To determine whether individuals with PMDD demonstrate a different luteal-phase sex hormone profile and ratio compared with asymptomatic controls.

NTC number:

Date: 29/6/26

To determine whether individuals with PMDD demonstrate a different luteal-phase sex hormone profile and ratio compared with asymptomatic controls.

Research question: Do individuals with PMDD have a lower progesterone-to-oestradiol ratio in the mid luteal phase compared with controls?

Please briefly describe the project's aims and methods.

Please include the primary ethical considerations and explain why this research is important.

Note: The summary of the protocol should concisely but clearly tell the Ethics Committee (in simple terms and in a way which would be understandable to a general audience) what you are broadly planning to do in your study. Your study will be reviewed by colleagues from different disciplines who will not be familiar with your specific field of research and it may also be reviewed by the lay members of the Research Ethics Committee; therefore avoid jargon and use simple terms. A helpful format may include a sentence or two about the background**/ "problem"** the research is addressing, why it is important, followed by a description of the basic design and target population. Think of it as a snapshot of your study.

Premenstrual dysphoric disorder (PMDD) is a severe cyclical mood disorder characterised with severe psychological and even physical symptoms occurring in the luteal phase (the 2 weeks before a women's menstrual cycle starts). It can have a huge impact on a women's life with 1 in 3 women attempting suicide with this disorder (hence the importance of the research). We know from research that the blood test levels of sex hormones e.g. progesterone and oestradiol are considered and known to be within 'normal' range in these patients.

Even though the full aetiology of PMDD is not known, theories related to genetics and lifestyle exist. The problem: it is postulated that PMDD may be related to an imbalance between progesterone and oestradiol and a low ratio between progesterone to oestradiol, rather than the absolute levels.

The aim of this project is to compare the ovarian hormone levels 7 days after ovulation and progesterone:oestradiol ratios between PMDD individuals and a control cohort (case control study).

Methods: In order to assess the aim, women aged 18-45 with regular periods and cycles (21-35 days) will be split into two groups – a PMDD group and a control group (with no PMDD symptoms).

The PMDD cohort will have already been diagnosed by a previous healthcare professional and are self-reporting a prior diagnosis. The initial screening meeting will be conducted by myself, a qualified GP, who will confirm the diagnosis of PMDD based on the answers given to questions in the screening questionnaire. Although participants may report a previous diagnosis of PMDD, confirmation will be undertaken to ensure that the participants meet the current diagnostic criteria.

All individuals that qualify to take part in the study will be given urine ovulation tests at their initial appointment. They will be asked to track their menstrual cycle and they will use a urine LH (leutinizing hormone) test to capture ovulation. One single venous blood sample test will be taken ideally 7 days (5-7 days is acceptable) after the LH spike (indicating ovulation) and tested for oestradiol and progesterone. This study involves a maximum of one blood sample per participant.

The primary outcome measure is:

1. Progesterone to oestradiol ratio

The main ethical considerations are related to a risk assessment in relation to data handling, potential upset when women are completing the questionnaires related to mood and small risks linked with taking blood samples (mild discomfort, bruising).

The risks will be considered and mitigated through:

1. clear patient participation information and knowledge that participation is voluntary
2. the ability of women to withdraw from the study at any time
3. the ability of women to ask questions to the principal investigator to clarify any research testing queries.
4. The formation of a distress protocol with appropriate signposting.
5. Clear following of GDPR and confidentiality law/rules in relation to data handling

Please provide a brief outline of your research project aims and objectives.

Aim - To establish whether individuals with PMDD have a different luteal-phase sex hormone profile and ratio compared with an asymptomatic control subset.

Objectives

1. Comparison of mid luteal oestradiol levels between controls and PMDD individuals
2. Comparison of mid luteal progesterone levels between controls and PMDD individuals
3. Comparison of mid luteal progesterone:oestradiol ratios between controls and PMDD individuals
4. Comparison of the neuroactive steroid pathway eg. Allopregnanolone, pregnenolone and compare levels between controls and PMDD individuals
5. Comparison of the inflammatory markers and compare levels between controls and PMDD individuals
6. Comparison of the serotonin pathway markers eg. Tryptophan, kynurenine ratio and compare levels between controls and PMDD individuals
7. To look at if any covariates exists in the data within the groups analysed in relation to e.g age, body mass, SSRI use, smoking status, alcohol intake

Please explain the number of participants you intend to include in your study and explain your recruitment strategy in detail (eg who will be recruited, how, where from; and expected availability of participants). If your study contains multiple parts eg interviews, focus groups, online questionnaires) please clearly explain the numbers and recruitment details for each of these cohorts (see help text).

An a- priori analysis was conducted using G* power for this study for the primary outcome of mid luteal progesterone to oestradiol ratio between 2 groups (PMDD versus controls) using a 2

tailed t test. Due to the in-person nature of the testing with a single cyclically timed blood draw and in order for this study to be conducted within the PhD time constraints, the study is powered to detect a large effect (Cohen's d = 0.9) with an alpha value of 0.05 and a statistical power of 80%. Based on this, it approximates 21 participants per group (total of 42 participants for both groups n=42). Accounting for an approximate 20% dropout rate, a total of 25 participants per group will be the aim for recruitment (n=50).

The recruitment strategy will include placement of advertising posters around the university, word of mouth, opportunistic sampling, social media advertisement (instagram, linkedin, facebook) of the project and influencer advertisement of the project via their social media. Furthermore, use of the advertising poster and advertisement via appropriate charities e.g. the PMDD project, International Association of Premenstrual Disorders (IAPMD). The interested parties will be directed to a generic study and or the principal investigators email address and or QR code linking to the participant information sheet (PIS) which will include the inclusion and exclusion criteria which are as follows below. Based on the below, participants will be recruited to either the PMDD or control group.

Each participant will provide one venous blood sample only and therefore each participant contributes a single measurement to the data set. The power analysis was conducted assuming independent observations, which is appropriate for this design.

The power analysis was carried out for the primary outcome only, which is the comparison of the mid-luteal progesterone to oestradiol ratio between the PMDD and control groups. The study is powered based on this single primary outcome and we have NOT adjusted the alpha value for multiple comparisons.

The control group will be recruited so that it is broadly comparable to the PMDD group in key characteristics e.g sex, age and BMI. These factors will also be recorded and considered in the statistical analysis.

Inclusion criteria

1. Aged 18-45 years old (adults but less than the typical perimenopausal age so there is not added bias/variable)
2. Regular menstrual cycles (21-35 days for the last 3 months)
3. Willing and able to
    - complete a questionnaire (no new clinical diagnosis given)

- Use home urine LH test strips to detect ovulation
- Attend a single in person on site in Lancaster mid luteal blood test sample appointment (approx 7 days post LH surge)

4. Provide written informed consent
5. Have sufficient English language skills to understand the patient information sheet
6. Be either in Group 1 or group 2
    - Group 1 (PMDD group ie case group) - self reported prior diagnosis of PMDD made by a healthcare professional (GP, psychiatrist, gynaecologist)
    - Group 2 (Control group ie comparison group) - no prior diagnosis of PMDD made by a healthcare professional and no history of clinically significant premenstrual mood symptoms on screening

Exclusion criteria

1. Current use or use in the last 3 months of hormonal contraception such as the combined oral contraceptive pill, the progestogen only pill, the implant, the injection, hormonal coil (Mirena, Kyleena) or patch/ring.
2. Current use or use in the last 3 months of any hormone replacement therapy (e.g. combined oestrogen and progesterone, oestrogen and Mirena coil combination, oestrogen alone if had a hysterectomy, or progesterone alone) or GnRH analogues use
3. On a selective serotonin reuptake inhibitor (SSRI) with a recent initiation or dose change within the last 3 months
4. Pregnant, breastfeeding or within 3 months post partum
5. Irregular menstrual cycles (outside the 21-35 day window)
6. Surgical menopause
7. Premature ovarian insufficiency
8. Other endocrine disorders e.g. uncontrolled thyroid disease, high prolactin, cushing's syndrome, polycystic ovarian syndrome)
9. Regular systemic steroid use or use of other medications known to affect sex hormone within the last 3 months
10. Severe mental health issues that impair consent or participation
11. Needle phobic so that venepuncture is intolerable
12. The use of the emergency contraceptive pill either 3 months prior to the study or during the study

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Once participants have confirmed their eligibility and have been provided with a Participant Information Sheet via email, we will book them in in person for an initial visit at the Human Performance Lab in Lancaster University's Sports Centre. This interview may also be carried out online via Microsoft teams if needed to allow flexibility for recruitment of participants. This Microsoft teams interview will NOT be recorded.

On arrival to the lab for this initial visit/ or online, participants will be given the Participant Information Sheet to read, which will be followed with an online Qualtrics consent form to sign to confirm they are happy to take part in the study. They will then complete the screening questionnaire pack which will include a brief demographic/health questionnaire (e.g. eligibility criteria, age, smoking status, medication use, hormone symptoms questions) to support analysis.
If when answering any of these questions, participants are found to be no longer eligible for the study, they will be informed and provided with a debrief sheet.

For those that screen positively and confirm their existing PMDD diagnosis on the screening questionnaire, they (the PMDD group) will be recruited into the PMDD arm of the group. The PMDD group will be identified for research purposes solely and it is not for diagnostic purposes. Control participants will be those without significant premenstrual symptoms confirmed by the screening questionnaire.

During the study, all participants will be asked to use ovulation strips to determine when they are due to ovulate. These strips are like a pregnancy test where the participant will use them at home in the morning and will be asked to urinate on them for three days before their expected date of ovulation (calculated from their preferred menstrual self-tracking application). This is required to determine when participants will visit the lab for their testing day. However, there is a chance that from this test participants discover they do not ovulate – this will exclude them from the study. Should this occur, these participants would be sent an email explaining that they can no longer take part in the study and which will provide them with resources and sources of support should they need them. Further, as an extra safeguarding layer, those excluded from the study, a follow up email 72 hours later to check on their wellbeing and again provide those additional resources will be done.

Participants will be recruited nationally across the wider UK. Once ovulation is identified, a single venous blood sample will be scheduled for ideally 7 days after the LH surge (5-7 days will suffice). Testing visits will be carried out in person in at Lancaster University either at the

Human Performance Lab or in other Medical School research labs appropriate for the study. Participants must be able to travel to Lancaster for testing. They will be asked to refrain from any alcohol and/or recreational drugs 24 hours before this visit.

The study is funded by Lancaster Medical School. No commercial organisations are involved in the funding and are not influencing how the study is designed.

Although participants will not receive any financial incentive from participating, they will all be offered the opportunity to receive a summary of their hormone profile blood test results and these results can help improve and benefit their understanding of their PMDD.

Null hypothesis: there is no difference in hormonal profile ratios between individuals with PMDD and control participants in the luteal phase.

As you have indicated that you are working with a vulnerable group please describe the intended participants, and why they are needed for this research.

The intended participants may be considered vulnerable because of their PMDD diagnosis – which is a mental health disorder and can involve significant mood issues. The Women's Health Strategy 2022 identified this condition requiring more research. This population is necessary to study as this quantitative study is important to help address and help us understand if any hormone imbalances or ratio discrepancies exist between controls and PMDD. This will help inform future management and individualized care in a subset of women who are often neglected from research.

You have selected that the research may involve personal sensitive topics that participants may not be willing to otherwise talk about. Please indicate what discomfort, inconvenience or harm could be caused to the participant and what steps you will take to mitigate or manage these situations.

Participants will not receive a clinical diagnosis as part of the study but will have a self-reported prior diagnosis of PMDD. A GP (Dr Milli Raizada) will complete the screening visits and will confirm this diagnosis via the questionnaire and will help classify patients into PMDD or control for research purposes.

Participants will access the Participant Information Sheet (PIS) via a QR code on advertising materials. The PIS will provide full details of the study, including eligibility criteria, study

procedures, and the nature of the questionnaires. Individuals who do not meet eligibility criteria will not proceed further and will not be required to contact the research team.

Participants will be informed in advance that the study involves questionnaires relating to menstrual cycles and mood symptoms, including questions about low mood, anxiety, irritability, and daily functioning. Some participants may consider these topics personal or emotionally sensitive. Participants will have sufficient time to consider this information before deciding whether to take part.

If participants feel uncomfortable answering any questions, they may skip individual items or withdraw from the study at any time without providing a reason

Participants will be required to provide information about their menstrual cycle, including cycle length and timing, and to use home urinary LH ovulation test kits to identify ovulation for scheduling the mid-luteal blood sample. Some participants may find this process inconvenient or may discover that ovulation cannot be confirmed during the study period. In this instance, participants may be excluded from further participation and will be informed in a sensitive manner. They will be reminded that the research team cannot provide medical diagnosis or advice and will be encouraged to consult their GP if they have concerns or questions.

The study includes a single venous blood sample, which may cause mild discomfort, bruising etc. Blood sampling will be conducted by trained staff using standard safety procedures. Participants will be monitored and offered rest and reassurance as needed.

Given that some participants may experience mood changes or psychological distress related to questionnaire completion, a distress protocol will be in place. If a participant becomes distressed, the researcher will pause the study activity, check in with the participant, and offer the option to take a break or withdrawal from the study. Participants will be provided with a debrief sheet containing contact details for appropriate support services (e.g GP, NHS 111, Samaritans).

All information provided by participants will be treated as confidential and used solely for eligibility screening and research purposes. Identifying information will be stored separately from research data.

Participants will be reminded that participation is entirely voluntary, and that their decision to participate, decline, or withdraw will not affect them.

Participants may request to view example questionnaires prior to attending the laboratory visit if they wish, to help them make an informed decision about participation.

You have indicated that you will collect identifying information from the participants. Please describe all the personal information that you gather for your study which might be used to identify your participants

I will ask participants to confirm their sex as this is a requirement for the study although they do not have to disclose how they identify unless they wish to inform me. I will be collecting age (in years), and this will be used in data analysis. Age will be provided in years, rather than date of birth, to help preserve participant anonymity. Participant IDs will be used to anonymise this information. Participants' email addresses will be used to contact them to organise a dates and times for the screening and test visits. Participants' email addresses will be stored separately from the participants research data.

Please describe how the data will be collected and stored.

Consent forms and questionnaires will be administered on paper in person before being stored in a locked filing cabinet that only the researcher and their supervisors Dr Chris Gaffney and Dr Jemma Kerns have access to. All data collected during testing e.g. blood analysis will be stored on a password-protected lab computer and uploaded to Lancaster University's OneDrive, and only myself and my supervisors will have access. Blood will be collected in vials and labelled with the participant ID; it will be stored at -80^{o}C in a freezer in the Biological Life Sciences (BLS) laboratories at Lancaster University until the analysis has been completed. The blood samples will then be disposed of in line with BLS laboratory regulations. Email addresses will only exist in Microsoft Outlook history and will not be stored in a database spreadsheet.

Fully anonymised and collated research data will be stored indefinitely on the Open Science Framework in line with open science best practice for future research. This data will not be attributable to any individual.

Please describe how long the data will be stored and who is responsible for the deletion of the data

Paper data will be digitised after study completion and then destroyed as per the university guidelines. All resultants digited data will be stored on OneDrive and will be stored securely for 10 years. The supervisors are responsible for the deletion of the data.

You stated that the study could induce psychological stress or anxiety, or cause humiliation or cause harm. Please describe the question(s) and situation(s) that could lead to these outcomes and explain how you will mitigate this.

Once the participants have confirmed their eligibility, they will be invited into the Human Performance Lab for a screening visit. This will require them to fill out a questionnaire pack that will ask questions regarding their general health; this may induce psychological stress if the assessed risk factors surrounding their general health require medical clearance before continuing with the study. If participants are required to gain medical clearance and do so, they will be able to continue with the study.

The questionnaire pack also contains questions surrounding participants menstrual cycle and asks them to disclose key timings of their cycle (menstruation and ovulation), which may cause psychological stress as this is personal information that may make some participants feel uncomfortable, however, they will be reminded that if they do feel uncomfortable, they are able to withdraw at any point without reason. Participants will also be asked to use ovulation test strips which they must urinate on for 3 days before their expected ovulation date. If participants do not get a positive test over the 3 days, it is possible that they are not ovulating which could be upsetting to some participants if unexpected – this excludes them from the study. If this occurs, participants will be sent a debrief email with resources and advised to speak with their GP if they are concerned as the researcher is not qualified to make any diagnoses of irregularities in the menstrual cycle.

During the study, there is a possibility that a participant needs to take the emergency contraceptive pill, if this is the case, they are no longer eligible for the study and must inform the researcher that they no longer wish to take part, but they do not have to provide the reasons.

During the testing visits, participants will have 10-15mls of bloods taken from their arm once to get their hormones, steroids +/- inflammatory markers tested. At the screening visit, each participant will be asked if they are okay with needles and blood and anyone with a phobia of

needles will be recommended not to take part in the study due to the anxiety and stress it could cause them.

If during the screening visit (psychological stress/anxiety caused by questionnaires) or testing visit (psychological stress/anxiety caused by questionnaires or bloods) the participant shows signs of psychological stress/anxiety, they will be recommended to take some time to pause the processes by stopping what it is they are doing at the time of distress. The participant will then be asked if they wish to continue with the study or if they wish to stop. If a participant wishes to stop, they will not be asked to explain why they wish to stop and will be given the option to either leave or to sit for some time (no more than the time of their booking due to time availability of the lab) if they need. They will be emailed the debrief sheet with additional links (specified in the distress protocol document) if they decide to withdraw from the study.

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You have indicated you are using intrusive procedure(s) such as attaching equipment, administering substances/ultrasound
, utilising ionising radiation, or using tissue.
Please describe the procedure(s), its methodology as well as any potential benefit to the participants.

Participants will also have their blood taken from their arm by myself (a doctor with over 18 years clinical experience) or a phlebotomy trained lab technician. The lab technician will sterilise the area before drawing blood using a needle into a vial where the blood will be collected and stored for later blood analysis. 10-20 ml of blood will be drawn for the analysis, 2-3 tubes of blood. Participants will be reassured throughout the process and monitored by the researcher and technician to ensure they are finding the experience within their comfort level.

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What are your dissemination plans? E.g publishing in PhD thesis, publishing in academic journal, presenting in a conference (talk or poster).

The findings of this research will be written up as a manuscript and published in an academic journal which will form one of my three papers towards my PhD thesis. I may present the findings of the study at a conference or disseminate the findings to members of the public

including social media, in the form of a poster, talk or an oral presentation should the opportunity arise. Further, discussion of the outcome of my work on social media, through podcast discussions, talks to community groups and media interaction may occur.

You have indicated site users have a reasonable expectation of privacy and therefore you will need to obtain consent to use their data for this project. Please explain how you propose to obtain consent.

When participants view the recruitment materials, they will scan a QR code, which will take them to a PDF of the participant information sheet. They will then be required to contact the researcher by email, and a screening visit will be arranged. The researcher will provide the Participant Information Sheet via email too and ask that the participant read it before agreeing to attend the screening visit. Participants will be asked to give informed consent at the screening visit via a consent form – they will have access to the information sheet at the screening if they wish to remind themselves of the study prior to signing consent. These consent forms will be stored securely in a locked cabinet in a secure building (Health innovation One) at Lancaster University and can only be accessed by the researcher and their supervisors.

You have stated that at least one member of the research team has links to the funder or organisations involved in this project. Please explain the relationship and how you will mitigate or manage this conflict of interest.

I as principal investigator, am a trustee of The PMDD Project charity, which helps support people with PMDD. The charity may be asked to use their social media platform and contacts to advertise the study (e.g sharing advertisement posters or links to the Participant Information Sheet).

I will not have access to any personal data or contact details held by the charity for the purposes of recruitment as all recruitment will be based on self-referral with interested individuals contacting the research team directly and participation is voluntary and optional which will be clearly stated in the advertisement. It will be made clear not participating will not have any adverse impact on the individual in respect to their relationship with the charity.

The Principal Investigator's trustee role will be declared to participants in the Participant Information Sheet. The research will be conducted independently of the charity and the charity

will have no involvement in study design, data collection, analysis, interpretation, or publication decisions at all.

Supervisory oversight will be provided by the academic supervisors Dr Christopher Gaffney and Dr Jemma Kerns to ensure transparency.

This ensures that any potential conflict of interest is appropriately managed and does not influence recruitment or participation

You have indicated that you will be gathering/working with special category data. Please confirm here how you will comply with data protection law (GDPR) for use of special category personal data.

Since GDPR recognises data concerning health as a special category of data, the data collected for this study may be considered to fall under this category. The data we will collect includes self-reported data around menstrual health, sex , height and weight and serum blood analysis. These data could be used to infer information about the health of participants. We will ensure that the processing of this data is lawful, fair and transparent, and complies with all the other principles and requirements of the UK GDPR. As such, we will only collect the minimum amount of data required to assess these variables to answer our research question. We will also ensure that the data is kept securely, and that anonymity is maintained. This research data will be associated with Participant ID codes, and not with any personally identifiable information. Further, the data will be securely stored in the research teams' folder on LU OneDrive for 10 years, and anonymised data will be available indefinitely on the Open Science Framework to facilitate open science practices.

To facilitate transparency, participants will provide fully informed consent prior to participating in the research. This will involve informing participants that we would like to collect this data in the Participant Information Sheet which participants will read before being asked to give consent to participate. We will inform participants in the information sheet, and when asking for consent, that if they do not wish to provide this information, they do not need to take part. Or, if they decide they no longer wish to provide this information after starting participation, they can do so at any time without providing reason.

How long will you retain the research data?

- The research data will be retained on Lancaster University's OneDrive server for 10 years. This data will only be accessible to the researcher and their supervisors. Fully anonymised data may also be stored indefinitely on the Open Science Framework for future use by other scientists.

How long and where will you store any personal and/or sensitive data?

- The eligibility criteria used for this study such as participants' age (in years), menstrual cycle details etc will be stored; this will be anonymised using participant identification numbers (IDs). This data is necessary as it will be used to match the participant group characteristics. Anonymised data will be stored on OneDrive for 10 years, only accessible by the researcher and their supervisors.

Please explain when and how you will anonymise data and delete any identifiable record?

Participants will be provided with a unique ID number when they provide consent. This ID number will allow each participant data to be anonymised and link all their data together. As the questionnaires, blood analysis need to be paired, the participants ID must be available until after data processing, after which any identifiable record will be deleted.