Participant Information Leaflet

A characterisation of immune cell phenotype and function across the menstrual cycle

PARTICIPANT INFORMATION LEAFLET

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We would like to invite you to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, family or your GP if you wish. If there is anything that you do not understand, or if you would like more information, please ask us.

First, we want to introduce you to the study and key facts. Then we will go through the study in more detail. You will be encouraged to ask us questions about the study and to take the time you need to consider participating. **Taking part in this study is entirely your choice.**

You will be compensated up to **£100** for your time, travel (where appropriate), and inconvenience.

Could I be eligible to take part?	
You must: - Be male or female (either menstruating or using oral hormonal contraception) - Be aged 18 to 35 years old - Be in good health - Have a body weight >50kg - Be willing and able to travel to the research facility in Oxford 9 times over ~2 months	You must not: - Have any significant medical conditions requiring regular medication - Be a smoker or regularly use nicotine-containing products - Be pregnant or breastfeeding - Iron deficient or take any prescribed iron supplementation

If you decide to join our study:

1. At screening you will give written consent for your participation in the study, and we will confirm your eligibility with a brief survey regarding your health and menstrual status (if applicable), a blood test and pregnancy testing.
2. We will then allocate you to your appropriate study group and inform you of your study schedule. This will involve 8 blood tests across approximately 2 months (i.e. roughly once a week). A maximum of 56ml will be taken at each blood test. You should feel no or very little effect from this.
3. *If* in the eumenorrheic female group, we will ask you to conduct daily at-home urine testing for Luteinizing Hormone (LH) around the mid-point of your cycle. The purpose of this is to test for a rise in LH which is known to happen 1-2 days before an egg is released from your ovary (ovulation). The results of this test will be used to help plan your study visits and confirm you have an ovulatory cycle. The study team will provide you with these tests and ask you to report back when they are positive.
4. Throughout the study you will be closely monitored by the study team with questionnaires and the tests mentioned above. In total you will need to attend the research facility on 9 occasions (1 screening visit, 8 study visits) over approximately 2 months, for about 30 minutes per visit.
5. You will be free to withdraw from the study at any time you wish.

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Definitions

Female	In this study, female refers to people assigned female sex at birth.
Male	In this study, male refers to people assigned male sex at birth.
Eumenorrheic Female	In this study, eumenorrheic females are people assigned female sex at birth who experience a regular, ovulatory menstrual cycle between 21-35 days.
Female using oral contraception	In this study, contraceptive users are people assigned female sex at birth, using an established form of oral contraception according to manufacturer's instructions.
Anaemia	A condition where the number of red blood cells or haemoglobin (Hb, carries oxygen) concentration is reduced. Anaemia can have many causes. In accordance with WHO[1] guidelines, in this study anaemia will be considered as Hb lower than 120g/L if female or 130g/L if male
Iron Deficiency Anaemia (IDA)	A condition where iron stores in the body are so low that erythropoiesis (generation of new red blood cells) has been reduced and oxygen in the blood (carried by haemoglobin) has also reduced. In accordance with WHO guidelines[2] in this study we will consider this as serum ferritin lower than 15µg/L AND Hb lower than 120g/L (female)/130g/L (male)
Iron Deficiency (IDef)	Defined as low iron stores in the body but has not significantly affected erythropoiesis or haemoglobin levels. IDef can develop into IDA if left untreated for a prolonged period of time. In accordance with WHO guidelines[3] in this study we will consider IDef as having serum ferritin lower than 15µg/L but crucially Hb higher than 120g/L (female)/130g/L (male)
HbA1c	HbA1c refers to glycated haemoglobin. This is a form of haemoglobin which is chemically linked to sugar. This can be used to assess an individuals average blood glucose (sugar) levels for the last 2-3 months.

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1. What is the purpose of the research?

For a long time, it has been thought that sex hormones (like oestrogen, progesterone and testosterone) may influence the function of a person's immune system. This is because some diseases are much more common in one sex rather than the other (like systemic lupus erythematous [SLE]) or may be more severe (like Covid-19). Some diseases also improve or deteriorate with changes in female sex hormones, for instance symptoms of SLE get worse during pregnancy.

Female sex hormone levels vary across the menstrual cycle in a predictable pattern which provides the opportunity to explore the effect of these changes on immune cells. This study aims to describe immune cells number, type and function in the blood at 4 time points in the menstrual cycle (corresponding with the peaks and troughs of different hormone levels), across 2 menstrual cycles. This will be compared to women taking an oral contraceptive pill and males.

This will represent an in-depth characterisation of the impact of female sex hormones and serve to provide real-world actionable insight relevant to multiple areas of medicine and healthcare.

2. Why have I been invited to take part?

You have been invited to take part because you are aged 18 – 35 years old, are healthy, and, if female, have a 'regular', ovulatory menstrual cycle or are using an oral contraceptive. Should the above conditions be met, you should be eligible to participate.

3. Do I have to take part?

No. There is no obligation to take part and participation is entirely voluntary. If you do decide to take part, you may withdraw at any time without giving a reason and without any penalty.

4. Can I take part?

To take part in the study, ALL of the following must apply to you:

- Be willing and able to give informed consent for participation in the study and are able to comply with the study protocol
- Be aged 18 to 35 years of age inclusive, at the time of signing the informed consent
- Be healthy as determined by participant and study team after completing screening survey
- Have a body weight greater than or equal to 50kg and body mass index (BMI) within the range 18.5 to 24.9 kilogram/meter squared (inclusive)
- Have sufficient English language ability to enable appropriate informed consent procedures to be conducted in English
- If female, have experienced your first period more than 3 years previously
  AND EITHER
  Have regular menstrual cycles (a cycle of length 21-35 days) with at least 9 bleeds in the last 12 months and have not used any form of oral contraception for more than 3 months
  OR

Consistently used the same oral contraceptive pill for more than 3 months

You cannot participate in the study if you:

- Have used immunosuppressant or immunomodulatory drugs in the 3 months prior to study entry that, in the opinion of the investigator, would interfere with the study
- Have existing metabolic/hormone disorders or any condition that, in the opinion of the investigator, would interfere with the study or impair interpretation of the study data
- Have a phobia of needles/blood
- Require assistance to get out of the house, help with routine tasks of daily living (such as shopping, meal preparation, housework) and/or in managing things like personal finances
- Have been pregnant, given birth or breast-fed in the last 12 months
- Are actively trying to conceive, within the next 3 months
- Use hormone modifying therapies, aside from established oral contraceptives that, in the opinion of the investigator, would interfere with the study
- Experience irregular periods
- Are unable to travel to Oxford Clinical Research Facilities over the next 3 months
- Present with Haemoglobin lower than 120g/L if female or 130g/L if male and/or serum ferritin lower than 15µg/L
- Currently take any prescribed iron supplements
- Regularly smoke or use nicotine containing products e.g. vapes
- Regularly consume more than 14 units of alcohol per week
- Use recreational drugs
- Present with HbA1c outside the healthy range 20 - 41 mmol/mol

5. What will happen to me if I take part?

Across the study you will have a maximum of 9 blood samples (one screening visit and 8 study visits) over approximately 2 months (i.e. roughly 1 per week)). *Figure 1* shows the visits you will need to attend if you join the study. The details of these visits are described below.

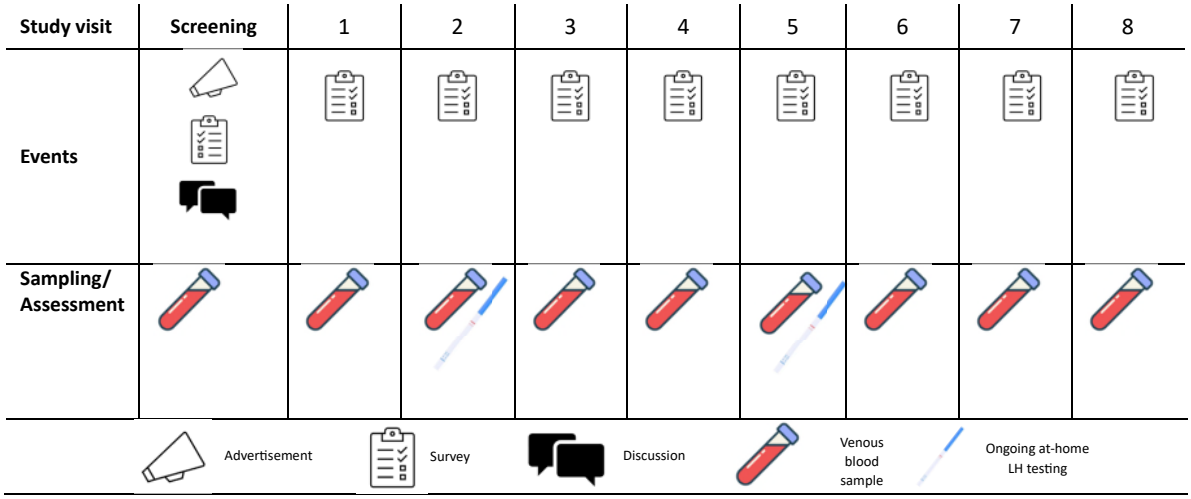

Figure 1: *Timeline of study procedures. Created in Microsoft Word.*

Questionnaire

At each sample point you will also be asked to complete a short questionnaire confirming which day in your cycle/pill you are at (if applicable), and other relevant information – e.g. illness, changes in medication.

Blood Tests

We will take blood samples to measure your sex hormone concentration (oestrogen, progesterone, testosterone), iron status and evaluate your immune system. The maximum volume of blood taken at each sample will be approximately 56mL (about 4 tablespoons). All visits will take place in the morning (7am-11am).

LH Testing

If you are in the eumenorrheic group, we will ask you to conduct at home urine testing for LH for up to 7 days in each cycle. LH is a hormone which surges approximately 36 hours before ovulation. We will use this test to confirm presence of ovulation and to help schedule study visits.

Temperature Monitoring

You will be provided with a thermometer to take home on your first study visit and asked to record your temperature at a consistent time each morning, daily throughout the study. This is used as another marker of ovulation as a rise in basal body temperature is expected in the luteal phase. This data will also allow for correlation of temperature with immune markers.

5.1. Screening

At your screening visit we will go through this document in detail, to ensure you understand what to expect if you decide to take part, and the risks involved. In particular, we will clarify exactly what procedures will be involved, how many times they will be performed, how often and what time commitment would be required from you (i.e. your 'study schedule'). You will receive full and comprehensive answers to any questions you might have.

This document will form a reference point for you and, if you do decide to take part, a member of the study team will ensure you have an accessible copy to keep (e.g. we can give you a paper copy or send it to you via email).

Confirmation of eligibility, including clinical tests, will take up to 45 minutes. If you are found to be ineligible, the study procedures will end at this point.

Confirmation of eligibility

The research team will go through a few administrative questions as well as confirming details about your eligibility through a health screening survey. If you are female this will involve answering questions about your menstrual cycle or contraceptive use.

Informed consent

Once you are happy that you fully understand what the study involves and before anything else takes place, the study team will ask you to sign a consent form. You will be given a copy of the consent form to take away and keep.

Clinical tests

If at this point you are happy and eligible to continue with the study a blood sample will be taken to assess your health parameters including iron status. If female, a urine pregnancy test will also be conducted.

Follow-up appointments

Following this visit the study team will let you know the results of your blood test. If you meet all eligibility criteria, we will send you a copy of your proposed study schedule. If you have any additional questions at this stage, please contact us by phone or email to discuss them.

If you are happy to enter the study, the study team will reach out to you to arrange your first visit as per your proposed study schedule.

If you are in the eumenorrheic female group, we will provide at-home urine test kits to measure Luteinizing Hormone (LH), which surges before ovulation. This helps confirm ovulation and plan your visit schedule. If you have cycle tracking data, we'll ask you to use the tests around ovulation (e.g. days 13–20 if you have a 30 day cycle) of your next cycle and share your data to improve sample timing predictions. If you don't have tracking data, you'll be asked to record one month's cycle data before continuing. Males and females taking oral contraception will not require LH testing or tracking.

What happens if any of the tests are abnormal?

Sometimes test results are outside the usual ranges for healthy individuals. Depending on the results, you may not be eligible for the study, and you may be advised to contact your GP for further tests or review.

What happens if I decide not to take part at this stage?

There is nothing else you need to do—taking part is entirely your choice. In this circumstance we will not perform any of the study procedures detailed below, but we will ask if you are willing to be contacted in the future to see if you are interested in taking part in any other studies. Consenting to this is entirely optional and you will be able to withdraw your consent at any time by contacting us.

5.2. Enrolment and Study group allocation

Following screening, confirmation of consent and once all eligibility criteria has been confirmed you will be considered enrolled in the study. You will be in one of the three study groups as follows:

1. **Eumenorrheic female**

2. **Female using oral contraception**
3. **Healthy male**

You will need to return to the study sites on 8 further occasions. The exact nature of your study schedule will depend on which group you are allocated to and, if applicable, your menstrual cycle length. The participant experience for each group is described in section 5.3 below.

5.3. Visits 1-8

Once allocated to your study group you will enter the main part of the study which will consist of a maximum of 8 blood samples across approximately two months. Whilst we will aim to conduct the study over 2 consecutive months it is possible this can be split into 2 separate months – e.g. due to illness/unable to attend testing etc. The exact study length will depend on your menstrual cycle length if you are in the eumenorrheic female group.

Eumenorrheic Females

Below, in *Figure 2*, is a diagram of the participant experience in the 'Eumenorrheic Female' group. This is based on the typical 30-day cycle. Most participants are likely to vary to some extent to this textbook menstrual cycle, which may change the dates of study visits and the time between them.

The first month after eligibility is confirmed eumenorrheic females will be asked to track their menstrual cycles, including LH testing. A positive LH test within this month will confirm ovulatory cycles and help improve prediction of menstrual cycle milestones for the sampling cycles.

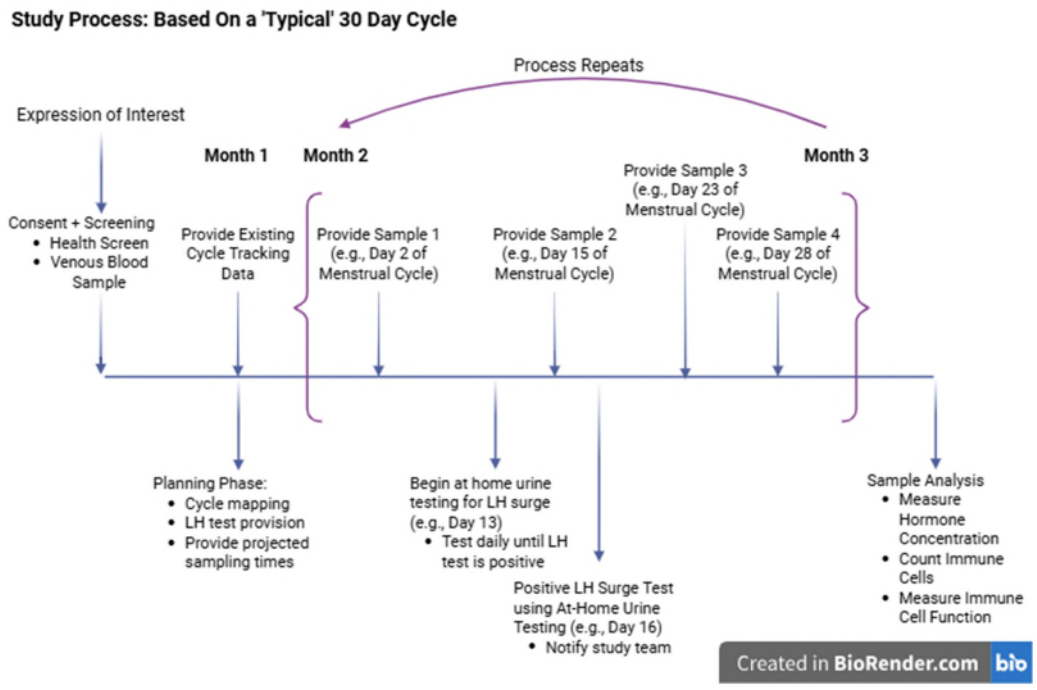

Figure 2: *Study overview modelled on a 'typical' 30-day cycle. Figure created using BioRender.com*

Females using Oral Contraception

Below, in *Figure 3*, is a diagram of the participant experience in the 'Female using Oral Contraception' group. This is based on a 28-day pill cycle and means the time points within this group will be predictable at the beginning of the study and will not vary. Samples are expected to be collected on Day 2, 13, 21 and 26 of each cycle.

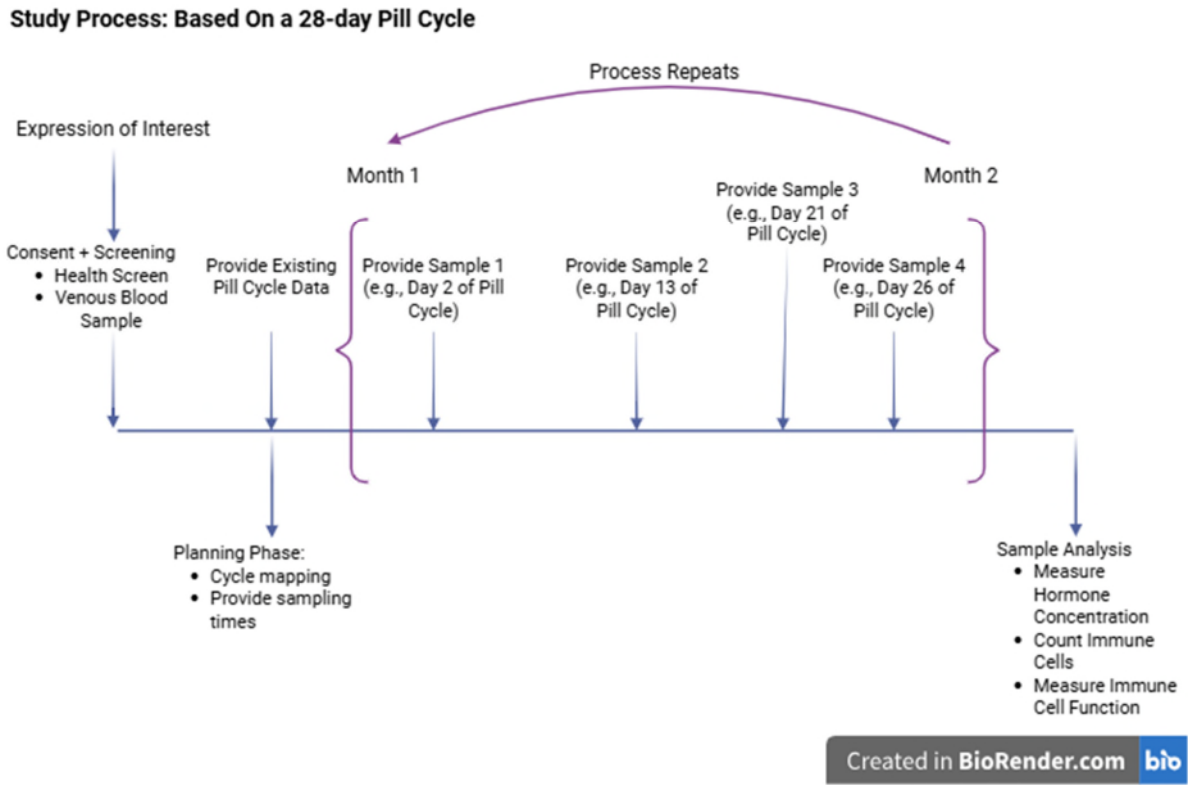

Figure 3: *Study overview modelled on a 28-day pill cycle.* *Figure created using BioRender.com*

Healthy Males

Below, in *Figure 4*, is a diagram of the participant experience in the 'Healthy Male' group. Each male will also be modelled on a 'textbook' 28-day pill cycle to mirror the contraceptive user group. Samples are expected to be collected on Day 2, 13, 21 and 26 of each modelled cycle.

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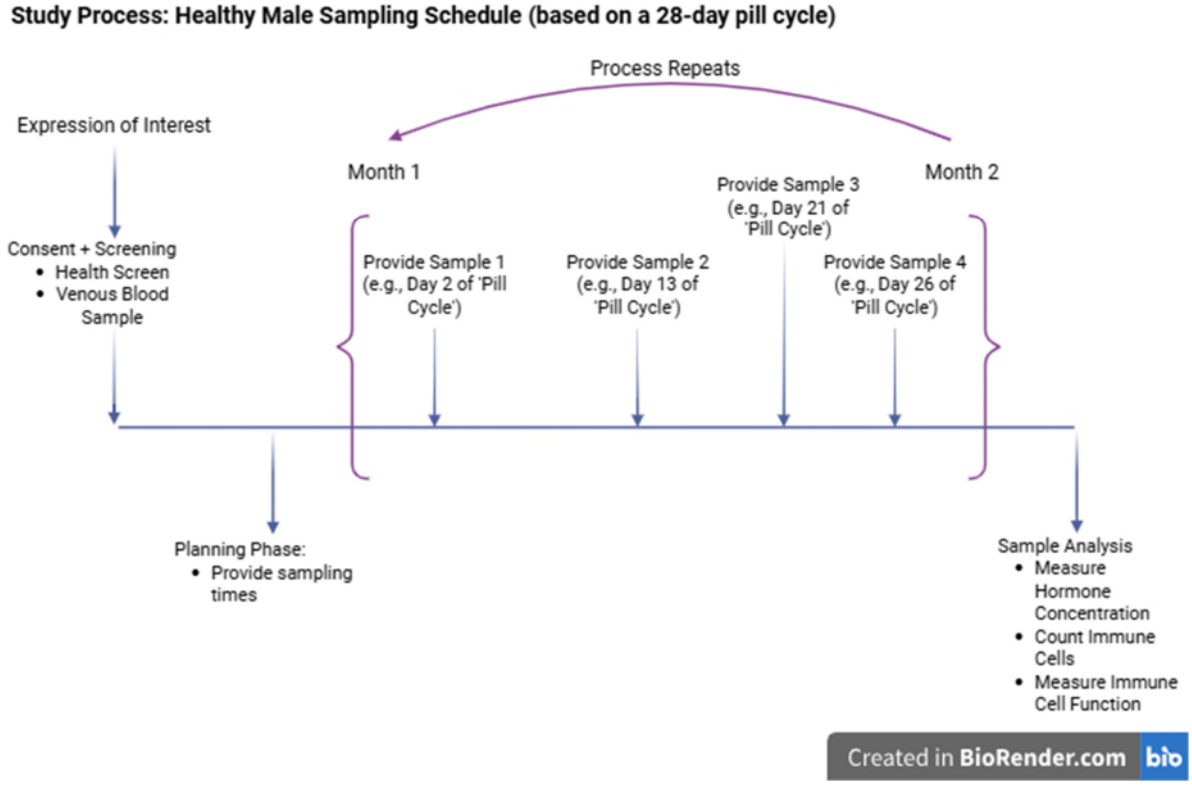

Figure 4: *Study overview modelled on a 28-day pill cycle.* *Figure created using BioRender.com*

End of the study

Your involvement in the study will end at the end of the last visit in your study schedule. You will have contact details for the study team in case there are any problems after this, however, none are anticipated. At the end of the study, if your blood markers from your final sample indicate you are anaemic, we will contact you to recommend you see your GP.

6. How do you decide when to take my blood samples?

The timing of blood sampling in this study is decided on an individual basis, depending which study group you are in.

Study Group 1: Eumenorrheic Female

Timing of blood samples will be decided on the basis of data provided on previous cycles, and on timing of 'milestone' menstrual cycle stages within the study.

We generally think of the menstrual cycle as composed of 4 phases, each of which is characterised by different concentrations of sex hormones. This is shown in the *Figure 5* below. In this study we will aim

to take blood samples from each of the phases of the menstrual cycle to observe immune cells with different sex hormone exposures.

Study Group 2: Female using Oral Contraceptive

People taking 28-day monophasic or multiphasic combined oral contraceptive pills will be modelled as 'textbook' 28-day pill cycles (as modelled in *Figure 3*), with their withdrawal bleed mimicking a menstrual period (i.eDay 1 will be considered as the first day no pill or a placebo pill is taken). This will mean the time points within this group will be predictable at the beginning of the study and will not vary. The testing days will therefore be day 2, 13, 21 and 26 of each cycle.

Study group 3: Healthy Male

Each male will be modelled on a textbook 28-day pill cycle (as modelled in *Figure 4*). This will mean the time points within this group will be predictable at the beginning of the study and will not vary.

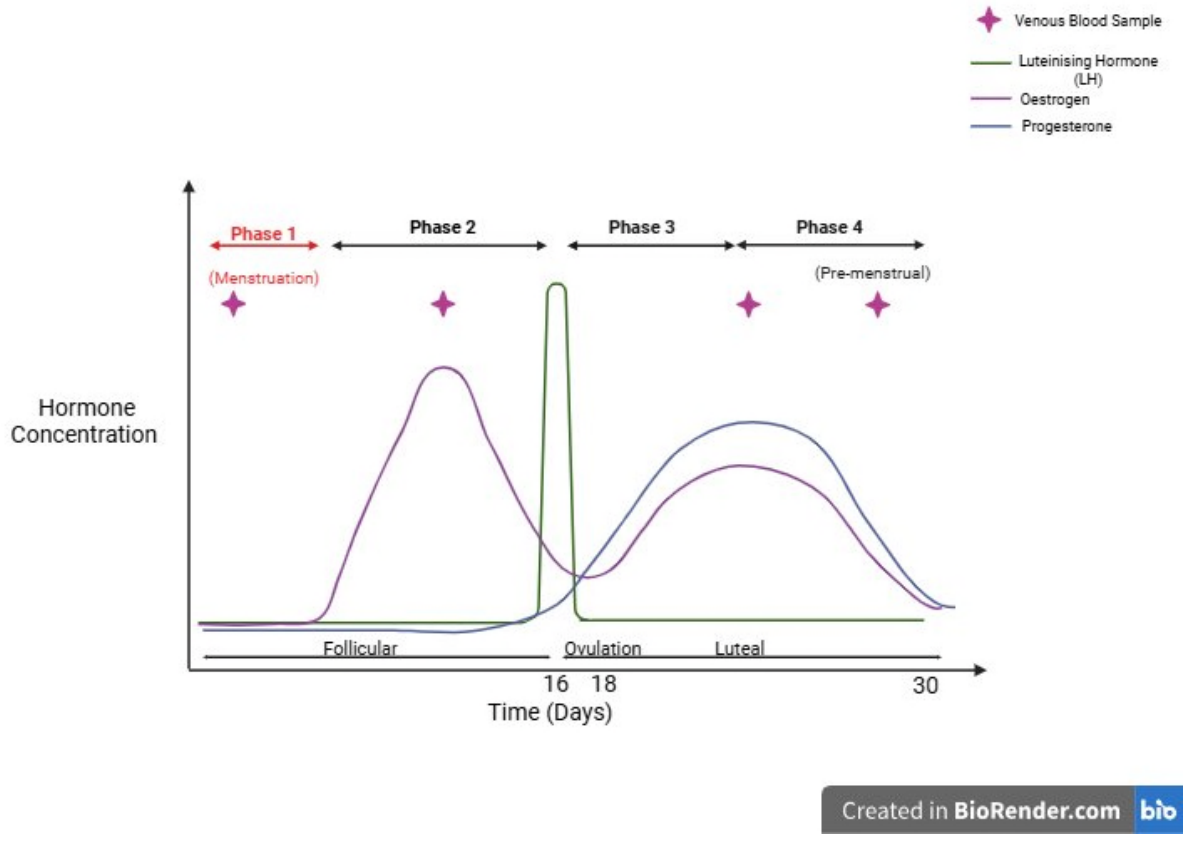

Figure 5: *Defined fluctuations in menstrual cycle based on average 30-day cycle.* *Figure created using BioRender.com*

7. What are my responsibilities?

It is important to consider whether you can commit to the entire study schedule, as far as possible.

If you take part in the study, we will ask you to avoid the following **on the day of blood sampling** prior to collection:

- Moderate/high intensity exercise
- Smoking of tobacco or cannabis or use of vapes
- Consumption of antihistamines
- Consumption of caffeine (e.g., coffee, tea or energy drinks)

We will ask you to inform us if you develop symptoms that may be consistent with infection (e.g. a high temperature, new continuous cough), as this may have an impact on your immune responses.

We ask that, if possible, in the 24 hours prior to a study visit you use paracetamol in preference to other forms of pain relief (e.g., ibuprofen or aspirin).

If in the eumenorrheic group, we will also ask you to continue tracking your cycle throughout the study and LH test using at home urine test kits (provided by the study team). We ask you to confirm with us when the LH test is positive via message and supply a photo of the test as evidence. This is to assist in scheduling your next visit.

8. Where will the study take place?

The study will take place in one of the Oxford Clinical Research facilities at the Nuffield Orthopaedic Centre (NOC) (OX3 7LD) and Churchill Hospital (OX3 7LE). We will confirm which with you. If these study sites are difficult for you to get to it may be possible for an alternative suitable location for sample collection to be arranged.

9. Are there any possible disadvantages or risks in taking part?

The disadvantages of taking part relate to the inconvenience of attending for study visits, and the small risk of adverse effects of the study procedures. You should consider the following risks before agreeing to take part. To minimise the risk of problems, all study procedures will be performed by experienced professionals using appropriate precautions and equipment.

9.1. Potential risks of blood sampling

Blood donation

The maximum volume of blood taken in this study would be 459ml which is just less than a regular blood donation (470ml). As the total volume taken across the study is equivalent to a standard blood donation it would not be expected to cause problems for an otherwise healthy volunteer. The blood donation itself requires the use of a needle into a vein of the arm, and this can cause minor bruising, tenderness, and occasionally feeling faint or fainting.

Iron deficiency/Anemia

We have specified a threshold of haemoglobin and serum ferritin that you must be above at your screening visit to be eligible to participate. This is in accordance with WHO guidelines. We will also assess your iron status at the end of the study, and recommend you seek advice from your GP if you fall outside normal ranges at either point.

10. Are there any benefits in taking part?

Individuals taking part in this study are unlikely to benefit directly from participation in this study. However, it is hoped that the information we gather from this study will help us to understand whether there are any differences in the immune system between males and females and within females across their menstrual cycle and on contraception. This information will help to understand sex-differences in disease states and might be used to inform treatment options in the future.

Whilst not the main purpose of the study the following indirect benefits may apply to individuals:

- Through study-specific screening individuals may benefit from the identification of previously unknown anaemia, iron-deficiency anaemia or iron deficiency. This will allow on-going investigation of its cause and appropriate management to be initiated through their GP. Iron-deficiency anaemia may cause symptoms such as tiredness, shortness of breath or reduced exercise tolerance and treatment should improve these.

- Through comprehensive tracking of menstrual cycles females may benefit from improved understanding and prediction of their own cycle. Those included in the study will have confirmed ovulatory cycles through measuring LH surges.

11. What happens if I don't want to carry on with the study at any point?

Participation is entirely voluntary. If you change your mind you can withdraw at any time without giving a reason and without penalty. If you withdraw from the study, any samples and data collected before your withdrawal will be used for research as detailed in this participant information leaflet, unless you specifically request otherwise. However, if any of your anonymised data has been incorporated into the study, it will not be withdrawn or erased in order to maintain the scientific integrity of the study. Should you withdraw mid-study, you will be compensated pro-rata according to the time spent.

12. Will I be reimbursed for taking part?

You will be compensated for your travel costs, time and inconvenience related to taking part in this study. The total amount of compensation you receive will depend on your degree of involvement:

- Per study visit: £12.50
- Full study involvement: £100 (involves 8 study visits with 8 venous blood samples)
- The screening visit will not be financially compensated as you will benefit from confirmation of health status or from identification of iron deficiency anaemia or iron deficiency

13. What will happen to any samples I give?

Blood samples we collect will be separated into cellular and non-cellular components and analysed in facilities based at NDORMS, University of Oxford. Cellular and acellular samples will be frozen and stored until analysis. After analysis cellular samples will be destroyed. Acellular samples may be stored for up to 5 years after study completion.

De-identified samples may be analysed in other hospitals, universities, non-profit institutions or commercial laboratories worldwide by other laboratories, including those overseas to gain further scientific insight.

We will ask for your consent for the use of your samples to be stored and potentially used in future ethically approved studies. If you agree to this, your anonymised samples will be used mainly by local researchers (if applicable), but ethically approved research projects may take place in hospitals, universities, non-profit institutions or commercial laboratories worldwide. If you agree to your samples being used in future research, your consent form will be held until the samples have been depleted or destroyed.

You, and any information about you, will not be directly identifiable as all data, including blood samples, will be labelled with a study code rather than your name. We will keep a linkage document with your name paired with your study code for 3 years after completion of the study, together with your consent form, as these are required for sample storage purposes.

14. What information will be collected and why is the collection of this information relevant for achieving the research objectives?

We will be using information provided by you to undertake this study and will use the minimum personally identifiable information possible. Consent forms will be held securely in locked filing cabinets at the University of Oxford for 3 years after publication of the study. Should you consent to being contacted about other studies in future, we will securely retain your contact information for up to 3 years after completion of this study, unless you request deletion before this. We will keep a copy of your consent form with this database, as your consent is our legal basis for re-contacting you under UK data protection law. Should you not consent to being contacted for future studies, we will delete your contact information upon conclusion of your involvement in the study. Bank details will be retained by the University finance team for 7 years for the purpose of audit.

Information we collect includes details about your health and medication history, and any data output from analysis of blood samples. This will be “de-identified” by assigning a unique study code to be used on all study documents and databases, which will be stored securely on encrypted university drives for 3 years after study publication, and only be accessible to study staff and personnel.

15. Will the research be published? Could I be identified from any publications or other research outputs?

The findings from the research may be written up as part of a DPhil thesis or masters dissertation and may form part of an academic publication or conference presentation. For thesis and dissertations, a

copy will be deposited in both print and online in the Oxford University Research Archive where it will be publicly available to facilitate its use in future research. Data in publications will be anonymised hence you would not be identified from publications or research outputs. Where such identifiable data might be made available in a research context, consent will be sought.

16. What will happen to my data?

The University of Oxford is the data controller with respect to your personal data and, as such, will determine how your personal data is used in the study.

The University will process your personal data for the purpose of the research outlined above. Research is a task that we perform in the public interest.

We will be using information provided by you in order to undertake this study and will use the minimum personally identifiable information possible. We will keep identifiable information about you for 3 years after the study has finished. We will also retain any research documents with personal information such as consent forms which will be held securely at the University of Oxford for 3 years after the end of the study.

UK Data protection regulation provides you with control over your personal data and how it is used. When you agree to your information being used in research, however, some of those rights may be limited in order for the research to be reliable and accurate. Further information about your rights with respect to your personal data is available from the University's Information Compliance web site at https://compliance.admin.ox.ac.uk/individual-rights.

You can find out more about how we use your information by contacting the study team (contact details at the top of this leaflet).

17. Who has reviewed this research?

This study has received ethics approval from a subcommittee of the University of Oxford Central University Research Ethics Committee. (MS IDREC 1072305)

18. Who is organising and funding this research?

This study is being organised by the Translational Pharmacology Group in the NDORMS department of University of Oxford. The funding will be sourced from the Lopez-Loreta Award and the Medical Sciences Internal Fund Pump Priming Award.

19. Who do I contact if I have a concern about the research or I wish to complain?

If a participant in research is ever considered to have suffered harm through their participation, the University has arrangements in place to provide for compensation. If you have a concern about any

aspect of this study, please contact translationalpharmacology@ndorms.ox.ac.uk and we will do our best to answer your query. We will acknowledge your concern within 10 working days and give you an indication of how it will be dealt with. If you remain unhappy or wish to make a formal complaint, please contact the University of Oxford Research Governance, Ethics & Assurance (RGEA) team at rgea.complaints@admin.ox.ac.uk or on 01865 616480.

20. Further information and contact details

If you would like to discuss the research with someone beforehand (or if you have any questions afterwards), please contact:

The Study Team: translationalpharmacology@ndorms.ox.ac.uk