. The heterogeneous nature of MF, including the presence of additional fluid components and blood clots, may result in dilution of red blood cells, explaining the observed underestimation of HbA1c. However further research is needed to verify this proposition. Taken together, these results suggest a reasonable agreement between MF and VB HbA1c, indicating potential utility of MF for HbA1c assessment. However, the presence of a significant mean difference where HbA1c values were consistently underestimated in MF compared to VB, suggests that normative values for VB may not be applied to MF measurements. Consequently, MF specific reference ranges would be required for clinical interpretation. As such, further research is also needed to test HbA1c values across a broader range of values and in different diseases and conditions.

In comparison, lipid parameters showed limited agreement. Concentrations from MF HDL and LDL were consistently lower than VB values and showed considerable variability between samples. These results suggest that MF-VB differences are generally larger than the change required to result in meaningful physiological or clinical effects. Together, these findings indicate that direct measurement of MF HDL and LDL are not a reliable measurement for individual level clinical use. While formulas could potentially adjust MF values, our model with the available variables explained less than half (41%) of the VB variance, so correction formulas may not enable clinical precision that is required.

Agreement between TC (MF) and TC (VB) was poor. The magnitude and variability of differences suggest that MF measurements are not adequate for individual clinical measurements. Importantly, limits of agreement generally exceeded what would be considered clinically meaningful. Direct comparisons are challenging as to date, limited research has explored lipid measurements in MF. However, research in MF composition such as differences in blood concentration, cellular material and viscosity has previously been noted which may explain inconsistencies in agreement observed in the present study (Tindal et al., 2024). Lipid concentrations are known to vary substantially between plasma, serum, and erythrocyte, and are particularly sensitive to processing conditions (Ishikawa et al., 2014; Loef et al., 2020). Given that MF contains cellular components and undergoes local tissue breakdown prior to collection, sample related shifts in lipid distribution fits with what we know about lipid biology (Loef et al., 2021). Interestingly though the only lipid biomarker that showed an increase in MF relative to VB was TG. This result would suggest that dilution may not be the only factor to explain the observed differences. A potential explanation for this result could be the addition of other components that make up MF that are not found in VB, such as endometrial tissue, reproductive tract excretions and cervical mucus. Cervical mucus, shed endometrium and other reproductive tract fluids contain a lipid fraction that includes TG (Liu et al., 2023; Zarezadeh et al.,2020). The presence of lipid sources outside the blood component of MF may be contributing to the elevated TG levels found in MF, thereby explaining why TG followed a different dilution pattern to other biomarkers. Together, the current findings suggest that MF TC and TG are not currently suitable replacements for VB measures at an individual POCT and further research is still required in this area.

Compared with individual lipid parameters, the TC:HDL ratio presented with improved agreement between MF and VB. A reason for this could be the presence of additional non-

blood components in MF, which may dilute systemic blood constituents and result in lower lipid concentrations in MF. When utilising a ratio (TC:HDL), this dilution effect may be proportionally balanced across both analytes, thereby reducing clear differences and improving agreement between TC and HDL. However, a small bias was still present and because there is no established MCID, firm conclusions cannot be made. Further research and validation are therefore needed for MF TC:HDL ratio.

With exception of HbA1c, across all biomarkers no absolute agreement was observed between MF and VB values. Therefore, the strength of these findings is insufficient to support MF as a reliable alterative for VB measurement at the individual diagnostic level for T2D and CVD risk assessment. However, MF may remain useful in situations where VB collection is impractical or undesirable, such as in large scale population studies, longitudinal monitoring, or settings where non-invasive sampling may improve participant engagement and accessibility.

The findings from the linear regression models, where we explored whether MF can predict VB differs from Naseri et al. (2019), who found that MF biomarkers could be used to predict VB concentrations. The pilot study of 20 paired samples, reported very strong linear relationships between MF and VB for several biomarkers, with high coefficients of determination, for example, of HbA1c ($r^2 = 0.80$-0.90), indicating strong predictive ability. The findings from this study also found significant associations between MF and VB biomarkers. Specifically, MF HbA1c was significantly associated with VB HbA1c, however the regression model explained only 44.5% of the variance ($R^2 = 0.445$), indicating that over half the variability observed in VB HbA1c remained unexplained by MF values, as well as other variables added to the model such as age, BMI and ethnicity. Similar trends were found in the lipid markers LDL, HDL, TC, and TG. However, the proportion of explained variance was

substantially lower across all models, suggesting that the models are not suitable for predicting VB. Most measures required a quadratic term, suggesting a non-linear relationship between MF and VB. As a result, neither model in this study was considered suitable for prediction. The contrast in explained variance clearly indicates that despite statistically significant relationships, MF biomarkers cannot reliably predict VB values at the individual level. This suggests that there are factors beyond MF and demographic factors (Age, Ethnicity and BMI) that influence the variance observed.

A few differences between the methods likely contribute to the weaker predictive performance observed in the present study compared with Naseri et al. (2019). Firstly, the current study analysed a much larger sample (n = 90 paired samples) compared with 20 paired samples in Naseri et al. (2019). The use of larger sample sizes reduces the influence of outliers and provided more stable estimates of model performance. Furthermore, the lower R^2 observed here may reflect a more accurate estimate of the true predictive relationship between MF and VB biomarkers. In smaller pilot studies, such as Naseri et al. (2019) strong linear relationships may be influenced by a limited number of highly correlated observations. Another difference relates to sample collection and processing. Naseri et al. (2019) analysed MF using DBS, whereas the present study utilised whole MF samples. Dried blood spot is known to stabilise analytes and limit degradation during transport and storage, whereas whole MF may be more susceptible to dilution, cellular breakdown, or enzymatic degradation prior to analysis (Malsagova et al., 2020; Salzmannet al., 2021). Additionally, Naseri et al. (2019) reported their menstrual blood samples contained no visible clots, whereas some samples in the present study did contain clots, which may have influenced biomarker measurements. These factors may contribute to increased variability and reduced predictive accuracy in whole MF measurements, thereby lowering the proportion of variance explained in the regression models. However, with only

Naseri et al. (2019) small pilot study and the larger present study having explored this relationship, the author recognises that further research is required to confirm these findings.

Differences in participants characteristics may also have contributed to the discrepancies observed between studies. Naseri et al. (2019) recruited a small, relatively uniform cohort of young healthy women, compared to the present study including a much larger more diverse population. Greater biological variability in age, BMI, and ethnicity may reduce the strength of linear prediction models, particularly when assessing biomarkers related to CVD and T2D. Together, these differences in population and methods provide an explanation for the reduced predictive performance of MF biomarkers in this study, despite the presence of statistical associations. We found that MF alone was not a strong enough predictor for VB, even after adjustment for age, ethnicity and BMI. Therefore, MF biomarkers cannot predict VB biomarker values. This may be due to important unmeasured variables such as MF composition and sample handling. An early study reported that approximately 36% of MF volume comprises of blood, with endometrial tissue, cervical mucus, and cell components making up the rest (Fraser et al., 1985). A more recent study similarly states that MF contains around one-third of blood (Wulandari & Hapsari, 2024). Given then substantial differences between MF and VB composition, dilution of systemic blood within MF may have contributed to lower biomarker concentrations in MF compared with VB. The effect of dilution may also increase inter-individual variations, as a result reducing the ability of MF to reliably reflect VB levels.

Secondary analysis was completed to understand if collection day impacted biomarker levels across the bleed period in both MF and VB. We found that MF biomarkers did not change over the bleed period, thus timing of MF collection did not influence biomarker concentrations. This is a strength that we explored as previous literature has noted variability in biomarker readings

throughout the MC, dependent on the phase due to hormonal fluctuations (Mumford et al., 2010; Vashishta et al., 2017). However, limited studies have specifically looked into biomarker concentrations during menstruation. This study is one of the first to find that timing of MF sample collection within a single menstrual cycle (days 1-2 vs 3-5) does not meaningfully affect differences between MF and VB for any biomarker, suggesting that MF-VB discrepancies are consistent across menstruation. However, it remains unclear whether this consistency is maintained across cycles within individuals. As such, further research is required to assess cycle-to-cycle variability and determine whether biomarkers levels remain stable within individuals over time. While these findings suggest that precise timing of sample collection within a cycle may not be necessary, variability across cycles may still influence the reliability of MF as a diagnostic method.

Overall, these findings suggest that, at a POCT individual level, MF cannot be used as an alternative to VB for assessing key biomarkers used in T2D and CVD risk screening at this point in time. This also highlights the need to critically consider the limitations of MF sampling itself. It remains unclear whether single MF sample is representative of an individual's typical physiological state, or whether substantial intra-individual variability exists across cycles. If biomarker concentrations fluctuate over time, this may reduce reliability and limit the ability of MF to accurately reflect underlying health status. Future work is needed to understand absolute agreement between the two sample types, alongside the reproducibility and reliability of MF measurements. This should include efforts to standardise collection methods, improve consistency of more variable biomarkers, and explore factors influencing differences between MF and VB. Further research in more diverse populations is also required to fully assess the clinical applicability of MF as an alternative to VB.

3.6 Conclusion

Menstrual fluid was relatively associated with VB for several key biomarkers. Despite being a practical minimally invasive alterative for venepuncture, no absolute agreement was observed between MF and VB. Therefore, MF cannot currently replace VB for POCT for CVD and T2D risk screening. Further research should explore specific reference ranges for biomarkers in MF, refine collection methods, understand the factors that alter MF composition in comparison with VB, and validate the measurement of biomarkers in MF in larger and diverse populations.

Chapter 4: Conclusion and Recommendations

4.1 Achievement of Aims and Hypotheses

The present study aimed to explore the level of agreement between menstrual fluid (MF) and venous blood (VB) for point-of-care testing (POCT) across key biomarkers associated with type 2 diabetes (T2D) and cardiovascular disease (CVD) risk in healthy premenopausal women in Aotearoa New Zealand. This chapter discusses how the findings address each objective and hypothesis of the study.

It was hypothesised that glycated hemoglobin (HbA1c) values measured from MF would show strong agreement with those from VB. This hypothesis was not achieved, as MF and VB HbA1c values showed strong relative associations but not adequate absolute agreement. However, the individual differences between samples fell within defined limits of agreement (LoA) considered clinically acceptable, and this biomarker displayed the strongest agreement among all biomarkers assessed. These findings suggest that HbA1c in MF has the most potential and can be measured reliability from MF in healthy premenopausal women, which is encouraging for future clinical and research applications.

Secondly, it was hypothesised that lipid profiles and C-reactive protein (CRP) values would show lower levels of agreement between MF and VB. This was reflected in several lipid parameters, such as low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), total cholesterol (TC), and TC:HDL ratio, whereby relative association were observed but absolute agreement was limited. Furthermore, CRP was removed from statistical analysis because most samples were below the lower limit of detection of the POCT device used by the study. While some consistency was observed, greater variability in these markers indicates that MF may not yet be a suitable substitute for VB sampling for lipid

measures. The lower agreement observed for lipid measures may partly be explained by MF samples having other components beyond blood that could potentially interfere with the concentrations of the lipids measured (Loef et al, 2021). The physiological differences could account for the variation observed between MF and VB measures.

The final objective explored how demographic factors influenced differences in VB biomarker measurements. The present study's hypothesis was that women with a high body mass index (BMI) and women who were older were more likely to have elevated lipid parameters and CRP. However, results did not meet the hypothesis. It was noted however, that inclusion of demographic variables showed subtle improvement in the linear regression predictive performance for a few biomarkers. These improvements did not demonstrate statistically significant predictions though. Overall, BMI and age were not significantly independent predictors of VB biomarker concentrations. These findings suggest that demographic factors had a limited influence on biomarker variability in this cohort. Within this healthy premenopausal cohort, demographic effects on biomarker differences were subtle, reinforcing the need for future studies with larger more diverse samples. In particular, actively recruiting individuals with T2D or CVD would enable more informative comparisons, and allow for categorisation of participants to assess diagnostic performance through sensitivity and specificity analyses.

4.2 Strengths

One main strength of the study is that to the best of our knowledge, it is among the first to look at the agreement between MF and VB biomarkers specifically in a healthy premenopausal population. Additionally, this study is one of the first studies to utilise whole blood analysis when comparing MF and VB samples. The biomarkers we explored are commonly used in

clinical practice to screen for T2D and CVD. Therefore, these results are directly relevant to how these tests are undertaken in clinical practice.

Another important strength to note is that paired MF and VB samples were collected at the same time. This allowed direct comparison of biomarker levels within the same person and reducing variability due to factors such as circadian rhythm, lifestyle and feeding. Additionally, all analyses were done using acute POCT device (COBAS B® 101 analyser) that is already widely available in clinics and requires very small volumes of blood. This means findings of this study could realistically inform future use of MF as a minimally invasive option for screening in everyday healthcare settings at a population level.

Finally, the study included a broad panel of biomarkers relevant to diabetes and cardiovascular health which covers key indicators used in clinical screening. This is combined with a sample size of 102 participants, which is relatively large for a lab-based study with paired sample collection, increasing study reliability. Even though there was no absolute agreement between biomarkers in MF and VB, the research design attested the feasibility of the procedure to collect MF in a clinical setting, showing it could potentially be used alongside VB as part of routine clinical practice.

4.3 Limitations

The participants included in this study were healthy premenopausal women, meaning they had low risk of comorbidities. This may have influenced the outcome of the demographic results and contributed to the subtle differences in biomarkers. Therefore, these findings only reflect a small group within the whole population, which do not relate to more diverse health

conditions and ages. Future research should explore a more representative higher risk population and a wider range of concentrations for those biomarkers.

There was limited ethnic grouping within this study. Although there was a range of participants with different ethnic backgrounds, the sample sizes of some ethnic groups were not represented well within the results. Participants were grouped roughly into two categories (European and Other). While this was necessary for statistical analysis it may have masked differences between individual ethnic groups. Future studies could look at more focused ethnic representation, to better understand the role ethnicity plays in biomarker measurements.

Finally, the composition of MF was not explored within this study. Several samples presented with collection challenges due to limited volume and presence of blood clots, which may have affected biomarker concentrations. The proportion between menstrual blood and other fluids also varied greatly among samples, which was easily noted while handling the samples. Most previous studies have used dried MF oppose to whole fluid which this study utilised. Therefore, considering whole MF composition in future studies could help understand more factors affecting biomarker levels and lead to an improvement in MF analysis and interpretation.

4.4 Recommendations and Future Directions for Research

This research provides an initial foundation for understanding the agreement between MF and VB biomarkers in healthy menstruating women. Additionally, the research highlights methodological and biological considerations that should be addressed before MF can be reliably translated into clinical or research practice. Future areas for research include:

- Longitudinal study designs should be prioritised. The cross-sectional study design of the present study limits the ability to interpret biomarker variability across the MC. Multiple samples collected across different cycles would allow for better understanding

of biomarker concentrations, hormonal fluctuations and identify intra-individual differences, which is crucial for determining whether MF measurements are stable enough for screening and diagnostic purposes. Understanding intra- and inter-cycle variations would help establish whether a single MF sample is representative of an individual's typical biomarker profile.

- Larger and more diverse cohorts, including individuals with T2D and CVD, to enable more clinically meaningful comparisons and assessment of diagnostic performance encompassing a wider range of concentrations for the target biomarkers. Further investigation into the reproducibility, validity, and reliability of MF sampling is also needed. Particularly, whether a single sample is representative of an individual's biomarker profile. Repeated sampling across cycles would help determine intra-individual variability and assess suitability for point-of-care testing (POCT).
- Expanding participant recruitment is required. The cohort in the present study included healthy premenopausal women, from Auckland, New Zealand. By extending recruitment across Aotearoa New Zealand and internationally, it would enable a wider demographic in age, ethnicity, and health status. This would improve ecological validity and clarify whether MF biomarkers preform similar across clinically relevant populations.
- Further exploration into MF composition is needed. Considerable variation in mucus content, clotting, and volume was noted between participants, with previous literature reporting differences in protein content, cellular composition, and viscosity compared to other biological fluids. These unique fluid characteristics may influence biomarker concentrations and measurement accuracy. A more comprehensive understanding of MF composition could inform development of optimal collection, handling, and methods, to improve data quality and consistency.

- Related to this, standardisation of MF collection, processing, and storage protocols is needed. For example, the use of dried blood spots (DBS) for biomarker analysis versus the use of whole blood biomarker analysis. Variations in current methodologies limits the ability to compare results across studies and makes interpretation of agreement with VB samples complicated. Establishing a consistent procedure for collection, processing and storage would allow meaningful comparison between studies.
- Future research should also consider other lifestyle and biological factors, including nutrition, contraceptive use, and physical activity on MF biomarkers. Accounting for these factors may enhance real world application and help determine whether MF biomarkers are suitable for clinical screening.
- Importantly, MF specific reference ranges will need to be established. Many existing "normal" thresholds are derived from mixed or male dominated populations and may not be appropriate for MF measurements. For example, HbA1c concentrations measured in MF tend to be lower than those measured in VB. This suggests that lower MF specific thresholds may be more appropriate. Rather than applying the current VB target for prediabetes (< 40 mmol/mol), a MF specific threshold slightly lower (e.g. 38.27 mmol/mol, based on the mean difference of -1.73 mmol/mol observed in this study) may better reflect MF physiology and improve clinical interpretation. Developing MF specific reference values could strengthen the potential of MF as a future diagnostic tool.
- Finally, consistency in terminology should also be addressed. Multiple terms are being used in the literature, such as menstrual blood or menstrual effluent. A range of terms can create confusion. The use of standardised terminology when referring to MF would improve clarity regarding the biological make up being measured and allow reliability when comparing between studies.

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Appendices

Appendix A: Participant Information Sheet

Participant Information Sheet

Our Flow: Understanding Menstrual Fluid Loss in Premenopausal Females

Lead [Researcher / Study Doctor]: Dr Claire Badenhorst

Study Site: Massey University Auckland

Contact phone number: (09)414 0800

Ethics committee ref.: Northern B

You are invited to take part in a study investigated health markers in menstrual fluid and total menstrual fluid lost during menstruation. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason, and it won't affect the care you receive. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is 9 pages long, including the Consent Form. Please make sure you have read and understood all the pages.

VOLUNTARY PARTICIPATION AND WITHDRAWAL FROM THIS STUDY

You are under no obligation to accept this invitation, participation in the study in voluntary. Should you choose to participate, you have the right to:

- Withdraw from the study at any time, even after providing consent (if you choose to withdraw you cannot withdraw your data from the analysis after the data collection has been completed).
- Ask any questions about the study at any time during participation.

What is the purpose of the study?

When we look at our healthcare systems there is a noticeable female disadvantage. For chronic lifestyle diseases (e.g., diabetes, cardiovascular disease), females are consistently undertreated.

Why is this?
It could be because you may have had a bad experience at the doctor's (e.g. discomfort, pain, or embarrassment), or you are experiencing increased financial and logistical strain, or maybe you consider the health and well-being of others above your own. But our health needs as females should be something we think about throughout our lives. This research is the first step to creating a new and exciting way to help females have increased access to healthcare and knowledge.

As females, we will menstruate ~9-12 times a year, and our menstrual blood is a valuable resource that could contain a lot of health information. This study will be the first to look at how menstrual blood compares to a normal blood test when we complete health screening for diabetes and cardiovascular disease. This will be the first step to our understanding if one day, having a period will allow us also to check in on our health and complete our own health testing in the privacy of our homes.

A second part of this study, if you are interested, is measuring menstrual fluid loss. We are interested to know the volume differences between females and volume differences between cycles (i.e. change each month) for an individual. Research in this area is minimal, and our understanding and discussions on what we consider normal may be a major barrier to us getting medical support when we need it. Understanding menstrual fluid loss will help us provide information and educational resources to females so that we can help increase our menstrual cycle knowledge and awareness.

How is the study designed?

Once you have read the information sheet and provided informed consent, the research team will schedule your first appointment during menstrual bleeding. Before this session your personal menstrual disc or cup will be sent out to you or can be picked up from Massey University.
In your first session, you will be asked to complete an online survey to collect general health information. We will ask about your age, ethnicity, diet, medical history, and reproductive health (including your current perception of menstrual fluid loss). The researchers will then complete a blood pressure measurement and a body composition assessment. We will measure your height, weight, muscle, and fat mass. This assessment will be done using a Bioelectrical Impedance Analyser (BIA). The researcher will then have you rest for 5 minutes before collecting a venous blood sample (~1-2 tsp in volume). After we have collected this blood sample, we will provide you with, a sample collection dish (e.g. kidney dish) and a blacked-out small tub. We will direct you to a private bathroom facility and ask that you change/ empty the menstrual disc/cup into the collection dish, then transfer some of the sample into the backed-out tub. Once the tub is sealed, you can wash the collection container and tub in warm water and reinsert the disc as required. We will ask that you leave the sample and collection dish in the bathroom. To finish we will ask you to complete a short survey (3 questions) before providing you with a $50 voucher.

If you are interested in the menstrual fluid volume study, please inform the research officer at study sign-up. At the end of the first session, you will be provided with a menstrual blood measurement kit (including a digital scale, and kidney dish), instructions on menstrual disc/cup insertion/wear, hygiene, and instructions on how to complete the data collection required for this part of the project.

During menstruation, we would like you to use the disc/cup continuously day and night. We ask that you remove the cup every ~6-8 hours (once in the morning and once at night). At each change, collect the disc/cup and all menstrual fluid into the kidney dish provided. You will then place the kidney dish on the digital scale, record the weight (grams), and take a picture of the scale measurement (grams) to

confirm the weight. You can then discard the menstrual contents as per your personal preference, wash the cup with warm water only, and replace the cup/disc. This will be repeated until menstrual bleeding has stopped for that cycle. You will also be asked to record any spillage of the contents of the menstrual cup/disc each day and night. To do this, you will provide a rating of menstrual cup leakage as non-existent, minimal, moderate, and significant at each measurement point. At the end of 3 cycles worth of data collection, a $50 voucher will be sent out to the participants as koha.

At the end of each menstrual bleed, we will again ask you to provide your perception of your menstrual fluid loss volumes and if you consider yourself a minimal, normal, or heavy menstrual bleeder.

You will be asked to monitor your menstrual symptoms for the 3 cycles when you are collecting data. The details that will be collected include:

- Timing: the day when the menstrual symptom occurred
- Type: The symptoms that you personally experience
- Intensity/Effect of symptoms: rating on a scale of mild, moderate, and severe of how the symptom affected you.

WHO CAN TAKE PART IN THE STUDY?

- Healthy females aged 18-40 years, both naturally menstruating females and hormonal contraceptive users who have a menstrual bleed
- Have access to a smartphone
- Willingness to use a menstrual cup or disc

What will my participation in the study involve?

First in-person session 45 minutes including online survey, blood pressure and body composition assessment, venous blood sample and menstrual fluid sample.

- If you choose to complete the second part of the study, total time will be: Approximately 10 minutes per day on menstrual bleeding days (3-7 days depending on person)
- Less than 5 minutes per day for 3 full menstrual cycles (online symptom tracking, recording body temperature, and providing the results of urinary ovulation test)
- Number of menstrual cycles: 3

At the end of both studies, we will also ask you to rate and provide comments on your perceptions of menstrual discs/cups and their use during the study. You will be asked to provide comments about

- 1) the study protocols and future recommendations,
- 2) what you learned about your menstrual cycle during the study, and
- 3) what educational resources you would like to receive and what information you would like to be provided in these resources.

WHAT WILL HAPPEN TO MY BLOOD SAMPLES?

The data collected for this project, both physical blood samples and online questionnaires, is confidential and will be de-identified and coded, so no individual or personal data will be identified. All your data will then be stored under this identification number, this is to maintain the confidentiality of the data and secure storage.

The menstrual blood samples will be analysed onsite at Massey University for markers of inflammation, cardiovascular disease, and diabetes. The remaining sample will treated to protect the DNA material and will be stored at Massey University. These stored samples will be used in future research looking to determine if menstrual blood can be used to screen for reproductive disorders (e.g. endometriosis,

PCOS) and reproductive cancers (e.g. cervical, ovarian and endometrial). All samples will be stored under participant identification number and will be stored for 10 years.

You may hold beliefs about a sacred and shared value of all or any tissue samples removed. The cultural issues associated with sending your samples overseas and/or storing your tissue should be discussed with your family/ whānau as appropriate. There are a range of views held by Māori around these issues; some iwi disagree with storage of samples citing whakapapa and advise their people to consult before participating in research where this occurs. However, it is acknowledged that individuals have the right to choose.

What are the possible risks of this study?

We do recognise that there are some potential psychological risks associated with participation. These include discomfort with body composition measurements and collection/recording of menstrual cycle data. To reduce any discomfort, we will:

- Complete the body composition assessment in a private room with a single researcher present. You will only need to remove your shoes and socks for this assessment.
- You will report your menstrual cycle data on your personal device and at a time/place that suits you (e.g., at home). This gives you the privacy to complete this data entry in a space that you feel comfortable in.

  We will provide private facilities for you to collect your sample in the lab. Containers will be blacked out and you will not have to return the sample to the researcher. You can leave it in the bathroom and once you have left, we will go in and retrieve it for you. This is to help reduce any discomfort you may have with this initial format of testing.

WHAT ARE THE POSSIBLE BENEFITS OF THIS STUDY?

You will be provided with your own Hello Period Ltd menstrual cup/disc and Massey resources (scale, kidney dish). You will be able to keep using your menstrual disc/cup during and after the study has been completed. You will be able to provide insights into the methods used in this study and contribute to building resources that may be used by health professionals to measure menstrual fluid loss. You will receive a report that summarises the main findings of the project. You will have access to your blood pressure and body composition data.

Will any costs be reimbursed?

There will be no cost for any of the procedures or assessments that are taken as part of this study. All participants will be provided with a $50 supermarket voucher at the in-person visit. Participants involved in the menstrual fluid volume study will be provided with all equipment that will remain as their personal property after study completion, valued at $100. In addition, at the end of 3 months of data collection a $50 supermarket voucher will be sent out to each individual to thank them for their support.

What if something goes wrong?

Blood tests results that require medical attention will be provided to you the participant so that you may take this information to your personal medical professional (e.g. GP).

If you were injured in this study, you would be eligible **to apply** for compensation from ACC just as you would be if you were injured in an accident at work or at home. This does not mean that your claim will automatically be accepted. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

What will happen to my information?

During this study the research staff will record information about you and your study participation. This includes your blood test results and survey responses. You will have access to a summary of the project findings after project completion and you will be given access to any personal data (body composition, blood pressure, and blood tests results) after the project completion.

Identifiable Information

Identifiable information is any data that could identify you (e.g. your name, date of birth, or address). Only the research team will have access to your identifiable information.

De-identified (Coded) Information

To make sure your personal information is kept confidential, information that identifies you will not be included in any report generated by the researcher. The researchers will keep a list linking your code with your name, so that you can be identified by your coded data if needed.

The results of the study may be published or presented, but not in a form that would reasonably be expected to identify you. Only group averages will be presented in reports and publications.

Future Research Using Your Information.

If you agree, your coded information may be used for future research related to menstrual fluid loss. If you agree, your coded information may also be used for other medical and/or scientific research that is unrelated to the current study. For both conditions, we will ask that you provide consent.

This future research may be conducted overseas. You will not be told when future research is undertaken using your information. Your information may be shared widely with other researchers or companies. Your information may also be added to information from other studies, to form much larger sets of data.

You will not get reports or other information about any / some research that is done using your information.

Your information may be used indefinitely for future research unless you withdraw your consent. However, it may be extremely difficult or impossible to access your information, or withdraw consent for its use, once your information has been shared for future research.

Security and Storage of Your Information.

Your identifiable information is held at Massey University during the study. After the study it is transferred to a secure archiving site and stored for at least 10 years, then destroyed. Your coded information will be entered into electronic case report forms and sent through a secure server to the sponsor. Coded study information will be kept by the sponsor in secure, cloud-based storage indefinitely. All storage will comply with local and/or international data security guidelines.

Risks.

Although efforts will be made to protect your privacy, absolute confidentiality of your information cannot be guaranteed. Even with coded and anonymised information, there is no guarantee that you cannot be identified. The risk of people accessing and misusing your information (e.g. making it harder for you to get or keep a job or health insurance) is currently very small, but may increase in the future as people find new ways of tracing information.

This research includes basic information such as your ethnic group, age range, and sex. It is possible that this research could one day help people in the same groups as you. However, it is also possible that research findings could be used inappropriately to support negative stereotypes, stigmatize, or discriminate against members of the same groups as you.

Rights to Access Your Information.

You have the right to request access to your information held by the research team. You also have the right to request that any information you disagree with is corrected.

If you have any questions about the collection and use of information about you, you should ask Dr Claire Badenhorst

Rights to Withdraw Your Information.

You may withdraw your consent for the collection and use of your information at any time, by informing lead researcher.

If you withdraw your consent, your study participation will end, and the study team will stop collecting information from you.

Information collected up until your withdrawal from the study will continue to be used and included in the study. This is to protect the quality of the study.

Ownership Rights.

Information from this study may lead to discoveries and inventions or the development of a commercial product. The rights to these will belong to Dr Claire Badenhorst. You and your family will not receive any financial benefits or compensation, nor have any rights in any developments, inventions, or other discoveries that might come from this information.

Māori Data Sovereignty

Māori data sovereignty is about protecting information or knowledge that is about (or comes from) Māori people. We recognise the taonga of the data collected for this study. To help protect this taonga:

We have consulted with Associate Professor Beven Erueti about the collection, ownership, and use of study data. In consideration of Article Two of Te Tiriti o Waitangi when referring to the term taonga (gift or prized possession), we acknowledge that this refers to both tangible and intangible possessions/assets. Hence bodily fluid as a tangible taonga needs to be regarded and valued in scientific research as a 'gift/prized possession'. Before consent, is given, a discussion on the on-site and at-home blood sample collection methods will be had with all participants. This is to make sure that the study protocols consider and respect your cultural values and practices. All Māori and non-Māori wahine will be encouraged to discuss with the researchers the methods of the projects. We want to be sure that all wahine in our study are empowered through the data collection and can flourish within their community and Whānau (Ōritetanga/Equity-Equality). We will provide you with details on how we will store the samples, and how we will be disposed of them. You will have the right to an act of Tikanga before any sample collection. This can be in any form that allows you to acknowledge the tapu nature of bodily fluids you have provided us e.g., karakia (blessing), whakatau (introduction), and tautoko (support). When giving consent you can select to have a Karakia with the disposal of remaining blood cells. This will be performed by Apirana Pewhairangi, the Senior Advisor Tikanga Māori and Māori Engagement at Massey University, Albany Campus.

We allow Māori organisations to access de-identified study data, for uses that may benefit Māori.

What happens after the study or if I change my mind?

If you wish to withdraw from the study, you can do so at any time by notifying Dr Claire Badenhorst. If you choose to withdraw you cannot withdraw your data from the analysis after the data collection has been completed unless specifically requested. Data will then be removed and disposed of appropriately.

Can I find out the results of the study?

You will receive a report that summarises the main findings at study completion.

Who is funding the study?

The project has been funded by the Health Research Council of New Zealand. This funding has been provided to the research team, led by Dr Claire Badenhorst at Massey University.

WHO HAS APPROVED THE STUDY?

This study has been approved by an independent group of people called a Health and Disability Ethics Committee (HDEC), who check that studies meet established ethical standards. The Northern B Committee has approved this study.

Who do I contact for more information or if I have concerns?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Dr Claire Badenhorst (School of Sport, Exercise, and Nutrition)
C.Badenhorst@massey.ac.nz
(09)414 0800

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050
Fax: 0800 2 SUPPORT (0800 2787 7678)
Email: advocacy@advocacy.org.nz
Website: https://www.advocacy.org.nz/

For Māori cultural support please contact:
Associate Professor Bevan Erueti
Phone: +6469516087
Email: B.Erueti@massey.ac.nz

You can also contact the health and disability ethics committee (HDEC) that approved this study on:
Email: hdecs@health.govt.nz
Phone: 0800 400 569 (Ministry of Health general enquiries)