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An Open-Label Trial Protocol to Test Menstrual Cycle Effects and Tolerance to Low-Dose Psilocybin in Healthy Menstruating Persons.

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Transparency Declaration

This manuscript is an honest, accurate and transparent account of the trial that will be conducted.

Keywords:

1. Menstrual cycle
2. Psilocybin
3. Pharmacokinetics
4. Pharmacodynamics
5. Electroencephalography

Abstract

Introduction: Research on the therapeutic benefits of psychedelics, particularly psilocybin, is becoming increasingly popular. Unfortunately, most psychedelic research overlooks the menstrual cycle as a biological variable, raising concerns about the safety and efficacy of findings for individuals who menstruate. Given the established effects of estradiol and progesterone (and metabolites) on serotonin receptor density and function, it is plausible that the subjective experience, efficacy, and side effect profile of psychedelics may vary significantly across the different phases of the menstrual cycle. Furthermore, the tolerance effects of psilocybin when taken on consecutive days are unknown. The PsiMen trial comprises two sub-studies: PsiMen-M, which will assess the potential for the menstrual cycle to affect the response to low-dose psilocybin in healthy menstruating persons, and PsiMen-T, which will test for potential tolerance effects.

Methods: Psilocybin (5 mg) will be administered during different menstrual cycle phases in 21 healthy participants for PsiMen-M, and 12 for PsiMen-T. Effects on subjective experience, brain/cognitive function, and pharmacokinetics will be measured by the Drug Effects Questionnaire, exploratory task-based electroencephalography (EEG), and blood plasma sampling. Safety and any clinically significant modifications of psilocybin will be monitored by laboratory and vital signs tests. Tolerance to psilocybin will be assessed by administering the drug for three consecutive days.

Conclusion: This study will determine whether low-dose psilocybin effects vary across the menstrual cycle, addressing a key gap in psychedelic research. Findings may inform the development of serotonergic treatments for menstrual-cycle-related mood disorders and improve the design of future psychedelic trials by accounting for hormonal fluctuations.

Introduction

Psychedelics and the serotonin system

Research on psychedelics has grown rapidly in recent years, particularly with respect to the classical serotonergic psychedelics such as psilocybin and lysergic acid diethylamide (LSD) as treatments for a range of neuropsychiatric conditions (1, 2). Classical psychedelics such as psilocybin have been shown to have a high binding affinity to serotonin receptors such as the 5-Hydroxytryptamine type 2A (5HT2A) receptor, a subtype of the serotonin receptor family (3). The 5HT2A receptor is believed to be the main receptor responsible for the striking psychological changes that occur after drug administration and more generally, the 5HT2A receptor is involved in many biological, neurological and psychiatric processes including cognition, appetite regulation, emotion, and mood (4, 5). The 5HT2A receptor is also implicated in the pathology of many cognitive and learning disorders including depression, schizophrenia and obsessive-compulsive disorder (6, 7).

Psilocybin is a lipophilic prodrug of psilocin. When ingested orally, it dephosphorylates to psilocin. Psilocin can cross the blood-brain barrier more readily than psilocybin (8) and acts as a partial agonist to the 5HT2A serotonin receptor (9). When Psilocin is bound to the 5HT2A receptor, it can potentially produce anti-depressive and anxiolytic effects (10, 11). Psilocybin has been studied in cellular, animal and human models all which have suggested that psilocybin has the ability to significantly alter brain dynamics (12-16)

Psilocybin is emerging as a more popular psychedelic agent than LSD in clinical research (17). Psilocybin's shorter duration of action, typically 4 to 6 hours compared to LSD's 8 to 12 hours, make it more practical for use in structured therapeutic sessions (18). Unlike LSD, which is fully synthetic, psilocybin is a naturally occurring compound found in various species of mushrooms, contributing to its perception as a more "organic" and traditional medicine (19). In the context of microdosing, psilocybin has taken over as the preferred substance by many users (20). Following early indications of potential effectiveness of psilocybin for mental health conditions (21, 22), there are now two Phase 3 drug development programmes investigating psilocybin therapy as a treatment for depression (23, 24). Indeed, the clinical development of psilocybin is at such an advanced stage that in 2025, MedSafe in New Zealand (25) set-up an authorised prescriber scheme to allow psychiatrists to prescribe psilocybin for treatment-resistant depression without a formal product approval (26). This was made possible by the fact that psilocybin is not a new chemical entity and can be manufactured to good manufacturing practices standards as a generic medicine.

The serotonin system and the menstrual cycle

Menstruation is a roughly monthly cycle regulated by the interaction of sex steroid hormones produced by the hypothalamus, pituitary and ovaries (27). A median healthy menstrual cycle length is 28 days, ranging between 21 to 35 days across most women (28). The cycle can be split into two main phases: the follicular and luteal phases. Each of these phases have their own physiological significance that are governed by changes in hormones (27). In particular, both 17β -estradiol (E2) and progesterone (P4) are low during the follicular phase, E2 is high during the ovulatory phase and both E2 and P4 are high during the luteal phase.

Fluctuations in sex steroids E2 and P4 (alongside their metabolites) modify the function and density of receptors and transporters of many neurotransmitter systems within the central nervous system (CNS), including the serotonergic systems (29). Interestingly, the 5HT_{2A} serotonin receptor that psychedelics (LSD, psilocybin) exert their main psychological effects through, is sensitive to sex steroid changes across the menstrual cycle (30, 31). Many pre-clinical studies have shown that fluctuating sex steroids alter related neurophysiology within the CNS. For example, Sumner and Fink (32), Sumner and Fink (33) and Rybaczyk, Bashaw (31) have shown that E2 injections increased 5HT_{2A} receptor density in female rat forebrain and nucleus accumbens. Alongside preclinical evidence, a Positron Emission Tomography (PET) clinical study conducted in menopausal women found similar results, showing an increase in 5HT_{2A} binding potential after participants were given an injection of E2 with micronised P4 (30). Moreover, a comparative gene expression analysis study found that E2 inhibited the expression of the SERT gene, which increased the overall availability of serotonin in the synaptic cleft (31).

Despite the effect of sex steroid hormones on the serotonin system, most psychedelic research has not accounted for the menstrual cycle as a biological variable, raising concerns about the generalisability and safety of findings for people who menstruate (34). Given the established effects of E2 and P4 on serotonin receptor density and function, it is plausible that the subjective experience, efficacy, and adverse event profile of psychedelics may vary significantly across the menstrual cycle. This means that not investigating the menstrual cycle in psychedelic clinical trials may be problematic considering the therapeutic importance placed on the intensity and quality of the acute psychedelic experience in determining clinical outcomes (22). Therefore, integrating menstrual cycle considerations into psychedelic research is not only overdue but important to ensuring both efficacy and safety for all participants.

Menstrual-cycle-related modification of neurotransmitter systems including serotonin is thought to underlie menstrual-cycle-related exacerbation of some of the most prevalent

neuropsychiatric conditions, including depression, anxiety, epilepsy, migraine, schizophrenia, substance-use disorders, eating disorders as well as conditions like premenstrual syndrome (PMS) and a more severe form, premenstrual dysphoric disorder (PMDD) (35-37). The health burden of PMS and PMDD is substantial, with limited treatment options, negatively affecting work productivity, social relationships, and overall wellbeing (38, 39). In the absence of tolerable, effective treatments, some individuals are turning to self-medication with psychedelic microdosing, particularly with psilocybin. While anecdotal accounts—such as Ayelet Waldman’s *A Really Good Day* (40) suggest benefit, no clinical trials support these practices, highlighting the urgent need for rigorous research into novel treatments for PMS and PMDD. Notably, Sumner (41) conducted a qualitative interview study of 15 women who microdosed psychedelics to self-treat their PMDD, all using psilocybin. Most of these women in the study claimed to feel benefits but there was the absence of a clear regimen being used.

An added challenge to understanding the role of the menstrual cycle in mediating the effect of psychedelic microdosing is tolerance with repeated dosing. Two protocols that have been commonly used by psilocybin microdosers are the Fadiman protocol of dosing every third day and the Stamets protocol of dosing five days in a row with two days break (42). The degree to which tolerance effects might occur is unknown and has not been explored with psilocybin in the literature, although informally users report that tolerance effects might occur. Studies from the 1950s show that tolerance effects do occur with LSD across multiple successive days of use and disappear rapidly on discontinuation (43, 44). To our knowledge, no studies, however, have been found investigating tolerance effects with psilocybin. Understanding the existence of tolerance effects will be important in providing information about potential microdosing regimens that could be tested in clinical populations such as those with severe PMS/PMDD.

Objectives of the trial

The core aim of this trial, named PsiMen, will be to address the underrepresentation of the menstrual cycle as a variable of interest in psychedelic research. Given that psilocybin influences the serotonin system, there is potential for the drug to have differing effects depending on the phase of the menstrual cycle. In addition, the tolerance of low dose psilocybin will also be assessed via administration on consecutive days. As such, the main objectives of this trial are to 1) To test for menstrual cycle effects in response to low doses of psilocybin and 2) To test for tolerance effects in response to low doses of psilocybin given on consecutive days.

Methods

Overall study design

PsiMen is an open-label Phase 1 trial with two separate sub-studies (Figure 1 and 2). All the onsite study visits will occur at the Clinical Research Centre at the Auckland University Grafton Campus in Auckland. Sub-study One (PsiMen-M) will assess the effects of low dose psilocybin across the menstrual cycle with a total of 21 participants. Sub-study Two (PsiMen-T) will assess the tolerance effects of low dose psilocybin with a total of 12 participants. Participants that are willing to take part in the trial will first undergo screening procedures. Once passed, participants may choose to enter one of the two cohorts. Both cohorts include a Baseline period during which participant will complete a Daily Record of Severity of Problems (DRSP) for at least two full menstrual cycles tracking participants symptoms during different phases of their cycle. The DRSP is the most widely used symptom scale in menstrual cycle research and is the currently recommended measurement tool for clinical trials in PMDD (45, 46). The baseline visits will be conducted at the participants follicular, ovulatory and late luteal stages of their menstrual cycle where EEG data will be recorded at visits termed VBF, VBO and VBL, respectively. If the participant continues to be eligible after the baseline phase, they will undergo the dosing phase of the study.

On a dosing study day participants will be administered a 5 mg fixed oral dose of psilocybin. The overall design and structure of a dosing day will be similar to a baseline study day with the addition of two extra visits and an overall longer study day encompassing EEG tasks, multiple pharmacokinetic/pharmacodynamic (PK/PD) sampling points, and a Drug Effects Questionnaire (DEQ). For PsiMen-M, participants will come in for dosing study visits during their follicular (VAF), ovulation (VAO) and late luteal (VAL). For PsiMen-T, participants will come in for three consecutive dosing visits during their follicular phase (VAF-1, VAF-2, VAF-3). On the last dosing visit for both cohorts, a follow-up semi-structured interview will be conducted to understand the participant experience during the trial.

The authors assert that all procedures contributing to the work in this trial comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the [Helsinki Declaration](#) of 1975, as revised in 2013. All procedures involving human subjects/patients were approved by Health and Disability Ethics Committee (HDEC) – reference - 2025 FULL 23149 and the Standing Committee on Therapeutic Trials (SCOTT) - Reference: 2025 SCOTT 23215.

Participants

The participants entering the study will be from the Auckland region within New Zealand. All participants will be recruited from the local community, through advertisements placed in local newspapers, noticeboards, and online using social media. A link to a pre-screening questionnaire will be placed in the advertisements allowing participants to contact the study team. Pre-screening/screening processes are discussed in more detail below. Participants that decide to take part in the trial must meet the inclusion/exclusion criteria (below) and adhere to the lifestyle changes for the duration of the trial (Tables 1 and 2). Participants will receive Koha (gift/donation) in recognition of the time and commitment with taking part in the trial.

Participant inclusion and exclusion criteria

Participant inclusion and exclusion criteria are summarised in Table 1. Participants recruited must not have any unstable medical or neurological conditions. An investigator may discontinue or withdraw a participant from the study (post screening) if they meet the exclusion criteria - either newly developed or not previously recognised. Recruited participants will have a healthy menstrual cycle, be within the reproductive age range of 18-46 years, consent to the study procedures, and to the lifestyle considerations (Table 2).

Ages 46 and above is typically perimenopausal, thus participants above that age range will not be recruited. Participants will be considered to have a healthy menstrual cycle if they have a consistent cycle between 25-35 days long that they can track. For heterosexually active persons of child-bearing potential, the participant must not be pregnant (confirmed by a pregnancy test) and agree to use non-hormonal contraception during the Dosing period. Persons using hormonal contraception, who are pregnant, are planning to get pregnant, or are lactating will be excluded.

Participants with a BMI < 18 and > 35 will be excluded. Psilocybin does have sympathomimetic effects (47) and therefore, participants with a history of cardiovascular disorders including abnormal heart rate or blood pressure will be excluded also. Participants with significant renal or hepatic impairment and or polycystic ovary syndrome will also be excluded. Participants must also have no risk of suicide, no confirmed diagnosis of (or first degree relative with) schizophrenia or other psychotic disorder, or bipolar I or II disorder. Participants recruited must also have no substance use disorders, not take CNS active medications, or have had any use of serotonergic psychedelic drugs (LSD, psilocybin, DMT, etc.) within the last 3 months. Hallucinogen Persisting Perception Disorder (HPPD) is a rare condition linked to ingestion of psychedelic drugs consumption and has only rarely been documented in clinical trials (48). The low dose to be used in PsiMen is unlikely to trigger HPPD - however, the risk of triggering HPPD can be reduced by screening participants for substance use and excluding recent psychedelic use.

Screening

PsiMen screening has three phases: a pre-screening questionnaire, a video call, and an onsite screening visit. The pre-screening questionnaire is composed of questions related to having a healthy menstrual cycle, any history of psychotic/bipolar disorders, major medical conditions, intention of pregnancy, and participant age. A participant information sheet (PIS) will be emailed to participants who meet the required criteria based on their responses, with a version specific to their selected cohort.

The PIS contains important information about the trial including its purpose, what is expected from the participant, lifestyle changes imposed by the trial (Table 2), and the overall trial protocol. Participants that are willing to continue with the study are then invited to participate in a screening video call. Before this video call, participant will have provided written consent and demographics data including age, handedness, biological sex, gender identity, occupation and highest educational qualification. Ethnicity information using the New Zealand Census questionnaire item and a complete recreational drug use history will be taken including whether the participant has microdosed psychedelics before and the date of last psychedelic use prior to the video call.

At the screening videocall, the participant's medical history will be taken by the researcher. Participants' medical history will cover their past and current medical conditions, any chronic illnesses, allergies, or other physical health issues. Reproductive/menstrual health will be assessed with the Reproductive Status Questionnaire (RSQ) (49). Participants will also be required to fill out a Big Five Inventory 2 (BFI-2) questionnaire. The BFI-2 questionnaire will provide a validated measure of the 'Big 5' personality traits including openness, conscientiousness, extraversion, agreeableness, and neuroticism (50). The researcher will also conduct a Columbia-Suicide Severity Rating Scale (C-SSRS). The C-SSRS will be performed not only at Screening but at each visit prior to discharge. A 'Lifetime' version will be employed for the screening videocall and a 'Since Last Visit' version used for other visits. Participants will also need to declare any concomitant medications and for each visit clarify any new concomitant medications in their daily/dose-day questionnaires or any variations to their usual medications. Additionally, at the videocall, the researcher will conduct a brief clinical interview taking psychiatric history through a New Zealand modified version of the NM-ASSIST, and the Alcohol Use Disorders Identification Test (AUDIT).

Should the participant be eligible to continue after this session they will be invited to attend their first visit session where onsite screening will be completed. Onsite screening will include physical measurement of height, weight, body mass index, vital signs, 12-lead ECG, drug/alcohol breathalyser test, urine drug screen, a serum pregnancy test and laboratory blood tests. The laboratory blood tests will include a complete blood count, liver function test, renal function test, thyroid function test, vitamin B12, sex steroid levels and iron studies. Screening results will be confirmed with a physician before participant is recruited

into the trial. If the participant is eligible after confirmation by a physician, they will enter the baseline phase (45, 46).

Baseline phase

During this phase, eligible participants will be required to track their menstrual cycle for two cycles while completing the DRSP. They will also come in for three baseline study visits: VBF, VBO and VBL. Menstrual cycle timing and ovulation testing to determine what exact days the participant will be studied is elaborated below ('Menstrual cycle timing and procedures'). During a baseline study visit, resting and task-based EEG will be recorded including a Visual Long-term Potentiation (LTP) Paradigm (51), Doors task (52), Loudness Dependence of the Auditory Evoked Potential (LDAEP) task (53), Visual Gamma Paradigm (54), Mismatched Negativity Paradigm (MMN) (55) and the faces task (56) will be completed (this schedule of activities is summarised in Tables 3a,3b, 4a and 4b). At the end of the Baseline period, eligibility against the inclusion and exclusion criteria will be again reconfirmed given DRSP data and Carolina Premenstrual Assessment Scoring System (C-PASS) (57).

Dosing phase

Laboratory Dosing Period will differ based on the selected cohort. PsiMen-M will involve a dosing period of three (+) menstrual cycles, with three dosing visits: VAF, VAO, and VAL with each dosing session occurring in a sperate menstrual cycle. PsiMen-T will involve a dosing period of one menstrual cycle with three consecutive dosing visits (VAF1/VAF2/VAF3) during the follicular phase to test for tolerance effects. During the dosing visits, 5 mg fixed dose oral capsules of Psilocybin will be administered to the participants. Psilocybin in capsules will be provided Nature MedTech. There is no placebo in this trial. On the last dosing visit, a follow-up semi-structured interview will be conducted by the investigator to understand the participant experience during the trial.

The PK/PD of 5 mg oral psilocybin have not been described in the literature. However, using a PK sampling scheme similar to that used by Holze, Becker (58) to investigate larger psilocybin doses is appropriate for PsiMen. During each dosing visit (except VAF2; VAF3 optional), 4 ml of blood will be collected into Lithium Heparin tubes at baseline (0) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, and 7 hours after drug administration. This will be used for PK analysis. The participant will be fasted when they come in for their study visit, and then take the oral capsule of psilocybin with water, followed by a standardised breakfast to ensure the drug absorbance rate stays consistent between participants (59). Participants are instructed not to drive or engage in any dangerous activities for a six-hour window following dosing. Each time PK samples will be taken, participants vital signs, Resting EEG, and DEQ will be measured.

The DEQ measures and EEG tasks will be used to measure psilocybin PD. While task based and resting EEG will provide an objective measure of PD, the DEQ will measure perceptual changes. Several previous studies have reliably shown dose-dependent effects of small doses of the psychedelic LSD on the DEQ that are amenable to PK/PD modelling (60-67). PsiMen will be unique in examining whether the DEQ is modified across the menstrual cycle when given low doses of psilocybin. While the DEQ can be affected by a placebo response, our within-subjects design will mitigate this potential confound.

Resting and task-based EEG will measure the CNS penetration of psilocybin directly and determine whether this is modified across the menstrual cycle (68-70). The EEG tasks will be the same as the baseline phase (dosing visits schedules of activities are summarised in Table 3a,3b, 4a and 4b).

Before discharge on the dosing visit, the participant will complete a 5-Dimensional Altered States of Consciousness questionnaire (5D-ASC). This validated questionnaire will be used to assess the participants' subjective experiences of psilocybin. The 5D-ASC is commonly used and measures specific experiences the participant may have undergone during an altered state (71). The Clinician-Administered Dissociative States Scale (CADSS) will also be used on dosing visits by the investigator to assess any present-state dissociative symptoms, acute stress or altered states (72) that may have been induced by the study drug.

Menstrual cycle effects of low dose psilocybin have never been tested and therefore PsiMen will determine the adverse event (AEs) profile of healthy persons who menstruate taking the drug. Whether the low dose of psilocybin causes any clinically significant modification of objective safety measurements in healthy persons who menstruate will also be recorded. Safety objectives will be fulfilled by measuring any adverse events or serious adverse events during the trial (Table 5c).

Menstrual cycle timing and procedures

Considering menses as Day 1 of the participant's menstrual cycle, follicular study visits will occur on Days 5-9 relative to menses, and ovulation visits will occur the day of, or day after a positive urine ovulation test. Participants will use urine home ovulation kits to check for ovulation starting 3-5 days prior to expected LH surge. Participants will receive a reminder on the evening before they are meant to start testing and will be given an instruction sheet, a box of tests, and an optional urine collection cup. Luteal measurements will occur on Days 8-12 after ovulation. Blood samples will be taken at each study visit to ensure the hormone levels match the menstrual cycle study visit timings.

Menstrual cycles vary in length and can be anovulatory in some of cycles (27). PsiMen will use the best practice for prospective visit scheduling and retrospective evaluation of visit timing success by using a combination of self-report of menses onset, urinary LH and serum sex hormone measurement (49, 62). An average cycle length will be taken across 3+ months of consecutive cycle tracking for each participant. Previous menstrual cycle studies within

our laboratory have suggested that some degree of individual flexibility in terms of scheduling clinic visits is required.

Menses and Ovulation anchor date

Day 1 of the follicular phase is the day of the onset of menstrual flow, not including spotting. For ovulation, the peak of the LH surge will be the first calendar day a positive test was received, which means ovulation should occur 24-36 hours after this surge (73). Estimated average cycle length will be considered to make ovulation anchor date vs luteal length estimations (74). For example, for cycles 25-31 days this will represent day -13.

Statistical and power calculations

PsiMen is an exploratory experimental medicine study and is not intended to evaluate efficacy. Given the large number of measures and dependent variables that will be available for analysis this does create a multiplicity issue. The approach taken will be to interpret any uncorrected statistically significant results as exploratory in need of confirmatory replication.

Given the within-subject design of this trial, the main statistical model will be a one-way repeated measures ANOVA (3x1) (parametric/Friedman test as appropriate). Where available, baseline measures will be entered into the model as covariates. Analyses will be conducted within a per-protocol framework, with a primary focus on complete-case analysis including only participants who complete all study visits. Where dropouts occur, participants will be replaced until the full analysis set of 21 participants for PsiMen-M and 12 for PsiMen-T is obtained. The sample size is based on pragmatics of recruitment and is similar in size to previous Phase 1 trials given the non-clinical outcome measurements. In terms of sensitivity analysis [35], with $\alpha = 0.05$, $(1 - \beta) = 0.8$, $r = 0.5$ there is sensitivity to effects of size $f = 0.28$ (Cohen's $d = 0.56$) which is a moderate effect size.

This is the first study to investigate low dose psilocybin across the healthy menstrual cycle, and as such, there are no previous effect size estimates to base power calculations on. Given the desire to also explore secondary and exploratory outcomes with maximum power, the sample size was based on pragmatic reasons such as cost and potential ability to recruit participants.

Outcomes and Hypotheses

Broadly, we hypothesise that psilocybin effects will vary as a function of the menstrual cycle phase and associated fluctuations in sex hormones. Psilocybin acts primarily as a 5-HT_{2A} receptor agonist, and both estradiol and progesterone are known to modulate serotonergic signalling, including serotonin receptor density (30, 75). We therefore predict that higher

estradiol states (ovulation phase) may be associated with relatively enhanced sensitivity to psilocybin, whereas low estradiol states (follicular phase) and higher progesterone states (the late luteal phase) may attenuate or alter these effects. However, all of our directional hypotheses remain tentative, given the limited empirical literature directly examining these interactions in humans, and the potential influence of dynamic processes such as receptor desensitisation and tolerance.

Our primary outcomes focus on subjective drug effects, assessed using the DEQ (Table 5a). We hypothesise that psilocybin will produce measurable subjective effects across all dosing sessions (PsiMen-M: VAF, VAO, VAL), with greater subjective intensity (higher DEQ scores) in higher estradiol states, and potential attenuation across consecutive dosing days (PsiMen-T: VAF1-3).

Secondary outcomes assess pharmacokinetics (Table 5b). Psilocybin PK profiles are expected to be comparable across the menstrual cycle, with variation primarily reflecting within-session pharmacokinetic dynamics rather than hormonal state.

Exploratory outcomes include EEG and behavioural measures (Table 5d). Psilocybin is expected to modulate neural indices of neuroplasticity, excitation-inhibition balance and serotonergic function, with effects potentially varying by menstrual phase. Specifically, markers of plasticity (LTP and MMN) may be enhanced under psilocybin during the dosing visits and further modulated by estradiol (76, 77), whereas measures related to inhibitory tone (visual gamma oscillations) may show enhanced modulation via GABAergic activity during progesterone-dominant phases (54, 78). Serotonergic indices (e.g. LDAEP) are expected to be sensitive to both psilocybin and estradiol variation, and thus would be expected to be the most enhanced during dosing visits and more so in the ovulation phase. At the behavioural level, psilocybin is expected to alter emotional processing (Faces task) and reward-seeking behaviours (Doors task), although the direction of these effects remain exploratory. Additional exploratory measures include: 5D-ASC, CADSS, BFI-2 (see list of abbreviations for all the acronyms used (Table 6)), and a semi-structured qualitative interview.

Discussion

There is a growing field of research on the menstrual cycle and its effects on the neurobiology and function of the CNS, but many clinical trials and pharmaceutical companies still overlook the menstrual cycle as a biological variable (34). Ignoring the potential for drug effects to be modified across the menstrual cycle is potentially hazardous, as drugs could not only have suboptimal efficacy across different phases of the menstrual cycle, but also increased side effects (30, 31, 34).

The goal of PsiMen is to assess both the tolerability and feasibility of low dose psilocybin across the menstrual cycle in healthy females. PsiMen will help determine whether effects of

low doses of psilocybin are altered across the different phases of the menstrual cycle and if taking the drug consecutively poses any tolerance effects. Until now, no clinical trials have investigated both PK and objective PD of a CNS drug across the key points of the menstrual cycle, making this trial significant globally as a proof-of-principle. Studies that account for the menstrual cycle are very rare for drugs that target the CNS. If studies incorporated the menstrual cycle as a biological variable and significant effects were found, overall therapeutic outcomes from CNS medicines for people who menstruate could be adapted and potentially improved.

In terms of future research, as a Phase 1 trial, this study will lay the groundwork for a future clinical trial of psilocybin as a treatment for severe PMS/PMDD. Severe PMS/PMDD affects 13-18% of persons who menstruate (36) and is in fact the leading health burden for persons who menstruate (ahead of endometriosis and PCOS which receive far more research funding and attention) (79). Despite this, there are very few treatment options available for PMDD and few clinical trials investigating new therapeutics.

Overall, PsiMen will contribute important knowledge on the menstrual cycle and its interaction with CNS drugs. Specifically considering the serotonin system which has known interactions with the menstrual cycle and hormone fluctuations. Psychedelics are a fast-growing area of mental health disorder research, making this a particularly timely study to improve how people with a menstrual cycle are included and treated in the potential new therapies.

Limitations Section

We note that the study has several limitations at the outset. Firstly, the study has no placebo or active placebo control condition. As such, subjective responses (e.g., the DEQ) might reflect expectancy effects. However, eliminating this possibility in a repeated measures design require an extra three dosing sessions per participant, rendering the study infeasible to complete due to participant burden. Moreover, for placebo effects to be a confounder in this design, they would need to manifest as an interaction between placebo effects and menstrual cycle phase; to our knowledge, there is no literature to suggest the existence of such an effect.

Secondly, formal counterbalancing is not implemented due to practical scheduling constraints. Instead, we are relying on natural counterbalancing to occur (as cycle timing and participant/researcher/facility availability allow) although we can in principle adjust scheduling to correct any major imbalances, if they emerge.

Thirdly, our target sample size (21 completed datasets for PsiMen-M and 12 for PsiMen-T) has been determined by pragmatic constraints, although it is similar in size to other Phase 1 experimental medicine trials. It is possible that we are not powered to see small menstrual cycle or tolerance effects. Detecting such effects would likely require a substantially larger

trial, which is difficult to justify based on the current literature. Additionally, as effect sizes get smaller the clinical significance is also reduced.

Finally, in terms of future applications, this study is targeting volunteers with a healthy menstrual cycle and mental health status. The extent to which these findings will apply to clinical populations would still require empirical determination.

Publication policy

This study is registered at ANZCTR (registration number: ACTRN12625000991415), and primary results will be available within 12 months of the study/sub-study's completion. Additionally, every effort will be made to publish results in peer-reviewed journals.

Statement of Ethics

This trial has gained Ethics approval from Health and Disability Ethics Committee (HDEC) – Reference: 2025 FULL 23149 and the Standing Committee on Therapeutic Trials (SCOTT) - Reference: 2025 SCOTT 23215. Recruitment for this trial commenced in August 2025. Informed written consent was obtained from participants to take part in the trial.

Conflict of Interest

The authors have no conflicts of interest to declare

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Data Availability statement

No datasets were generated or analysed for this protocol article. All the materials that are associated with this protocol are described within this manuscript.

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Figure Legend

Figure 1: Schematic summarising the study design of the PsiMen-T cohort in accordance with the participant menstrual cycle, including the trial days that participants will be required to attend onsite visits.

Figure 2: Schematic summarising the study design of the PsiMen-M cohort in accordance with the participant menstrual cycle, including the trial days that participants will be required onsite.

Table 1: Participant inclusion and exclusion criteria

Inclusion criteria	
Consent and Demography	• Ability to understand, read and comprehend English language • Provision of signed and dated informed consent form. • Stated willingness to comply with all study procedures and availability for the duration of the study. • Agreement to adhere to Lifestyle Considerations throughout the trial duration – see Table 2. • Aged between 18-46 years
Reproductive	To have regular menstrual cycles, this includes participants that: • Self-reports knowing when it should be even if only general terms (e.g. usually in the last or first week of a month) • Reports occasionally or never missing this expected timing • Demonstrates trackability using ovulation testing for at least 2/3 cycle in Baseline phase • Menstruation has a defined start and finish day (some spotting is ok) • Average menstrual cycle length between 25-35 days. For heterosexually active persons of child-bearing potential, the participant must agree to use non-hormonal contraception during the Dosing period. Methods of contraception include: • Intra-uterine device (IUD) containing copper • Female sterilisation (e.g. bilateral tubal ligation ('clipping or tying tubes')) • Be in an exclusive relationship with a sterile male (vasectomy) • Use of prophylactic condoms
Exclusion criteria	
Mental health diagnosis	• Any item 1-13 of the PSST scoring above "mild" and any item A-E scoring above mild. • Any current psychiatric diagnosis. • Confirmed diagnosis of (or first degree relative with) schizophrenia or other

	psychotic disorder, or bipolar I or II disorder.
Current risk	• Risk of suicide as determined by The Columbia-Suicide Severity Rating Scale (C-SSRS).
Drug and substance use	• Substance dependence in the previous 6 months as assessed by clinical interview with a New Zealand modified version of the NM-ASSIST. • Problematic use of alcohol defined as a score on the AUDIT of 16 or greater. • Regular use of any CNS active medications in the last three months • Use of hormonal contraceptives/steroid hormones in the last three months • Regular use of any medication/supplements deemed to be contraindicating as judged by a study physician. • Treatment with another investigational drug /intervention within the last 3 months. • Use of serotonergic psychedelic drugs (LSD, psilocybin, DMT, etc.) in the last 3 months.
Physical health	• BMI <18 and > 35. • Planned or current pregnancy or lactation. • Cardiovascular conditions including abnormal heart rate or blood pressure to be checked at screening. A threshold of exceeding 160 mmHg (systolic) and 90 mmHg (diastolic), averaged across three assessments taken on the screening day will be used. Participants with well-managed hypertension would not be excluded. • Abnormal 12-lead ECG as judged by a study physician.

Table 2: Lifestyle considerations participants must adhere to for the duration of the trial.

Lifestyle considerations	
Caffeine	Abstain from caffeine/nicotine consumption on a dosing day to avoid over stimulation
Alcohol	Abstain from alcohol for 24 hours before the start of each visit. A breathalyser test will be performed at each visit. A failed breath alcohol test (>0) may lead to withdrawal at the discretion of an Investigator.
Recreational drugs	Abstain from recreational drugs for the duration of the study. A urine drug screen will be taken at Screening, Baseline and Dosing periods. A failed drug test during the trials may lead to withdrawal at the discretion of an Investigator.
Tobacco	Participants who use tobacco products will be instructed that use of nicotine-containing products (including nicotine patches) will not be permitted while they are at the study site.
Fasting	Attend VAF1, VAF2, VAF3, VAO and VAL sessions fasted. Food will be supplied at the trial-site.

Table 3a: Schedule of activities through the trial PsiMen-T cohort

Procedures	Screening (Remote) Day -28 to 0	Screening (V1) Day -28 to 0	Baseline Period (Cycles 1-2)	Baseline Visits (VBO/VBF/VBL)	Lab Dosing Period (Cycle 3)	Lab Dosing Visits (VAF1-3)	Last Lab Dosing Visit	Follow-up (30 +/- 7 days)
Informed consent	X							
Demographics	X							
Medical history (including RSQ)	X							
Psychiatric screening ^a	X							
C-SSRS	X	X		X		X		
Concomitant medications	X	X	X	X	X	X		
Personality inventory (BFI-2)		X						
Height		X					X	
Weight		X					X	
Laboratory tests		X						
12-lead ECG		X					X	
Pregnancy tests		X		X (VBO/VBL only)				
Hormone levels		X		X		X		
Vital Signs		X		X		X		
Drug / Breathalyzer test		X						
DRSP		X-----X						
Adverse Events						X		

Home ovulation testing			X		X			
Eligibility confirmation		X	X					
EEG ^b				X		X		
Drug intervention						X		
Pharmacokinetic samples ^c						X		
Pharmacodynamic measures ^d						X		
5D-ASC						X		
CADSS						X		
Semi-structured Interview							X	
Follow-up interview								X
	a: PSST, MINI Screen, AUDIT, NM-ASSIST and Clinical Interview b: Resting EEG eyes-open, eyes-closed, long-term potentiation, doors task, LDAEP, visual gamma, faces task. c: PK samples at baseline and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7 hours after drug administration. Actual time will be recorded. d: VAS measures (Drug Effects Questionnaire), vital signs, resting EEG measured after each PK sample-point							

Table 3b: Schedule of activities through the trial PsiMen-M cohort

Procedures	Screening (Remote) Day 28 to 0	Screening (V1) Day -28 to 0	Baseline Period (Cycles 1-2)	Baseline Visits (VBO/VBF/VBL)	Lab Dosing Period (Cycle 3-5)	Lab Dosing Visits (VAF/VAO/VAL)	Last Lab Dosing Visit	Follow-up (30 +/- 7 days)
Informed consent	X							
Demographics	X							
Medical history (including RSQ)	X							
Psychiatric screening ^a	X							
C-SSRS	X	X		X		X		
Concomitant medications	X	X	X	X	X	X		
Personality inventory (BFI-2)		X						
Height		X					X	
Weight		X					X	
Laboratory tests		X						
12-lead ECG		X					X	
Pregnancy tests		X		X (VBO/VBL only)		X (VAO/VAL only)		
Hormone levels		X		X		X		
Vital Signs		X		X		X		

Drug / Breathalyzer test		X						
DRSP		X-----X						
Adverse Events						X		
Home ovulation testing			X		X			
Eligibility confirmation		X	X					
EEG ^b				X		X		
Drug intervention						X		
Pharmacokinetic samples ^c						X		
Pharmacodynamic measures ^d						X		
5D-ASC						X		
CADSS						X		
Semi-structured Interview							X	
Follow-up interview								X
a: PSST, MINI Screen, AUDIT, NM-ASSIST and Clinical Interview b: Resting EEG eyes-open, eyes-closed, long-term potentiation, doors task, LDAEP, visual gamma, faces task. c: PK samples at baseline and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7 hours after drug administration. Actual time will be recorded. d: VAS measures (Drug Effects Questionnaire), vital signs, resting EEG measured after each PK sample-point								

Table 4a. Timeline of the data collection procedures on a baseline trial day.

Hour of Day	Session: VBF, VBO, VBL
1-2pm	Participant arrival ▪ Medical check ▪ Hormone level measure via blood sample ▪ EEG Capping ▪ EEG tasks: LTP, MMN, LDAEP, Doors, Faces, Visual Gamma, Resting (Eyes open and closed)

Table 4b. Timeline of the data collection procedures on a dosing trial day.

Hour of Day	Time (mins)	Session VAF(1,3), VAO, VAL	VAF2,
8am	-60	Participant arrival - Medical check (C-SSRS, Concomitant medications, New Diagnoses, Breathalyser, Urine test for drugs and pregnancy) - Breakfast - Cannula - EEG Capping - Vital signs - Hormone level measure via blood sample - Baseline PK and DEQ - Resting EEG (Eyes closed and open)	Participant arrival - Medical check (C-SSRS, Concomitant medications, New Diagnoses, Breathalyser) - Breakfast - Vital signs sample - Baseline DEQ
9am	0	Dose of Psilocybin	Dose of Psilocybin
	15	DEQ, PK, Vitals, Resting EEG	DEQ, Vitals
	30	DEQ, PK, Vitals, Resting EEG	DEQ, Vitals
	45	DEQ, PK, Vitals, Resting EEG	DEQ, Vitals
10am	60	DEQ, PK, Vitals, Resting EEG	DEQ, Vitals
	90	DEQ, PK, Vitals, Resting EEG	DEQ, Vitals
11am	120	- DEQ, PK, Vitals, Resting EEG - EEG – LTP	DEQ, Vitals
	150	- DEQ, PK, Vitals, Resting EEG - EEG –MMN	DEQ, Vitals
12pm	180	- DEQ, PK, Vitals, Resting EEG - EEG –LDAEP / Door's task	DEQ, Vitals
	210	- DEQ, PK, Vitals, Resting EEG - EEG –Faces task - Visual Gamma task	DEQ, Vitals
1pm	240	- DEQ, PK, Vitals, Resting EEG - EEG –Visual Gamma task	DEQ, Vitals
2pm	300	DEQ, PK, Vitals, Resting EEG	DEQ, Vitals
3pm	360	- DEQ, PK, Vitals, Resting EEG - Remove EEG cap 5D-ASC	- DEQ, Vitals 5D-ASC

4pm	420	▪ DEQ, PK, Vitals ▪ Participant discharge	▪ DEQ,Vitals ▪ Participant discharge
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Table 5a. PsiMen primary measures

Outcome domain	Measure	Definition
Menstrual cycle effects in response to study drug	Drug Effects Questionnaire- Collected during all dosing sessions	Significant changes in response to study drug between VAF1 / VAL / VAO.
Tolerance effects in response to study drug given on consecutive days (VAF1/VAF2/VAF3)	Drug Effects Questionnaire	Significant changes in response to study drug between VAF1/VAF2/VAF3 sessions

Table 5b. PsiMen secondary measures

Outcome domain	Measure	Scale
Plasma pharmacokinetics of the study drug	Pharmacokinetic samples will be taken at Baseline, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6,7 hours during VAF1/ VAF2/ VAF3/ VAO/ VAL.	Quantification by liquid chromatography mass spectrometry
Pharmacodynamic response associated the study drug	Pharmacodynamic measurements will be taken at Baseline, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6,7 hours during VAF1/ VAF2/ VAF3/ VAO/ VAL.	Vital signs (Blood pressure, heart rate, aural temperature), 5 min resting state EEG and Drug effects questionnaire

Table 5c. Safety measures

Outcome domain	Measure	Scale
Adverse event profile the study drug	Frequency and severity of AEs (and any SAEs)	Tabulation of AEs by severity and SAE listing from the baseline

Table 5d. Exploratory measures

Outcome domain	Measure	Scale
EEG	The EEG LTP paradigm will be measured at VBF/ VBO/VBL/VAF1/VAF3/VAO/VAL. Only these sessions are used to prevent habituation effects. EEG Doors task will be measured at VBF/ VBO/ VBL /VAF1 /VAO /VAL. EEG LDAEP task measured at VBF/VBO/VBL/VAF1/VAO/VAL. An EEG visual gamma paradigm will be measured at VBF/VBO/VBL/VAF1/VAO/VAL. An ERP faces paradigm will be measured at VBF/VBO/VBL/VAF1/ VAO/ VAL.	Recorded with 64 channel EEG: • Visual LTP • Door's task, • LDAEP • Visually induced gamma • ERP faces task
Personality trait modification	BFI-2 measured at Screening	BFI-2 60 items on Likert 1–5, to measure main personality traits.
Participant's subjective psychological experience being part of the trial and receiving the study drug	5D-ASC measured at the end of each dosing session (VAF1/ VAF2/VAF3/VAO/VAL) A semi-structured interview will be conducted at the last dosing session	94 item self-report questionnaire measuring subjective effects of altered states during the psychedelic experience Conducted with the participant to record their subjective experiences of being involved in the trial.
Investigators impression of participants intoxication/ dissociation during the session.	CADSS is recorded by an investigator at each dosing session	CADSS includes a 23 self-report, and 4 clinician rated item scored on a Likert scale (0-4) that measures dissociative symptoms

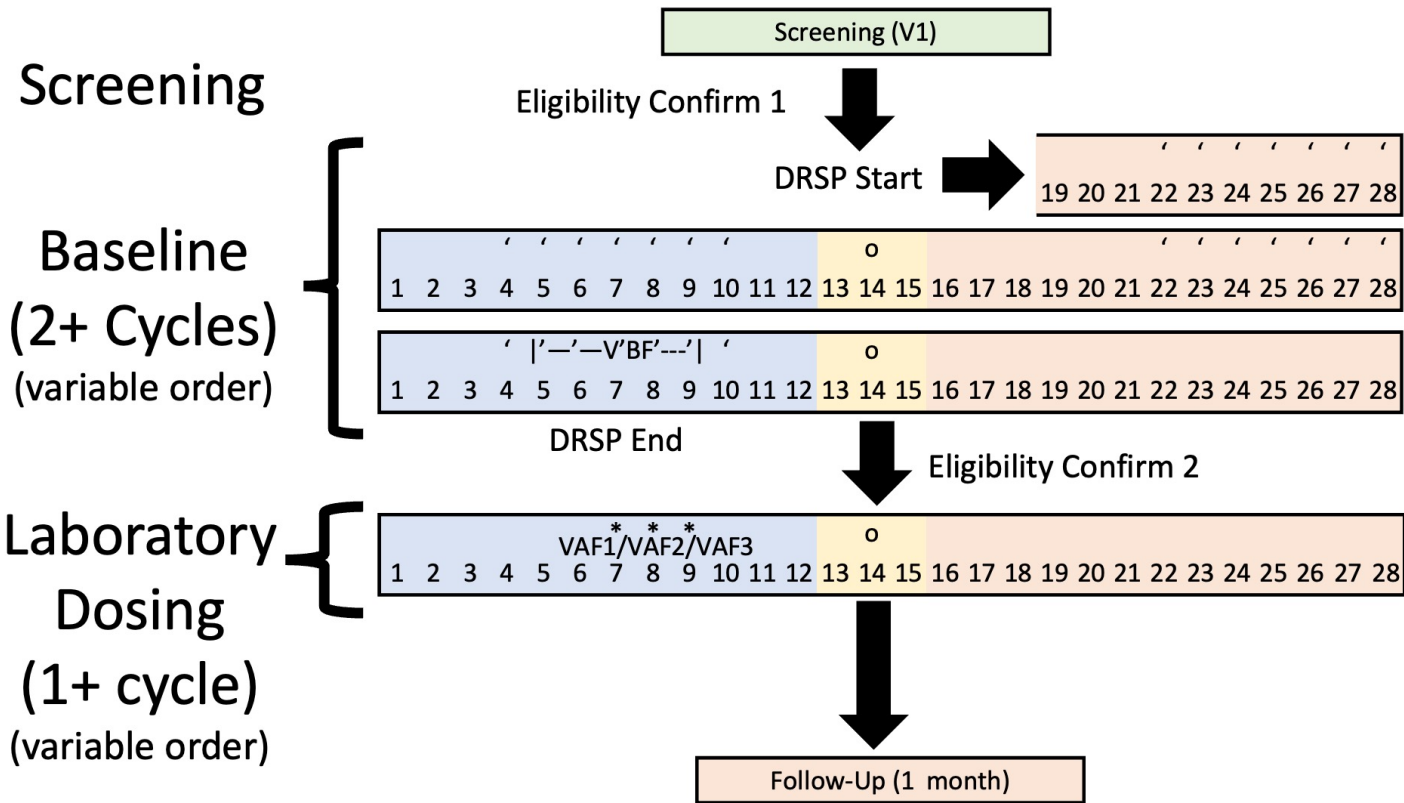
Table 6: List of abbreviations

AE	Adverse Event
ALLO	Allopregnanolone
ANZCTR	Australian New Zealand Clinical Trials Registry
AUDIT	Alcohol Use Disorders Identification Test
BFI-2	Big Five Inventory 2
CADDS	Clinician Administered Dissociative States Scale
CF	Consent Form
C-PASS	Carolina Premenstrual Assessment Scoring System
CRF	Case Report Form
C-SSRS	Columbia Suicide Severity Rating Scale
DEQ	Drug Effects Questionnaire
DMC	Data monitoring committee
DRSP	Daily Record of Severity of Problems
DSM-5	Diagnostic and Statistical Manual
EEG	Electroencephalography
E2	Estradiol
GABA	Gamma – aminobutyric Acid
GCP	Good Clinical Practice
HDEC	Health and Disability Ethics Committee
IANZ	International Accreditation New Zealand
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
KDEF	Karolinska Directed Emotional Faces
LDAEP	Loudness Dependence of the Auditory Evoked Potential
LH	Luteinizing Hormone
LSD	Lysergic acid diethylamide

LTP	Long-Term Potentiation
MINI	Mini International Neuropsychiatric Interview
MMN	Mismatched Negativity Paradigm
NM-ASSIST	NIDA (National Institute of Drug Abuse) Modified Alcohol, Smoking and Substance Involvement Screening Test
P4	Progesterone
PD	Pharmacodynamic
PI	Principal Investigator
PIS	Participant Information Sheet
PK	Pharmacokinetic
PMDD	Premenstrual Dysphoric Disorder
PMS	Premenstrual Syndrome
PSST	Premenstrual Symptoms Screening Tool
QA	Quality Assurance
QC	Quality Control
REDCap	Research Electronic Data Capture
RSQ	Reproductive Status Questionnaire
SAE	Serious Adverse Event
SCOTT	Standing Committee on Therapeutic Trials
VAS	Visual Analogue Scales
VAF	Visit Active Follicular
VAO	Visit Active Ovulatory
VAL	Visit Active Luteal
VBF	Visit Baseline Follicular
VBO	Visit Baseline Ovulatory
VBL	Visit Baseline Luteal

Figure 1

Schema: PsiMen-T Study Design



Notes:

A = Active Drug sessions; B = Baseline no Drug sessions

VPF = Visit Baseline Follicular; VAF1 = Visit Active Follicular 1; VAF2 = Visit Active Follicular 2; VAF3 = Visit Active Follicular 3; VAF1/VAF2/VAF3 occur on consecutive days

Women will start DRSP as soon Eligibility Confirmation 1 complete not at menses (Day 1). See Section 4.1.1.

Cycle timings are idealised. See Section 4.2 for details and algorithms

Key

' = C-PASS diagnostic timepoint

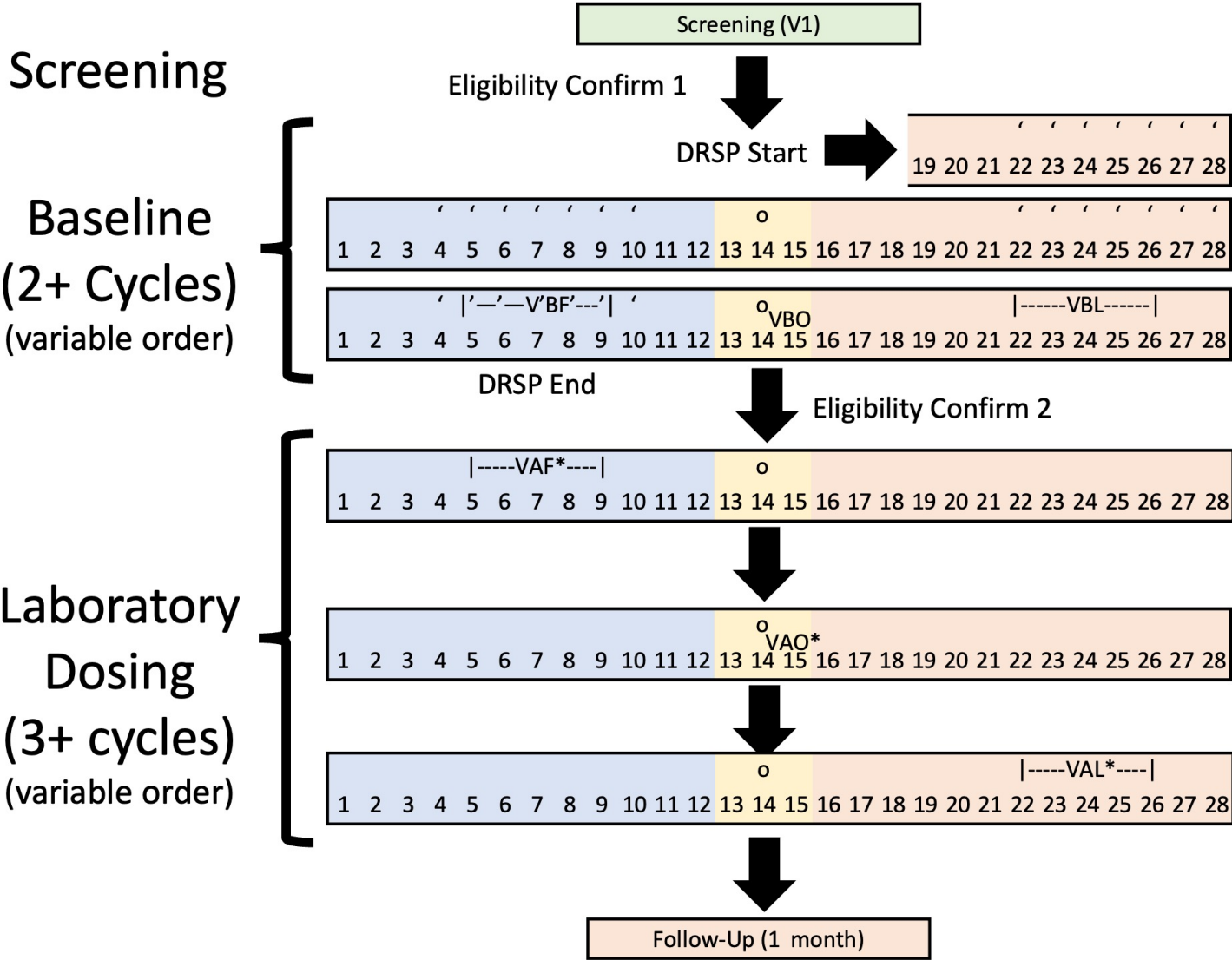
VX = Visit X

* = Dose

o = ovulation

Figure 2

Schema: PsiMen-M Study Design



Notes:

A = Active Drug sessions; B = Baseline no Drug sessions

VBF = Visit Baseline Follicular; VBO = Visit Baseline Ovulatory; VBL = Visit Baseline Luteal; VAF = Visit Active Follicular, VAO = Visit Active Ovulatory; VAL = Visit Active Luteal. Baseline sessions will be distributed over the two baseline sessions.

Women will start DRSP as soon Eligibility Confirmation 1 complete not at menses (Day 1). See Section 4.1.1.

Cycle timings are idealised. See Section 4.2 for details and algorithms

Key

' = C-PASS diagnostic timepoint
VX = Visit X
* = Dose
o = ovulation