Northwell Health

RESEARCH PROTOCOL

Protocol Title:	ROSE II: Pilot study to analyze menstrual blood to predict endometriosis
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Date Revised:	06/20/2023 v2
IRB Number:	22-0346

Guidelines for Preparing a Research Protocol

Instructions:

- You do not need to complete this document if you are submitting an *Application for Exemption* or *Application for a Chart Review*.
- Do not use this template if:
  - Your study involves an FDA regulated product. In this case, use the *Clinical Trial Protocol Template.*
  - Your study has a protocol from a sponsor or cooperative group. In this case, use the *Protocol Plus*.
  - Your study is a registry or repository for data and/or samples, In this case, use *Protocol Template – Registry Studies.* .
- If a section of this protocol is not applicable, please indicate such.
- Do not delete any of the text contained within this document.
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- Start by entering study information into the table above, according to these rules:
  - Protocol Title: Include the full protocol title as listed on the application.
  - Investigator: include the principal investigator's name as listed on the application form
  - Date Revised: Indicate the date at which the protocol was last revised
  - IRB Number: Indicate the assigned IRB number, when known. At initial submission, this row will be left blank.
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1. PREVIOUS STUDY HISTORY

Has this study ever been reviewed and rejected/disapproved by another IRB prior to submission to this IRB?

☐X No ☐ Yes – if yes, please explain:

2. BRIEF SUMMARY OF RESEARCH

- *The summary should be written in **language intelligible to a moderately educated, non-scientific layperson**.*
- *It should contain a clear statement of the rationale and hypothesis of your study, a concise description of the methodology, with an emphasis on what will happen to the subjects, and a discussion of the results.*
- *This section should be ½ page*

Study title: ROSE II: Pilot study to analyze menstrual blood to predict endometriosis

Study investigators:
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Study sites: Northwell Health, including clinical sites and the Feinstein Institutes for Medical Research

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INVESTIGATIONAL PRODUCT
The product consists of the analysis of menstrual blood and an algorithm (menstrual global (MG) score) for predicting endometriosis based on gene and/or protein expression analysis of selected cell types present in menstrual blood, as well as the cellular profile or composition of menstrual blood.

Intended use / purpose

This product will be used as a diagnostic test to screen for the likelihood of endometriosis in menstruating adults with symptoms suggestive of endometriosis. The purpose of the test will be to guide clinical decisions regarding: 1) whether to undergo diagnostic laparoscopy to confirm the presence of endometriosis (currently, the gold standard for diagnosing endometriosis) and/or 2) how to guide the choice of hormonal or other therapies to treat symptoms of endometriosis. **Note**: the current definition of endometriosis requires pathological analysis of lesions removed at the time of surgery (Tomassetti et al, 2021PMID: 34693033). For the purposes of this study, this product will not guide any clinical decisions for the participants. The purpose of the study is to confirm our prior results using scRNA-Seq.

Assay description

The assays are conducted on the cells and tissue present in menstrual blood. Upon receipt of the menstrual sample in the diagnostic laboratory, endometrial tissue fragments present in the sample will be digested with collagenase and DNAse I to release single cells that reflect the endometrium. After depletion of neutrophils and red cells, the remaining cells will be analyzed in multiple ways:

1. Flow cytometric analysis
2. Real time quantitative polymerase chain reaction (RT-qPCR) analysis
3. Single cell RNA sequencing (with or without cell-specific enrichment) following cell fixation

Genotyping will be performed using subject DNA derived from the menstrual blood or a cheek swab.

Peripheral Blood analysis

In addition, we may ask participants for a peripheral blood sample (10ml [for plasma] or about 2 teaspoons) following venipuncture. Blood will be separated to collect plasma, which will be analyzed for multiple analytes associated with inflammation and senescence using ELISA, MSD, and/or other platforms (Somalogics).

System description

1) Diagnostic flow cytometry will be carried out on a calibrated BD Fortessa analytic flow cytometer or similar analytic flow cytometer (calibrated).

2) RT-qPCR will be performed using the Roche Lightcycler 480 (or similar qPCR machine).

3) Single cell RNA sequencing will be carried out using 10X Genomics 1 10 Genomic Chromium X cell separator, followed by sequencing on an Illumina Next Gen 500 sequencing platform (or similar sequencing equipment).

Comparator products/methods
There are no existing commercially available products for the diagnostic analysis of cells or tissues present in menstrual effluent for endometriosis or any other condition. To our knowledge this is no commercially available product for predicting endometriosis using peripheral blood or other biological specimens (other than the analysis of ectopic endometriosis lesions themselves). Currently, definitive diagnosis of endometriosis requires laparoscopic surgery and pathological analysis of the removed ectopic lesions.

Abstract
Endometriosis is a chronic, complex, and common gynecologic disorder characterized by the growth of endometrial-like tissues outside of the uterus that is accompanied by inflammation. One of the most frustrating problems for those with endometriosis is the long delay before being diagnosed, which can be up to 7-10 years. One of the reasons for this delay is that definitive diagnosis requires invasive surgery (Tomassetti et al, 2021 PMID: 34690084). There are no approved non-invasive methods for predicting endometriosis. This study will assess the analysis of fresh menstrual blood as a non-invasive predictor of endometriosis. This approach is based on the numerous reports documenting the differences in the endometrium of women with and without endometriosis; this endometrium is shed each month as menstrual blood which can be easily collected and analyzed. Through this prospective, non-interventional pilot study we propose to collect and analyze menstrual blood from healthy controls without chronic symptoms of endometriosis and symptomatic women who have significant and chronic symptoms suggestive of endometriosis and are considered by their healthcare providers to be candidates for diagnostic laparoscopic surgery in the coming months (as part of their standard care). Menstrual blood from controls and symptomatic cases will be analyzed using single cell RNA-sequencing to develop a panel of biomarkers that can be developed into a screening test or diagnostic test for endometriosis.

The primary objectives of this study are to confirm the results of our recent single cell RNA sequencing (scRNA-Seq) analysis of menstrual effluent obtained from healthy controls vs. endometriosis (and symptomatic subjects) obtained through IRB 13-376 and to develop a screening/ diagnostic algorithm (menstrual global (MG) score) based on the data to be used to predict endometriosis in symptomatic patients.

The secondary objective is to assess the reproducibility of the scRNA-Sequencing data using menstrual blood collected across different menstrual cycles among a subset of controls and/or cases (symptomatic patients).

3. INTRODUCTION/BACKGROUND MATERIAL/PRELIMINARY STUDIES AND SIGNIFICANCE

- *Describe and provide the results of previous work by yourself or others, including animal studies, laboratory studies, pilot studies, pre-clinical and/or clinical studies involving the compound or device to be studied.*
- *Include information as to why you are conducting the study and how the study differs from what has been previously researched, including what the knowledge gaps are.*
- *Describe the importance of the knowledge expected to result*

Endometriosis is considered a common, complex, and chronic disease with a spectrum of subtypes and clinical presentations. In 2021 an international working group representing four international societies focused on endometriosis (American Association of Gynecologic Laparoscopists (AAGL), European Society for Gynecological Endoscopy (ESGE), European Society of Human Reproduction and Embryology (ESHRE) and World Endometriosis Society (WES)) collaboratively defined endometriosis "as a disease characterized by the presence of endometrium-like epithelium and/or stroma outside the endometrium and myometrium, usually with an associated inflammatory process" (International working group of AAGL, ESGE, ESHRE and WES; Tomassetti et al, 2021 PMID: 34690084). In addition, the group defined endometriosis subtypes and locations (International working group of AAGL, ESGE, ESHRE and WES; Tomassetti et al, 2021 PMID: 34690084). This diagnosis relies solely on invasive laparoscopic surgery (or laparotomy) and excision of lesions for pathological confirmation as a gold-standard diagnostic. There are numerous studies supporting the delay in diagnosing endometriosis – with ranges spanning up to 7-10 years between the onset of symptoms and diagnosis (Ballard 2006, PMID: 17070183; Dunselman 2014, PMID: 24435778; Johnston et al, 2015, PMID: 25624305). The reasons for this delay are numerous – however, it is clear that many delay or forgo the invasive diagnostic procedure. Thus, many patients and healthcare providers are left without a diagnosis for several years (or forever). Lack of an early diagnosis also prevents early and subtype-specific treatment strategies and prevents the implementation of clinical trials for assessing the effectiveness of various early treatment strategies.

Our group has identified numerous distinct features of menstrual blood in those with surgically confirmed endometriosis vs. healthy controls (Warren et al, 2018 PMID: 30134794; Nayyar et al, 2020; Shih et al, 2022 MedRxiv). The scientific premise for studying menstrual effluent are the well-described differences in the eutopic endometrium (the cells lining the inner space of the uterus where fertilized eggs implant) of women with endometriosis vs. healthy controls – which mainly point to increased inflammation in the setting of endometriosis (Brosens et al, 2012, PMID: 22417665; Liu and Lang, 2011, PMID: 21455119; Drury et al, 2018, PMC6052567; Chehna-Patel et al 2010, PMID: 20236630; Bulun et al, 2006, PMID:

16406281; Aghajanova and Giudice, 2011, PMID: 21063030). In addition, only animals that menstruate are known to develop endometriosis (e.g. humans, non-human primates, elephant shrews, and some types of bats) and endometriosis can be facility in non-human primates by delivering shed endometrial lining intraperitoneally in recipient animals. Finally, the most commonly accepted theory for the pathogenesis of endometriosis is Sampson's theory of retrograde menstruation (described in 1929) that describes the delivery of menstrual tissues to the peritoneal cavity via the fallopian tubes with each menstrual cycle. While it is known that over 90% of women experience retrograde menstruation only about 10% of females of reproductive age develop endometriosis. Thus, there must be other contributing factors.

Numerous studies support the existence of alterations within the endometrium in patients with endometriosis, and which we have shown can be analyzed by evaluating menstrual blood (Warren et al, 2018 PMID: 30134794; Nayyar et al, 2020). This pilot study will analyze the menstrual blood collected from women who have chronic symptoms indicative of endometriosis (who are expected to undergo diagnostic laparoscopic surgery) and compare the results with the menstrual blood collected from women who do not have these symptoms (controls) to confirm the differences that we have previously found in patients with endometriosis vs. controls (Shih et al BMC Med, 2022). These differences will be used to develop a rapid, non-invasive diagnostic product to assist in diagnosing endometriosis; this product will identify those with a high likelihood of endometriosis so that they are encouraged to undergo definitive diagnostic laparoscopic surgery in a timely manner. At a minimum this approach is expected to significantly shorten the common diagnosis delay of 7-10 years. In addition, we propose to compare repeat menstrual samples from the same subjects to confirm the reproducibility of our findings and to compare menstrual samples before and after treatment (surgical and non-surgical). This will provide a method to determine whether menstrual blood analyses can be used to track patient responses to therapy and manage these treatments.

Use in patient management

Clearly, an earlier, rapid, and non-invasive diagnostic for endometriosis is warranted based on the published evidence of prolonged delays in the time between symptoms and diagnosis. This pattern of delay in diagnosis is partly due to the need for a laparoscopic surgery, which patients are often reluctant to pursue due to the expense, discomfort, and fear of invasive surgical procedures. In addition, there are no methods for measuring endometriosis patients' responses to therapies. Almost all non-surgical treatment options are hormone-based. There is little evidence these therapies revert or slow disease progression and many patients claim that the treatments are worse than the disease. Our approach will likely provide some insight into the pathogenesis of disease and hopefully this can be leveraged to personalize treatments. Also, the numerous subclasses of

endometriosis are based on surgical findings and these have had a limited influence on better understanding the disease. The approach to using menstrual blood to evaluate various subclasses of disease along with clinical information may improve our understanding of the disease so that better treatment options can be developed.

Prognostic use
Several reports have defined differences in the endometrium of women with and without endometriosis. These cell-based, biochemical, hormonal, and gene expression differences are the foundation for studies focused on analyzing menstrual effluent for screening or diagnosing endometriosis. Our prior studies support significant differences in stromal cells of women with and without endometriosis that can be leveraged to develop a diagnostic or screening tool for identifying this condition (Warren et al, 2018 PMID: 30134794; Nayyar et al, 2020, Shih et al, BMC Med 2022).

4. OBJECTIVE(S)/SPECIFIC AIMS AND HYPOTHESES

- *A concise statement of the goal(s) of the current study.*
- *The rationale for and specific objectives of the study.*
- *The goals and the hypothesis to be tested should be stated.*

Primary objectives
The primary objectives of this study are to confirm the results of our recent single cell RNA sequencing (scRNA-Seq) analysis of menstrual effluent obtained from healthy controls vs. endometriosis (and symptomatic) subjects obtained through IRB #13-376 (as described in Shih et al, 2022 and to develop a screening/diagnostic algorithm (menstrual global (MG) score) based on the data to be used to predict endometriosis in symptomatic patients.

Secondary objectives
The secondary objective of this pilot study is to assess the reproducibility of the scRNA-Seq data using menstrual blood collected across different menstrual cycles among a subset of controls and cases (symptomatic patients).

Additional objectives
We will translate the scRNA-Seq data into a predictive diagnostic product based on scRNA-Seq analysis of menstrual blood. Using emergent and prior scRNA-Seq data, we will establish the most cost-effective methods of measuring the parameters contained within the screening/ diagnostic algorithm (MG score) determined in the primary objective.

In addition, in cases where peripheral blood is collected and processed to isolate plasma, numerous analytes will be evaluated to identify a signature associated with endometriosis. We may use information on inflammation or senescence markers in menstrual blood or peripheral blood to explore the utility in contributing to a diagnostic algorithm.

5. RESOURCES AVAILABLE TO CONDUCT THE HUMAN RESEARCH

- *Explain the feasibility of meeting recruitment goals of this project and demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period*
    - *How many potential subjects do you have access to?*
- *Describe your process to ensure that all persons assisting with the trial are adequately informed about the protocol and their trial related duties and functions*

Patient population

The cohort will be made of up to 185 menstruating individuals in total; up to 80 control subjects who self-report the general absence of symptoms of endometriosis and up to 105 symptomatic cases who report chronic symptoms of endometriosis (defined below) but who have not yet been diagnosed and are seeking physician evaluation for their symptoms.

Controls: Control subjects will be recruited via the 'control' pool of participants in the ROSE (Research OutSmarts Endometriosis) study (IRB# 13-376). Control subjects (who generally do not experience symptoms of endometriosis – see below) will be consented for this study. Control subjects will complete a gynecologic/medical information form, demographics form, and quality of life form (SF-36). Control subjects must be willing to provide up to four separate menstrual samples (using a menstrual collection kit mailed to their home [with detailed collection, packing and shipping instructions]) within 1-12 months of consenting/enrolling; each sample will be obtained in separate menstrual cycles (usually in different months). A menstrual collection form will be completed with each sample collection. The second menstrual blood sample will be used to assess the reproducibility of the 'diagnostic test' over subsequent cycles. Controls may be asked to provide two additional menstrual samples if the first or second sample does not meet sample criteria. The data of the patients will be pseudonymized by a study-specific patient ID (see section 9.4). Control subject IDs will be linked to the subjects' original ROSE study IDs.

Symptomatic cases: To be eligible, symptomatic subjects must be considered by their physician a candidate for laparoscopic surgery or a laparotomy procedure for the detection of endometriosis (which will occur as part of standard of care –

laparoscopic surgery is not part of research). *Symptomatic cases must self-report experiencing at least three of the following six symptoms for the past 4-5 months: 1) chronic pelvic pain, 2) painful menses, 3) pain during intercourse, 4) pain going the bathroom, 5) abdominal bloating, 6) missed days of work, school, athletic, social, and/or other activities due to related pain and discomfort

The symptomatic cases (with chronic symptoms of endometriosis, described above) will be recruited from the participating OBGYN sites at Northwell Health and outside of Northwell Health (to be added after initiating study at Northwell Health). Symptomatic case recruitment rates are based on reasonable planning expectations. The site investigators and their designated coordinators will continuously compare the actual and expected recruitment rates and make every effort to ensure that they are as closely matched as possible. If the investigator anticipates major problems with recruitment or a delay in expected completion date, the site investigator will discuss this with the executing CRO/Northwell as early as possible.

Symptomatic subjects must be willing to provide up to four menstrual samples (using a menstrual collection kit mailed to their home [with detailed collection, packing and shipping instructions]). Symptomatic participants must provide at least one menstrual sample within 1-2 months of consenting/enrolling PRIOR to scheduled laparoscopic surgery. Additional menstrual sample(s) will be requested if the first sample does not meet sample criteria and there is time prior to planned surgery. In addition, symptomatic patients will be asked to provide optional menstrual blood sample(s) within 3 months post- surgery if they were diagnosed with endometriosis.

Symptomatic subject IDs will be linked to the subjects' original ROSE study IDs – in cases where the symptomatic subjects are referred via the original ROSE study.

Training
All study staff are CITI-trained and participate in additional training throughout the year regarding human subject research guidelines and regulations, as well as the policies of the IRB/HRPP office at Northwell Health. Also, pertinent staff will have full knowledge of study parameters, protocol, and consent.

6. RECRUITMENT METHODS

- *Describe the source of potential subjects*
- *Describe the methods that will be used to identify potential subjects*
- *Describe any materials that will be used to recruit subjects. A copy of any advertisements (flyers, radio scripts, etc.) should be submitted along with the protocol.*

- *If monetary compensation is to be offered, this should be indicated in the protocol*

All participants must be >18 years of age or <40 years of age (18 years to 40 years of age) and be willing to provide 1-4 menstrual effluent (ME) samples and must fall in inclusion/exclusion criteria (as noted in section 7).
Symptomatic cases: The symptomatic cases (with chronic symptoms of endometriosis and pending surgery) will be recruited from the participating OBGYN sites at Northwell Health and outside of Northwell Health (to be added after initiating study at Northwell Health). A recruitment flyer will be posted in laparoscopic surgeons' offices of participating Northwell (and non-Northwell) collaborating laparoscopic surgeons to recruit patients who are being evaluated for endometriosis diagnosis by surgery (patients must have symptoms of endometriosis and being evaluated for laparoscopic surgery, which is part of their standard care). In addition, surgeons may discuss the study with potential participants. Additionally, social media/online information/print information highlighting the ROSE study may be used to recruit patients who are suffering with symptoms and contemplating surgical diagnosis. Finally, some symptomatic subjects who are considering diagnostic surgery may be recruited via the original ROSE study.

Recruitment materials: The ROSE II Study flyer is attached.

Controls: Control subjects will be recruited via the 'control' pool of participants in the ROSE (Research OutSmarts Endometriosis) study (IRB# 13-376) as well as word of mouth from recent study media attention. Note: the ROSE study participants (IRB# 13-376) consent to allow re-contact). Additionally, controls may be recruited via social media/online/print stories featuring the ROSE study

Reimbursement to participants: Participants will be compensated $50 for each menstrual blood collection (that meets a minimum volume of 3.0-3.5 mL or just less than ¾ of a teaspoon) using a menstrual cup (the study provides a free Diva Cup, but participants may choose to use their own menstrual cup) and/or $25 for each menstrual blood collection using a menstrual sponge/pad (that meets a minimum saturation of 40%). Participants who provide a peripheral blood sample will receive $25.

7. ELIGIBILITY CRITERIA

- *Describe the characteristics of the subject population, including their anticipated number, age, ranges, sex, ethnic background, and health status. Identify the criteria for inclusion or exclusion of any subpopulation.*

- *Explain the rationale for the involvement of special classes of subjects, such as fetuses, pregnant women, children, prisoners or other institutionalized individuals, or others who are likely to be vulnerable. You cannot include these populations in your research, unless you indicate such in the protocol*
- *Similarly, detail exclusionary criteria: age limits, special populations (minors, pregnant women, decisionally impaired), use of concomitant medications, subjects with other diseases, severity of illness, etc.*

Controls

Control participants (those with a general absence of chronic symptoms of endometriosis) will be recruited from the ongoing ROSE study via the ROSE coordinating team.

Inclusion criteria:

Age 18-40 years

Continue to get periods regularly, i.e. menstruating and have uterus

Have more than a light flow (<4ml/8hr peak flow – subject is soaking less than 1 thin pad or light tampon in 4 hours at peak flow)

General absence of symptoms suggestive of endometriosis (1 symptom allowed):

1) chronic pelvic pain, 2) painful menses, 3) pain during intercourse, 4) pain going the bathroom, or 5) abdominal bloating, BUT MUST NOT report missed days of work, school, athletic, social, and/or other activities due to related pain and discomfort.

Willingness to provide up to four menstrual samples across four separate menstrual cycles.

Willingness to provide a DNA sample (obtained via menstrual blood or cheek swab)

Symptomatic Cases

Symptomatic subject must report experiencing at least three of the following six symptoms for the past 4-5 months: 1) chronic pelvic pain, 2) painful menses, 3) pain during intercourse, 4) pain going the bathroom, 5) abdominal bloating, 6) missed days of work, school, athletic, social, and/or other activities due to related pain and discomfort.

Patients with persistent symptoms of endometriosis who are being evaluated by laparoscopic surgeons for the definitive diagnosis of endometriosis will be referred to the coordinators for study information, screening, and enrollment.

Inclusion criteria:

Age 18-40 years

Continue to get periods regularly, i.e. menstruating and have uterus

More than light flow (<4ml/8hr peak flow – subject is soaking less than 1 thin pad or light tampon in 4 hours at peak flow)
Consistently experiencing chronic symptoms of endometriosis* - without definitive diagnosis
Seeking physician evaluation; is considered by the physician to be a candidate for laparoscopic surgery within the next 1½-4 months
Willingness to provide at least one menstrual sample prior to planned surgery
Surgical and pathologic confirmation of endometriosis (or not) at laparoscopy
OPTIONAL: participants may provide additional pre-op & a post-op menstrual sample(s)
([if diagnosed with endo] for assessing patient response to therapy (non-surgical and surgical)
Willingness to provide a DNA sample (obtained via menstrual blood or cheek swab)

Participant (control and symptomatic) exclusion criteria
>18 years of age or <40 years of age (participants must be 18 years to 40 years of age)
Prior surgical diagnosis of endometriosis, with histological confirmation

No uterus or no menstruation
Inability to collect menstrual sample
Participants with recently placed IUD or other intrauterine devices (within 6 months), will need GYN approval for menstrual cup collections
IUD or other intrauterine devices that prevent menstrual sample collections because they block menstruation
Light menstrual flow (<4ml/8hr peak flow – subject is soaking less than 1 thin pad or light tampon in 4 hours at peak flow)

8. NUMBER OF SUBJECTS

- *Indicate the total number of subjects to be accrued locally. If applicable, distinguish between the number of subjects who are expected to be pre-screened, enrolled (consent obtained), randomized and complete the research procedures.*
- *If your study includes different cohorts, include the total number of subjects in each cohort.*
- *If this is multisite study, include total number of subjects across all sites.*

The cohort will enroll a total of up to 185 menstruating individuals; up to **80 control subjects** who self-report the general absence of symptoms of

endometriosis and up to **105 symptomatic cases** who report chronic symptoms* of endometriosis but who have not yet been diagnosed and are seeking physician evaluation for their symptoms.

9. STUDY TIMELINES

- *Describe the duration of an individuals participation in the study*
- *Describe the duration anticipated to enroll all study subjects*
- *The estimated date of study completion*

Time of involvement per patient

Control participants will be involved in the study for a minimum of approximately 2 months or until they collect and ship their two menstrual samples (obtained during two separate menstrual cycles) for analysis. Once the second sample passes quality control, then their active participation will end. If the second sample does not pass quality control, the control subjects may be asked to provide additional menstrual collections (up to four collections). Study participation could last up to approximately 12 months (maximum length of participation) for control subjects who would like to provide an additional menstrual sample for reproducibility studies to compare results of menstrual analyses across cycles.

Symptomatic cases will be involved in the study for a minimum of 2½ months or until they collect and ship their menstrual sample for analysis and undergo laparoscopic surgery (part of their standard care) and share their surgical and pathology results. Study participation for symptomatic cases could last up to approximately 6 months post-laparoscopy surgery, if symptomatic cases collect and ship a post-surgical menstrual sample (although this is not mandatory for the study).

Study duration

Active participation of affected/symptomatic cases in this study will last for approximately 2-4 months from the time of enrollment. Control subjects will actively participate for approximately 2-6 months from the time of enrollment. Samples and data will be held indefinitely.

The study endpoint is 2 years from start to include data analysis.

10. ENDPOINTS

- *Describe the primary and secondary study endpoints*
- *Describe any primary or secondary safety endpoints*

Primary endpoint: directly measure and compare test characteristics of the screening/diagnostic test (as determined by the MG score) to predict the presence

of endometriosis in symptomatic patients (who undergo definitive diagnostic laparoscopy)

Secondary endpoint: assess the reproducibility of the screening/diagnostic test (MG score) in subset of subjects

For primary and secondary acceptable criteria includes successful isolation of cells with viability >60% and single cell RNA-Seq analyses; minimum number of cells to be analyzed ≥1000). Peripheral blood plasma samples will be assessed for hundreds of analytes using multiplex ELISAs, MSDs or other platforms (Somalogics).

Planned analyses

Primary study endpoint: directly measure and compare test characteristics of the screening/diagnostic test (as determined by the MG score) to predict the presence of endometriosis in symptomatic patients (who undergo definitive diagnostic laparoscopy).

Bioinformatic analyses will be performed to determine whether scRNA-Seq data collected from the samples described in this study are similar to our previous study that enrolled controls, symptomatic patients, and those previously diagnosed with endometriosis. The major features previously observed were the increase in B cells among endometriosis and symptomatic patients (vs. controls), the decrease in uterine natural killer cells among endometriosis and symptomatic patients (vs. controls), as well as significantly differentially expressed genes among various cell types found in menstrual effluent tissues (e.g. stromal cells)**.**

Secondary study endpoints assess the reproducibility of the screening/diagnostic test (MG score) in subset of subjects.

Paired analyses will be performed to assess reproducibility of profiles, including cell type differences and gene expression scores in menstrual effluent tissues obtained from control (and symptomatic) participants who provide more than one sample for the study.

11. RESEARCH PROCEDURES

- *Include a detailed description of all procedures to be performed on the research subject and the schedule for each procedure.*

- *Include any screening procedures for eligibility and/or baseline diagnostic tests*
- *Include procedures being performed to monitor subjects for safety or minimize risks*
- *Include information about drug washout periods*
- *If drugs or biologics are being administered provide information on dosing and route of administration*
- *Clearly indicate which procedures are only being conducted for research purposes.*
- *If any specimens will be used for this research, explain whether they are being collected specifically for research purposes.*
- *Describe any source records that will be used to collect data about subjects*
- *Indicate the data to be collected, including long term follow-up*

Overview of study procedures

Procedure	Summary
Subject recruitment	Recruitment of patients according to the eligibility criteria; up to 80 controls and up to 105 symptomatic patients (who are pending laparoscopic surgery) will be enrolled.
Sample collection	Collection of menstrual blood (min 3-3.5ml, max approx. 20mL per collection) and subsequent processing to yield cells that will be analyzed. Control subjects will consent to providing up to four menstrual blood samples across four different cycles. Symptomatic patients will consent to providing up to four menstrual blood samples; at least one menstrual blood sample prior to their scheduled laparoscopic surgery (part of their standard care). Symptomatic patients may provide optional menstrual blood sample(s) 1-6 months post laparoscopic surgery. In addition, control subjects and symptomatic subjects who are local to the NY metro area may be asked to donate one peripheral blood sample (10 mL or about 2 teaspoons). We will arrange to have participants' peripheral blood taken wherever it is most convenient for them: either at a blood drawing service center at Northwell Health, or by having a blood drawing service perform the service at the subjects' home or place of work. For symptomatic subjects, the samples will be collected prior to surgery. Lastly, discarded tissue from surgeries may be collected. We might access tissue samples and data collected at any point of the participants' care.

Data collection	Collection of individual data: i) demographics, medical and gynecologic history, medication use, and quality of life assessment; and ii) information related to each menstrual blood collection (time of collection, pain, and pain medication during collection). Symptomatic patient data will also include standard-of-care diagnosis and follow-up, as well as surgical and pathology reports. This data will be obtained after subjects complete an Authorization for Medical Record request. Additionally, laparoscopic surgeons performing diagnostic surgery on symptomatic subjects to definitively diagnose endometriosis will complete a surgical form documenting laparoscopic surgery. surgical case and complete/submit the surgical form
Central sample testing (Feinstein Institutes for Medical Research)	Processing of menstrual blood and assessment by: 1. Flow cytometric analysis or other protein assessment methods. 2. Real time quantitative polymerase chain reaction (RT-qPCR) analysis 3. Single cell RNA sequencing (with or without cell-specific enrichment) following cell processing/preparation (as described above) Processing of peripheral blood for plasma for assessment of inflammatory and senescence-associated markers. Processing of cheek cells or menstrual blood for DNA, this information is required for single cell RNA sequencing of menstrual blood (to identify subjects based on genotype).

STUDY PROCEDURES: SAMPLE COLLECTION

Patient population

The cohort will be made of up to 185 menstruating individuals in total; up to 80 control subjects who self-report the general absence of symptoms of endometriosis and up to 105 symptomatic cases who report chronic symptoms of endometriosis (defined below) but who have not yet been diagnosed and are seeking physician evaluation for their symptoms.

Controls:

-Complete interest/screening form and consent process; enroll in study
-Complete demographics (including mailing/shipping address), gynecologic and medical questionnaire, including medication/hormone usage, and quality of life (SF-36) form

-Receive menstrual blood collection kits (mailed or priority shipped), with detailed instructions for collecting menstrual blood and shipping menstrual sample to the lab

-Provide menstrual blood samples (2 within months 1-3)

Collect menstrual blood according to menstrual cup/sponge collection kit instructions

Complete menstrual collection form (includes pain scale/score, hormones, pain meds, as well as menstrual collection times) with each menstrual blood collection

Ship menstrual blood with completed menstrual collection form to lab according to instructions (for each collection)

-May provide a peripheral blood sample from the forearm (10mL or 2 teaspoons) from local participants once during menstruation

-Provide a DNA sample (obtained either via menstrual blood or cheek swab)

Step 1: Pilot Schedule of Visits for CONTROLS

CONTROLS Recruited from ROSE study or from those interested in joining the ROSE study	Screening/ Inclusion	At home ME collection	At home ME collection (optional)
Time	Baseline	Month 1	Month 2 or 3
Inclusion/Exclusion Criteria -Interest/enrollment form	X		
Consent	X		
Health, quality of life (SF-36), demographics and medical/gynecologic questionnaires	X		
Menstrual collection, with completion of menstrual collection form; may include peripheral blood collection at home or local phlebotomy		X X (peripheral blood only)	X

<u>Symptomatic cases</u>:

-Complete interest/screening form and consent process; enroll in study

-Complete authorization form for collection of pathology report and operative notes

-Complete demographics (including mailing/shipping address), gynecologic and medical questionnaire, including medication/hormone usage, and quality of life (SF-36) form

-Receive menstrual blood collection kits (mailed or priority shipped), with detailed instructions for collecting menstrual blood and shipping menstrual sample to the lab

-Provide menstrual blood sample(s) (at least 1 time prior to laparoscopic surgery and optional: 1 or more collections within 6 months post-surgery)

Collect menstrual blood according to menstrual cup/sponge collection kit instructions

Complete menstrual collection form (includes pain scale/score, hormones, pain meds, as well as menstrual collection times) with each menstrual blood collection

Ship menstrual blood with completed menstrual collection form to lab according to instructions (for each collection)

-May provide a peripheral blood sample from the forearm (10mL or 2 teaspoons) from local participants once during menstruation

-Surgical form and pathology report (post-laparoscopic surgery) shared with study (via Authorization for medical records to obtain pathology report and operative reports

-Post-op evaluation by physician; symptomatic patients will repeat SF-36 form within 6 months post-surgery

-Provide a DNA sample (obtained either via menstrual blood or cheek swab)

Step 1: Pilot Schedule of Visits for Symptomatic CASES

SYMPTOMATIC CASES	Screening/ Inclusion Visit	At home ME Collection	At home ME Collection (optional)	Laparoscopic Surgery/ Evaluation	Post-Laparoscopic Evaluation	At home ME Collection
Time	Baseline	Up to 4-5wks post-baseline	Up to 10 wks post-baseline	Up to 12 wks post baseline	Up to 6 wks post surgery	Up to 3 months post surgery
Inclusion/Exclusion Criteria	X					
Consent	X					
Health, quality of life (SF-36), demographics & medical/gynecologic questionnaires	X				X (SF-36)	
Menstrual collection, with completion of menstrual collection form; may include peripheral blood collection at home or local phlebotomy		X X (peripheral blood only)	X			X
Pathology report				X		
Post op eval form (surgeon)					X	

Participant and sample/form identification

Every participant enrolled will be pseudonymized by and assigned to a multi-part number as a
patient ID. It consists of the study abbreviation XX, the site number and a consecutive patient number, e. g. XX02001 [CP = study abbreviation, 02 = site number (2 digits), 001
= patient number (X digits)]. Each sample and data form will contain a subject ID and a sample ID/form ID. The use of this patient ID in the eCRF in the electronic data capture system allows a clear attribution of each sample and data capture form to a single subject. Menstrual blood collection containers and forms will be pre-labeled with the subject ID and sample/form ID.

A separate subject identification list will be maintained allowing the tracking of the participants by
the sample ID. This list will remain at the coordinating site (Feinstein).

STUDY MATERIALS

Menstrual sample collection
Following consent, subjects will complete an online questionnaire to identify the method of menstrual collections (sponge vs. menstrual cup), as well as cup size, if applicable, as well as a demographics form to provide their mailing address and contact information for shipping/mailing 'at home' menstrual collection kits. Subjects may receive their collection kits by mail/priority shipping or at the study site. Menstrual sample collections will be done by study participants at home (or the site of their choice) using the menstrual collection kit (provided by the study) following detailed instructions. After each menstrual collection, subjects will pack and ship their collected menstrual sample using the shipping box and FedEx label provided by the study (a courier may be used for local subjects). Subjects will also complete a sample collection form at the time of each menstrual collection, which will be included in the shipment with their menstrual sample according to the instructions. The menstrual collection kits will be delivered by commercial overnight delivery services to testing laboratories at Northwell Health. Note: for local participants, a courier may be used to transport the sample to the lab.

DNA sample: DNA will be isolated from either menstrual blood or a cheek swab.

Peripheral blood sample: One peripheral blood sample (10mL or about 2 teaspoons, using purple top EDTA tube) may be obtained from local participants. We will arrange to have blood drawn wherever it is most convenient for the participant: either at a blood drawing service center at Northwell Health, or by having a blood drawing service come to the subjects' home or place of work. Once delivered to the lab, the blood will be centrifuged, and plasma will be collected and analyzed.

Surgical samples (optional): Discarded tissue from surgeries may be collected. Laparoscopic Surgery is often part of the treatment for women with endometriosis. Commonly during such surgery, the endometriosis tissue is destroyed using laser therapy or electrical energy. In contrast, some surgeons perform a surgical procedure in which ectopic endometrial tissue nodules are excised intact, without the use of any energy (cold scissor excision). In this procedure, because the tissue is not destroyed, it is possible to use discard tissue samples for banking.

We might access tissue samples and data collected at any point of the participants' care (prior to or after signature date) and related to her condition that are placed into long-term storage by the Pathology Department at Northwell Health or at other approved healthcare facilities. In cases where tissue specimens and related clinical or medical data are stored outside of Northwell Health, a ROSE Authorization for medical records form (IRB-approved) will be completed by the subject to obtain their expressed permission to collect the specimen/data.

Collected tissue samples will be:

1) snap frozen in liquid nitrogen as soon as possible after collection at a Northwell surgical site or
2) Placed in appropriate buffer or
3) Any other mechanism to safely preserve the integrity of the biospecimen, such as paraffin block

Materials to be provided by the study site

The central study site at Northwell Health will provide menstrual sample collection kits (with detailed instructions) to participants; ROSE coordinators will be available for answering questions regarding the menstrual sample collections 7 days a week. These collection kits for menstrual effluent consist of the following items:

- Detailed instructions
- Menstrual cup (DivaCup menstrual cup, size 0, 1 or 2 depending on patient size; patient may use their own menstrual cup) or menstrual sponge, depending on the subject's familiarity with menstrual cups
- Castille soap towelettes - 2 (PDI D41900), for sponge collections only
- Collection cup (Fisher, Cat# 13-711-56) (wrapped in gel blanket; **refrigerate upon receipt**)

containing antibiotics (100×, 10,000 units penicillin, 10mg streptomycin, Life Technologies, #15140-122) and antimycotics (Normocin Cat# ant-nr-2, InvivoGen) – (**refrigerate upon receipt**)

Collection cup for sponge sample will contain clear Hank's solution

- Gel Blanket (United States Plastic Corp, Cat#7524) to wrap sample vial/cup (**refrigerate upon receipt**)
- Gel Pouch (**refrigerate upon receipt**)
- Blue freezer block (**freeze upon receipt**) (Uline, Cat#S-18257)

- 2 Biohazard Bags (double bag) (Fisher Cat# 22-310-408) – for containing menstrual sample for shipment to the lab (USDOT and IATA compliant)
- Disposable non-latex (nitrile) gloves, size M (Cardinal Health Medical Products, Cat #8897NB); L (Cardinal Health Medical Products, Cat #8898NB) or Size S (Cardinal Health Medical Products, Cat #8896NB)– for sponge collections only (*vinyl gloves can be substituted)*
- Menstrual sample collection form (to be completed after collecting and submitted with menstrual sample)
- Return shipment box (keep the styrofoam box inside the outer cardboard box with UN3373 label affixed on side panel) – with FedEx label area (Uline, Cat#S-9903)

In cases where menstrual blood does not yield DNA, a cheek swab will be sent to the subject via mail to collect a cheek swab for DNA isolation.

The study site will provide online (REDCap) surgical evaluation and patient follow-up forms to be completed by the surgeons.

The study site will provide online questionnaires for subjects to complete (demographics, medical/gynecologic history (including a listing of medications), and quality of life).

Shipping and storage instructions

Menstrual collection kits will be mailed to subjects (or provided by the site) with detailed instructions for **Returning Menstrual Samples (see below):**

- Keep the styrofoam box inside the outer cardboard box with UN3373 label affixed on side panel
- Place blue frozen block on the bottom of the Styrofoam box (which is inside cardboard box).
- Place the refrigerated gel pouch on top of the frozen blue block
- Place the collection container containing menstrual sample (wrapped in gel blanket) on top of the gel pouch
- Place the Styrofoam lid on inner Styrofoam box
- Place the completed ME specimen collection form (below) on top of the Styrofoam box
- Close and seal the outer cardboard box
- Adhere the pre-paid FedEx label (included with kit) to the top of the outer cardboard box
- Call FedEx in the morning at 1-800-463-3339 (GOFedEx) for pick up. Otherwise, if convenient, bring the completed box to the nearest FedEx.
- Package is a FedEx Priority Overnight to return to the Feinstein Institutes.
- Note: a courier may be used for local participants.

If you have questions, call 516-562-3636

Safety and environmental concerns/instructions

Safety: For specimen handling and in particular for the preparation of aliquots, the local safety regulations shall be applied to rule out any possible biological hazard.

Environmental: All biological specimens and collection containers will be disposed according to Northwell guidelines (in biohazard bags). All kit components returned to the laboratory for testing will be disposed of according to local and state/federal guidelines. The menstrual collection cup is made of silicone; it is re-usable for up to one year (according to the manufacturer's guidelines and therefore, more environmentally friendly than disposable feminine hygiene products.

Sample processing

The date and time of receiving and processing menstrual blood specimens (including menstrual blood serum, cells, etc) will be recorded. Menstrual blood samples will arrive at the Feinstein laboratory and will be immediately processed to: 1) isolate the liquid portion ('serum', which will be frozen (in tubes with de-identified codes)and used in future studies); 2) collect and cryopreserve a small portion of whole menstrual blood (which will be used in future studies); 3) grow menstrual blood-derived stromal cells (which will be frozen (in tubes with de-identified codes)and used to assess decidualization and estrogen and/or progesterone sensitivity); and 4) fix, digest, process and perform single cell RNA-Sequencing analyses, qPCR, and/or flow cytometry or live/dead cell analyses.

DNA will be isolated from menstrual blood or cheek swab using standard methods and genotyped.

Peripheral blood will be centrifuged and cell-free plasma will be collected, aliquoted (into tubes with de-identified codes) and frozen at -80C until analyses.

Sample identification

Barcode-labels will be provided by the Boas Biorepository for each menstrual blood sample. Each Subject ID will be linked to specimen ID labels and subject-specific data ID labels (for subject collection forms, questionnaires, etc).

All sample tubes (including cryotubes) and culture flasks will be labeled with specimen IDs and specimen type.

Sample storage

Labeled cryotubes or microfuge tubes (labeled with subject IDs) containing cheek swab/buccal DNA (for genotyping), whole menstrual blood for cryopreservation, the 'serum' or liquid phase of the menstrual blood, and the processed menstrual blood for single cell RNA-Sequencing, qPCR, flow cytometry, and live/dead analyses will be frozen as soon as possible. Similarly, plasma will be stored in tubes labeled with subject IDs

FORMS TO BE COMPLETED BY PARTICIPANTS

Adverse events
Controls and Symptomatic Cases: also see section 14 potential risks to subjects
Expected adverse events:
Potential breach of confidentiality: Study investigators, ROSE coordinators and participating surgeons will do their best to protect subjects from breach of confidentiality. The data will be kept in locked filing cabinets in a locked office; online data will be kept in a HIPAA compliant REDCap database.

Difficulty inserting and/or removing the menstrual cup.

All adverse events (AEs) that occur as a result of the menstrual blood collection communicated by the subjects must be recorded in the eCRF. Severe adverse events (SAEs) must be reported to coordinating site.
The following data is collected on adverse events:
- Start and end date and time
- Severity (mild, moderate, severe, serious)
- Relation to the menstrual blood collection
- Action taken
- Outcome
- Discontinuation from the study
- Reporting responsibilities

Follow-up events
Controls and Symptomatic Cases:
During the study, participants will be contacted by phone/text to remind them about menstrual collections and to provide instructions and answer questions. Following study participation study staff may contact participants to follow up on incomplete questions or for questions related to the CRFs.

Diagnostic classification
Symptomatic Cases:
Determination of control vs. symptomatic cases will be determined by the ROSE study coordinators based on interest/enrollment form and referring physician (for

symptomatic cases); all subjects will be considered either a control (n=40) or a symptomatic case (n=60) at the time of consent.

Site investigators (collaborating laparoscopic surgeons) are responsible for verifying the final diagnosis status (yes/no) of symptomatic participants who undergo laparoscopy and laparotomy procedures for suspicion of endometriosis – this decision will be based on surgical reports and pathology reports following the procedure.

12. STATISTICAL ANALYSIS

- *Describe how your data will be used to test the hypotheses.*
- *State clearly what variables will be tested and what statistical tests will be used.*
- *Include sample size calculations.*
- *If this is a pilot study, state which variables will be examined for hypothesis generation in later studies.*

This is a pilot study

Exploratory analysis

One of the purposes of this study is to establish a menstrual global (MG) score that will provide a prediction of endometriosis based on an algorithm developed by using the scRNA-Seq data along with other analyses of the menstrual effluent (e.g. flow cytometry and qPCR). In addition, the peripheral blood sample will be used to explore whether markers of inflammation and/or senescence can be found in the peripheral blood during menstruation.

Sample testing at Feinstein Institutes:
All menstrual samples that meet criteria for processing will be processed for single cell RNA-Sequencing and analyses, as well as flow cytometry and qPCR using standard operating procedures developed by the lab. All peripheral blood plasma samples will be analyzed by multiplexed ELISA, MSD or other platforms (e.g., Somalogics) for hundreds of analytes.

11.1.2 Data analyses
Computational biologists will be responsible for analyzing scRNA-Seq data and working with Drs. Gregersen and Metz to identify biomarkers to be considered for the diagnostic. Analytes from plasma will be examined for differences in profiles

among endometriosis cases and controls (vs. non-endometriosis cases with symptoms).

Monitoring Entity Responsibilities

Evaluating the progress of the study on an ongoing basis including periodic assessments of data quality, participant recruitment, accrual and retention, participant risk vs. benefit, and other factors that can affect the outcome.

Considering the impact of factors external to the study when new information, such as scientific or therapeutic developments becomes available that may affect safety of participants, their willingness to participate in the study or the ethics and conduct of the study.

STATISTICAL METHODS

Sample size and justification

This is a pilot study and therefore, there is no formal sample size calculation. The sample size was determined by prior work by the Gregersen-Metz lab team, in consultation with Dr. Marty Lesser, PhD (biostatistician) and feasibility. This proposed study is designed to obtain information on prevalence of endometriosis among symptomatic patients, as well as specificity and sensitivity. We expect that at least 60% of symptomatic subjects to be diagnosed as having endometriosis (via laparoscopic surgery); hence, we are including up to 105 symptomatic patients and up to 80 controls. The data from this study will hopefully be used for a pivotal trial (this study will provide the sample size estimates for the future pivotal trial).

13. SPECIMEN BANKING

- *If specimens will be banked for future research, describe where the specimens will be stored, how long they will be stored, how they will be accessed and who will have access to the specimens*
- *List the information that will be stored with each specimen, including how specimens are labeled/coded*
- *Describe the procedures to release the specimens, including: the process to request release, approvals required for release, who can obtain the specimens, and the information to be provided with the specimens.*

As described in the consent, menstrual blood and isolated cells (as well as DNA and RNA) will be stored indefinitely in the Boas Center Biorepository – but only after de-identification. Only de-identified samples will be used in the lab or shared with collaborators. The PIs on the study will determine with whom the de-identified samples will be shared.

The de-identification process will be based on Boas Center Biorepository (BCB) standard operating procedures. Patient confidentiality will be protected by the sophisticated coding/de-identification and tracking system governing specimens and data collection – which is offered through the TDP and BCB at Northwell Health. All data is stored in a REDCap database that is firewalled and HIPAA compliant. All computers are password protected and access to the REDCap database is password protected.

Sharing of Samples:
The PIs on the study will determine with whom the de-identified samples will be shared. If we share any samples with other researchers, then we will remove all identifiers such as name or date of birth before sharing them.

14. DATA MANAGEMENT AND CONFIDENTIALITY

- *Describe the data and specimens to be sent out or received. As applicable, describe:*
  - *What information will be included in that data or associated with the specimens?*
  - *Where and how data and specimens will be stored?*
  - *How long the data will be stored?*
  - *Who will have access to the data?*
  - *Who is responsible for receipt or transmission of data and specimens?*
- *Describe the steps that will be taken to secure the data during storage, use and transmission.*

Sample de-identification will be based on Boas Center Biorepository (BCB) standard operating procedures. All samples will be stored in the Boas Center Biorepository indefinitely, as described above. Patient confidentiality will be protected by the sophisticated coding/de-identification and tracking system governing specimens and data collection – which is offered through the TDP and BCB at Northwell Health. All data is stored in a REDCap database that is firewalled and HIPAA compliant. All computers are password protected and access to the REDCap database is password protected.

Subject information: Similar to ROSE, all ROSE II study data (including consents and questionnaires, medical authorization forms, etc) will be stored in a REDCap database that is firewalled and HIPAA compliant. All computers are password protected and access to the REDCap database is password protected. Limited non-electronic files are maintained in the coordinator's office (which is locked) in locked filing cabinets.

Specimen handling, storage, transport, and disposal
Subject specimens and all materials coming into contact with them will be handled as if potentially infectious under BSL2+ conditions and disposed of with proper precautions and in accordance with the US Occupational Safety and Health Administration's standard on blood-borne pathogens (29CFR 1910.1030). All specimens will be shipped by priority shipping according to IATA guidelines (or via courier for local specimens) at 4-10 °C and stored onsite before use at 4-10°C; a portion of menstrual blood (not processed) will be cryopreserved onsite in liquid nitrogen.

Source and number of specimens
Source: Menstrual blood will be collected using a vaginally inserted menstrual collection cup (provided by the study) or an external menstrual collection sponge (provided by the study) which is placed atop a pad. Menstrual blood, collected on day 1 or 2 or 3 of the menstrual cycle when flow is highest (peak flow) and when menstrual blood is bright red blood (not brown). All subjects will provide at least one menstrual sample; control subjects will provide 2 during different cycles (i.e. different months); symptomatic subjects will provide at least one menstrual sample prior to surgery and if diagnosed with endometriosis, may provide a second menstrual sample. Menstrual collections will occur at home (or location chosen by the subject) using a menstrual collection kit provided by the study.

Peripheral blood may be collected from the forearm via venipuncture during the bleeding phase of the menstrual cycle.

Collection method: Menstrual blood will be collected using a menstrual cup (provided by the study or the study participant) or a novel menstrual sponge (provided by the study) that is used externally, affixed to a menstrual pad. Peripheral blood, may be collected from the arm via venipuncture during the bleeding phase of the menstrual cycle

Volume: A minimum volume of approximately 3.0-3.5 mL (and up to 20mL) of menstrual blood will be prospectively collected at each time point from each subject; a sponge saturation image is provided to participants to estimate approximately 3.0-3.5 mL; minimum=40% saturation. Peripheral blood: 10mL or 2 teaspoons will be collected once.

Collection times: Menstrual blood: typically 5-8 hrs for one single collection, but overall collection time can vary depending on menstrual flow; maximum 12 hr collection time. Collections are a 'one time' event per cycle, menstrual samples are not added to one another. Controls and symptomatic cases can provide up to four menstrual samples. Controls will collect their menstrual blood samples (during peak flow, typically on days 1 or 2) at different cycles (up to four different months) to assess reproducibility of analyses. Symptomatic cases will provide at least one menstrual sample prior to surgery (during peak flow, typically on days 1 or 2) and symptomatic cases may provide an additional menstrual sample(s) prior to their diagnostic surgery if time permits; also symptomatic cases who are diagnosed with endometriosis may provide menstrual sample(s) after surgery during peak flow, typically on days 1 or 2.

Peripheral blood may be collected once from local participants during the bleeding phase of the menstrual cycle. For symptomatic participants, blood will be obtained prior to surgery.

Collection site: All participants will collect their own menstrual blood at their chosen location(s) (home or work or elsewhere).

Peripheral blood specimens (obtained from the forearm) will be collected at home for local subjects via LabFly or local phlebotomy clinics within Northwell Health.

Number of menstrual blood specimens: up to 4 maximum.
Control subjects will consent to provide 2 menstrual samples (at two different menstrual cycles) for validation of repeat samples across different menstrual cycles. If the original volume is insufficient for processing, participants may provide two additional samples.
Symptomatic subjects will consent to provide at least one menstrual sample prior to their diagnostic surgery and optional additional samples in other menstrual cycles before planned surgery, if time permits. Also, symptomatic subjects who are diagnosed with endometriosis during their participation in this study and undergo treatment (hormone-based, non-hormone-based or surgery) will be asked to provide a menstrual blood sample within 1- 6 months post-surgery to evaluate the effects of treatment on menstrual blood analyses. Note: If the original volume is insufficient for processing, participants may provide an additional sample.

Number of peripheral blood specimens: up to 1 maximum; only local subjects will be asked to provide a menstrual sample.

Specimen inclusion criteria
Sample testing for the endpoints of this study will be performed using fresh specimens delivered to the lab within 60 hrs of collection. Fresh menstrual blood will be processed for single cell-RNA sequencing and other related analyses. A small portion of menstrual blood will be frozen; menstrual blood will be plated to grow (and freeze) stromal cells to assess decidualization capacity and estrogen/progesterone

responsiveness in vitro. Testing of frozen and stored specimens will be performed within the limits of valid data on sample stability.

Specimen exclusion criteria

Specimens will be excluded if

- samples below 3.0 mL (or sponge appears less than 40% saturated)
- samples arriving more than 60 hrs post collection
- samples appear to have been frozen or exposed to >4C for more than 12 hours.

Data management

Data quality will be assured according to established procedures determined by the study monitor referred by the Office of Clinical Research. Electronic consistency checks will identify potential data errors at the time of data entry. Unclear, incomplete, or illogical data will be queried for participant clarification. The combination of electronic and manual data review will ensure data quality and integrity before the data are declared final and released to biostatistics for analysis.

15. DATA AND SAFETY MONITORING PLAN

A specific data and safety monitoring plan is only required for greater than minimal risk research. For guidance on creating this plan, please see the Guidance Document *on the HRPP website.*

Part I – this part should be completed for all studies that require a DSMP.
Part II – This part should be completed when your study needs a Data and Safety Monitoring Board or Committee (DSMB/C) as part of your Data and Safety Monitoring Plan.

Part I: Elements of the Data and Safety Monitoring Plan

- *Indicate who will perform the data and safety monitoring for this study.*
- *Justify your choice of monitor, in terms of assessed risk to the research subject's health and well being. In studies where the monitor is independent of the study staff, indicate the individual's credentials, relationship to the PI, and rationale for selection*
- *List the specific items that will be monitored for safety (e.g. adverse events, protocol compliance, etc)*
- *Indicate the frequency at which accumulated safety and data information (items listed in # above) will be reviewed by the monitor (s) or the DSMB/C.*
- *Where applicable, describe rules which will guide interruption or alteration of the study design.*
- *Where applicable, indicate dose selection procedures that will be used to minimize toxicity.*
- *Should a temporary or permanent suspension of your study occur, in addition to the IRB, indicate to whom will you report the occurrence.*

Data and safety monitoring

This is not an interventional study. In order to safeguard against risks to subjects, the study will adhere to the strict DSMP outlined below.

All interest forms, consents and forms/data will be captured/stored in a HIPAA compliant, study-specific REDCap database.

An external data monitor referred by the Office of Clinical Research will monitor the overall data of the study and ensure that it is collected and stored in accordance with federal regulations, ICH Good Clinical Practice (GCP) guidelines, and the CRO's standard operating procedures. The monitor will review study records to confirm that study data and documents are complete and accurate. The CRO, its authorized agents, and appropriate regulatory authorities shall be granted direct access to all study-related databases and documents to perform this assessment.

The study monitoring will consist of an independent data monitor serving as the study monitor responsible for executing the data and safety monitoring plan (DSMP). The independent data monitor has extensive expertise in clinical trial data management while not having any scientific, financial, or other conflict of interests related to the study.

The study data monitor will be responsible for evaluating study and the PIs will be responsible for the documentation and reporting of events meeting the criteria and definition of an adverse event, serious adverse event, unanticipated adverse device effects, and unanticipated problems involving risks to subjects or others in this study.

The study monitor, PIs, the coordinators and the REDCap database manager will review the study data every 6-8 months, as needed, starting from the first enrolled subject, until the completion of enrollment and all study procedures by participants. These reviews will be documented and stored within the regulatory binder. During these reviews, they will evaluate the study data to determine if aspects of the study need to be changed or stopped. In addition, they will review any and all deviations, adverse events, and unanticipated problems that may occur to determine their relatedness to the study, their severity, and whether they require study changes. In addition, any unanticipated problems or instances of non-compliance will be reported to the IRB as per their reporting requirements. If any protocol changes are needed, an investigator will submit a modification request to the IRB. Protocol changes will not be implemented prior to IRB approval unless necessary to eliminate apparent immediate hazards to the research subjects. In such a case, the IRB will be promptly informed of the change following implementation.

In the unlikely event that two grade 3 or higher adverse events (as per the Common Terminology Criteria for Adverse Events (CTCAE)) occur that are deemed at least possibly related to study participation, the medical monitor will notify the principal investigator(s) and a meeting will occur to determine how to best proceed, including the development of risk mitigation strategies to decrease the severity and/or likelihood of the adverse events from reoccurring.

The study participants will be evaluated for any possible adverse events from the time consent is obtained until the last subject exits the study or completion of enrollment and all study procedures. The principal investigator(s) will serve as the point of contacts to which the study team will report adverse events since they will be responsible for ongoing study monitoring. In addition to being assessed by the principal investigators, the event will be sent to the independent medical monitor for review and concurrence.

The Principal Investigator(s) will be responsible for ensuring participants' safety on a daily basis. The PIs are responsible for reporting Serious Adverse Events and Unanticipated Problems to our Institutional Review Board (IRB)/Office of Human Research Participant Protection (HRPP) at Northwell Health, as required. The study coordinators carefully follow each subject. Any questions regarding subject safety are immediately communicated to the PIs, who are responsible and all questions regarding potential risks (anticipated and unanticipated are discussed as soon as possible with the Northwell IRB/HRPP.

Frequency of Data and Safety Monitoring
According to the DSMP, the study monitor will review the aggregate study data every 6-8 months, as needed, starting from the first enrolled participant. In addition, more frequent reviews may occur in the unlikely event that two CTCAE grade 3 or higher adverse events occur that are deemed at least possibly related to the study. Safety monitoring will stop upon completion of enrollment and all study procedures by participants. The PIs will be informed of any serious adverse events as soon as they occur by the study coordinators, collaborating physicians, and/or the study monitor. As described above, the PIs will report the Serious Adverse Events to the Northwell Health and to the study sponsor as soon as possible (within 48 hrs) after becoming aware of the event. The PIs will report the Serious Adverse Events and Unanticipated Problems to their IRB/HRPP within 5 business days of becoming aware of the event, according to Northwell Health policies.

Content of Data and Safety Monitoring Report
At the scheduled monitoring meetings, the independent monitor will be provided a report including, but not limited to, the following: summary of study progress in terms of IRB-approved changes and/or updates; details of study enrollment;

anticipated timelines for completion of enrollment and study procedures in respects to study milestones; inventory of protocol deviations, protocol exceptions, adverse events (including serious adverse events), unanticipated adverse device effects, unanticipated problems involving risks to subjects or others, instances of non-compliance, and/or subject complaints; and any data analyses that may have occurred. Upon review of the report and an assessment of the study's current risks and data management strategies for potential changes, the medical monitor will provide recommendations and a decision for study continuation.

Part II: Data and Safety Monitoring Board or Committee

- *When appropriate, attach a description of the DSMB.*
- *Provide the number of members and area of professional expertise.*
- *Provide confirmation that the members of the board are all independent of the study.*

Monitoring Body Membership and Affiliation
The DSMP will be independently developed and monitored by a monitor referred by the Office of Clinical Research.

All individuals participating in this study will be assessed for conflicts of interest through the Northwell Health compliance program (required as part of the IRB/HRPP review and approval process). Management plans will be provided, as needed.

16. WITHDRAWAL OF SUBJECTS

- *Describe anticipated circumstances under which subjects will be withdrawn from the research without their consent*
- *Describe procedures for orderly termination*
- *Describe procedures that will be followed when subjects withdraw from the research, including partial withdrawal from procedures with continued data collection.*

Subject withdrawal criteria
All study subjects are free to stop their participation in the study and withdraw their consent at any time without providing reasons and without any influence on their treatment or therapy in the future. In such cases the collection of the subject's personal information will stop, but the information already collected will be kept and used to guarantee the validity of the study and to comply with regulatory requirements. Study subjects have a right to access, and request correction of, their

personal information, ask for it to be deleted as well as receive a copy of their data in a common electronic format. Similarly, if specimens have been processed and evaluated prior to subject withdrawal, samples/sample analyses will be unable to be removed from the study. If symptomatic patients do not undergo laparoscopic surgery as part of their standard care, they will be withdrawn from the study.

Major reasons for discontinuation of subject participation in the study
There are two major reasons for participant discontinuation in the study. 1) Withdrawal of Informed Consent by the study participant, i. e. active discontinuation of study participant because they no longer wish to participate in the study or 2) Withdrawal by investigator, i. e. due to occurrence patient non-compliance or for any reason deemed necessary by the site investigators (e.g. symptomatic participant chooses not to undergo laparoscopic surgery).

17. RISKS TO SUBJECTS

- *Describe any potential risks and discomforts to the subject (physical, psychological, social, legal, or other) and assess their likelihood and seriousness and whether side effects are reversible. Where appropriate, describe alternative treatments and procedures that might be advantageous to the subjects.*
- *Include risks to others , like sexual partners (if appropriate)*
- *Discuss why the risks to subjects are reasonable in relation to the anticipated benefits and in relation to the importance of the knowledge that may reasonably be expected to results*
- *Describe the procedures for protecting against or minimizing any potential risks, including risks to confidentiality, and assess their likely effectiveness.*

Potential Risks for Participants

Breach of confidentiality: The main risk of this study for those subjects providing menstrual blood is the potential for breach of confidentiality. As described below, we will make every effort to assure this risk is minimized – mainly by de-identification methods using bar-coded labels and use of a password and firewalled protected databases. This risk and our efforts to protect against this risk are described in the consent. All subject data is securely stored in a HIPAA-compliant password-protected REDCap database maintained behind a firewall on one of the Northwell Health IT servers. Access to this database is limited to a few individuals who are trained in human subjects' research. This study will be monitored by Drs. Metz and Gregersen.

There is also a risk that samples obtained for this study are not received by the clinical coordinators or biorepository at the Feinstein Institutes of Medical Research due to either an error with a Northwell courier, FedEx, or the USPS.

Participants are encouraged to alert study coordinators of received kits as well as pending sample arrival to diminish the possibility of loss or mishap. Coordinators will check tracking numbers where appropriate.

Menstrual blood collections: In most cases, menstrual blood is collected using a menstrual cup (DivaCup®), which is safe when used as directed (see Diva International website). The DivaCup is provided by the study (as described in the consent) and given to all research participants. Subjects may choose to use their own menstrual cup. A recent meta-analysis reviewed the safety and efficacy of menstrual cups (van Eijk et al, *Lancet Public Health* 2019) and reported no adverse effects on vaginal flora and that 76% of subjects wished to consider using menstrual cups after completing the study. Van Eijk et al reported that professional assistance was needed for removing the cup in 2 cases (of over >1000 subjects). Consistent with this, we found that in rare cases women have difficulty inserting the menstrual cup or removing the menstrual cup after insertion. Subjects can contact study coordinators 24hours/day 7 days per week via the study phone; in addition, women can visit their physicians or local ER if they have difficulty removing the cup. Removing the menstrual cup for the first time can cause anxiety and make it more difficult to insert and/or remove. For patients with pelvic pain or other reasons that prevent them from using menstrual cups (or they simply don't want to try menstrual cups), externally placed menstrual sponges/pads will be offered for the collection of ME. In cases where women choose to collect their menstrual effluent using an externally placed menstrual sponge, there are no known risks associated with the sponge.

Peripheral blood collection: The risks of having peripheral blood drawn from the arm include some pain when the needle goes in and a small risk of bruising and/or infection at that site. Some people get lightheaded, nauseous, or faint.

Genetic Research: There is a small possibility that in the course of the research we may become aware of unexpected information that may be relevant to the health or healthcare of a participant. If this finding is considered to be something one can take steps to address, and the accuracy of the research results can be verified, then we will seek permission from the IRB to share this result with the participant. Study staff are trained for working on genetic research studies with humans and handling human genetic information safely. They will take all necessary precautions to maintain privacy and confidentiality with genetic research. We take privacy very seriously and take extensive measures to protect the participant's personal information and identity. Even with strong protections in place there is a small risk that the confidentiality of personal information or information learned from the genetic sample could become known by unauthorized individuals.

Definitions, Collection and Reporting of Adverse Events (AEs), Serious Adverse Events (SAEs) and Unanticipated Problems (UPs)

We do not anticipate experiencing adverse events, serious adverse events, or unanticipated problems beyond the expected anticipated risks below.

Although women have the option of using an external menstrual sponge/pad, some women may choose to use the menstrual cup. In some cases, a woman may have difficulty inserting and/or removing the menstrual cup after insertion. This can cause anxiety and make it more difficult to insert and/or remove. The study coordinators recruiting and consenting subjects for ME collections are well-trained in working with research participants and with the use of the DivaCup, as well as other menstrual cups (if preferred by the subjects) and menstrual sponges. The study coordinators are available to speak with participants 7 days a week via study phones. They can explain the use thoroughly before and after the consent process. A user's guide is provided with the cup (and sponge) and is available online. A customer care team at DivaCup provides numerous FAQ sheets, videos, and medical information regarding the DivaCup and they are available to answer questions by phone and email. Finally, the consent reads that if they are unable to remove the cup they should contact the study coordinators first and if unavailable go to the emergency room or an urgent care center for help removing the cup (this would be extremely rare).

All of the above described 'risks' are considered to be anticipated. The investigators and the Feinstein Institutes for Medical Research at Northwell Health will follow institutional policies derived from sections of 45 CFR 46, US Food and Drug Administration (FDA) regulations, including the January 2009 guidance, and the Office for Human Research Protections (OHRP), including the January 15, 2007 guidance. The investigators will work closely with the IRB/Human Research Protection Program (HRPP) of the Feinstein/Northwell Health with respect to reporting adverse events (AEs), serious adverse events (SAEs) and unanticipated problems (UPs). The IRB/HRPP at Northwell Health is fully accredited by the Association for Accreditation of Human Research Protection Programs (AAHRPP) (FWA# 00002505). AAHRPP accreditation indicates that our organization follows rigorous standards for ethics, quality, and protections for human research. The PIs will follow the terms and procedures described on the website of our IRB/HRPP at Northwell:

Protection against Study Risks
Informed Consent Process

Control research participants will be recruited through the ROSE (Research OutSmarts Endometriosis) study at the Feinstein Institutes (see websites). Symptomatic cases will be mainly recruited through collaborating physicians' practices. They may also be recruited via the original ROSE study (as symptomatic patients planning their diagnostic surgery) and via social media/online/print stories featuring the ROSE study. The consent process informs each research participant about the study, indicates the participation is completely voluntary and she has the right to stop at any time. Risks are outlined in the informed consent document and described orally during the consent process. All risks will be described in detail by the study staff. Pparticipant study IDs will be linked to prior ROSE study via REDCap.

Protection Against Risks

The collection of data and menstrual effluent, as described in the main consent is a 'minimal risk' study.

There is a risk to privacy and confidentiality. We take privacy very seriously and take extensive measures to protect the participant's personal information and identity. Even with strong protections in place there is a small risk that the confidentiality of personal information or information learned from the genetic sample could become known by unauthorized individuals. The collected biospecimens and data will be de-identified prior to storage in the Boas Center Biorepository or staff processing and isolation of stromal fibroblast cells (SFCs), according to the Northwell's IRB-approved operating procedures and Boas Center Biorepository (BCB) standard operating procedures. Patient confidentiality will be protected by the sophisticated coding/de-identification and tracking system governing specimens and data collection – which is offered through the TDP and BCB at Northwell Health. All data is stored in a REDCap database that is firewalled and HIPAA compliant. All computers are password protected and access to the REDCap database is password protected. Coordinators' offices are locked and filing cabinets in those offices are locked.

18. RESEARCH RELATED HARM/INJURY

- *Describe the availability of medical or psychological resources that subjects might need as a result of anticipated problems that may be known to be associated with the research.*
- *If the research is greater than minimal risk, explain any medical treatments that are available if research-related injury occurs, who will provide it, what will be provided, and who will pay for it.*

N/A

19. POTENTIAL BENEFIT TO SUBJECTS

- *Explain what benefits might be derived from participation in the study, noting in particular the benefit over standard treatment (e.g. a once-a-day administration instead of four times a day, an oral formulation over an IV administration).*
- *Also state if there are no known benefits to subjects, but detail the value of knowledge to be gained*

Potential Benefits for Participants
There are no direct benefits received by subjects participating in this study. However, we expect that the study results will contribute to generalizable knowledge and hopefully, will contribute to the development of a non-invasive diagnostic for endometriosis based on menstrual effluent. Currently, endometriosis is definitively diagnosed by laparoscopic surgery with pathologic confirmation. As a result, many women delay their diagnostic surgery. A non-invasive diagnostic for endometriosis may significantly reduce the time between symptoms and diagnosis and thus improve clinical outcomes for women/teens suffering with this condition in the future.

20. PROVISIONS TO PROTECT PRIVACY INTERESTS OF SUBJECTS

- *Describe the methods used to identify potential research subjects, obtain consent and gather information about subjects to ensure that their privacy is not invaded.*
- *In addition consider privacy protections that may be needed due to communications with subjects (such as phone messages or mail).*

The symptomatic participants will be mainly recruited through surgeons. Control participants and some symptomatic participants (who are planning their diagnostic surgery) will be recruited through IRB 13-376 and by word of mouth, as well as online/social media/print stories highlighting the ROSE study. Consent will be obtained via Northwell's secure REDCap application.

The participant will opt to allow text messaging in their consent form. All packages received from participants will be stripped of identifying markers and will disposed of in Northwell shred bins.

Participants will mainly interact with ROSE II coordinators via phone, text, and email – their privacy will be protected provided they choose to talk while in a

private setting. If meeting in person, ROSE II coordinators will provide a quiet, private space.

Protection of Confidentiality

There is a risk to subjects' privacy and confidentiality. We take privacy very seriously and take extensive measures to protect the participant's personal information and identity. Even with strong protections in place there is a small risk that the confidentiality of personal information or information learned from the genetic sample could become known by unauthorized individuals. The collected biospecimens and data will be de-identified prior to storage or staff processing, according to the Northwell's IRB-approved operating procedures, as well as the ROSE study standard operating procedures and the Boas Center Biorepository (BCB) standard operating procedures. Patient confidentiality will be protected by the sophisticated coding/de-identification and tracking system governing specimens and data collection – which is offered through the BCB at Northwell Health. All data is stored in a REDCap database that is firewalled and HIPAA compliant. All computers are password protected and access to the REDCap database is password protected. Only a limited number of staff (who are trained) have access to the REDCap database. **In the consent, we ask study participants for their permission to receive 'study reminder' text messages.** Finally, all study staff are CITI-trained and participate in additional training throughout the year regarding human subject research guidelines and regulations, as well as the policies of the IRB/HRPP office at Northwell Health.

REDCap (Research Electronic Data Capture) data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the study team, under the direction of the PI. REDCap data is housed on virtual servers at Northwell Datacenters (Yonkers, Westbury) requiring badge access and only accessible by members of Northwell's infrastructure team. The application and the database are backed up regularly in accordance with Northwell IT policy. Team members access REDCap through a secure login page using Northwell Active Directory credentials. All traffic between the web browser and the application is encrypted. REDCap was developed specifically around HIPAA-Security guidelines and is recommended to Northwell Health's researchers by our Clinical Research Service, Research Compliance Office, and Institutional Review Board. REDCap has been disseminated for use locally at other institutions and currently supports more than 6400 active institutional partners and other institutions in more than 150 countries (www.projectredcap.org).

21. COSTS TO SUBJECTS

- *Describe any foreseeable costs that subjects may incur through participation in the research*
- *Indicate whether research procedures will be billed to insurance or paid for by the research study.*

There is no cost for study participation.

22. PAYMENT TO SUBJECTS

- *Describe the amount of payment to subjects, in what form payment will be received and the timing of the payments.*

Reimbursement to participants: Participants will be compensated $50 for each menstrual blood collection using a menstrual cup (the study provides a free Diva Cup, but participants may choose to use their own menstrual cup) and/or $25 for each menstrual blood collection using a menstrual sponge/pad. Participants who provide a peripheral blood sample will receive $25. Women with severe chronic pelvic pain really can't use the cup – so we offer an option that allows them to participate. These payments are not substantial. The rationale for paying $50 is due to the training time and awkwardness of menstrual cup use for first-time users. The cup is a $35-40 item. Interestingly, many first-time users end up liking the cup so we give them this opportunity to save money on future menstrual products.

23. CONSENT PROCESS

If obtaining consent for this study, describe:

- *Who will be obtaining consent*
- *Where consent will be obtained*
- *Any waiting period available between informing the prospective participant and obtaining consent*
- *Steps that will be taken to assure the participants' understanding*
- *Any tools that will be utilized during the consent process*
- *Information about how the consent will be documented in writing. If using a standard consent form, indicate such.*
- *Procedures for maintaining informed consent.*

Informed Consent

Eligible subjects may participate in the study only after providing IRB-approved informed consent for participation using an online (REDCap) portal (or paper consent form, if portal is not available). Before prospective participants provide informed consent, they will be informed about the study (purpose, procedures, study risks, and study benefits, including the risks associated with specimen collection). Participants will be informed if new information becomes available that may impact their decision to continue participation.

Participant enrollment and Informed Consent

All potential participants, including controls and symptomatic cases, will complete the online ROSE II Study interest form; this will be evaluated by coordinators to confirm study eligibility

Referred participants will be screened for inclusion; included if they meet criteria and excluded if they don't meet criteria.

A screening and an enrollment log on REDCap will be used to track potential participants and consented/enrolled participants.

Eligible subjects/patients will be informed about the study objectives and the nature of the study by the ROSE II coordinators.

Patients will receive information describing the potential risks and discomforts, and a description of the procedures required for study participation.

Subjects/patients are allowed to withdraw from the study at any time without any disadvantages (and without explanation). Eligible subjects who consent to participate in the study have to sign the Informed Consent form (online via REDCap). Participants cannot participate in study procedures before completing the consent process. All patients will receive a PDF copy of the signed Informed Consent form. The original signed Informed Consent will be maintained in a REDCap database at the study site.

In the state of NY, any participants under the age of 18 are considered children. If your study involves children, additional information should be provided to describe:

- *How parental permission will be obtained*
- *From how many parents will parental permission be obtained*
- *Whether permission will be obtained from individuals other than parents, and if so, who will be allowed to provide permission. The process used to determine these individual's authority to consent for the child should be provided*
- *Whether or not assent will be obtained from the child*
- *How will assent be documented*
- *Whether child subjects may be expected to attain legal age to consent to the procedures for research prior to the completion of their participation in the research. If so, describe the process that will be used to obtain their legal consent to continue participation in the study. Indicate what will occur if consent is not obtained from the now-adult subjects.*

N/A

If the study involves cognitively impaired adults, additional information should be provided to describe:

- *The process to determine whether an individual is capable of consent*
- *Indicate who will make this assessment*
- *The plan should indicate that documentation of the determination and assessment will be placed in the medical record, when applicable, in addition to the research record.*
- *If permission of a legally authorized representative will be obtained,*
  - *list the individuals from who permission will be obtained in order of priority*
  - *Describe the process for assent of subjects; indicate whether assent will be required of all, some or none of the subjects. If some, which subjects will be required to assent and which will not.*
  - *If assent will not be obtained from some or all subjects, provide an explanation as to why not*
  - *Describe whether assent will be documented and the process to document assent*
  - *Indicate if the subject could regain capacity and at what point you would obtain their consent for continued participation in the study*

N/A

If the study will enroll non-English speaking subjects:

- *Indicate what language(s) other than English are understood by prospective subjects or representatives*
- *Indicate whether or not consent forms will be translated into a language other than English*
- *Describe the process to ensure that the oral and written information provided to those subjects will be in that language*
- *If non-English speaking subjects will be excluded, provide a justification for doing so*

Due to the nature of the sample collection being menstrual effluent, the decision was made to enroll English speaking participants only. Having had experience consenting, completing questionnaires, and receiving menstrual effluent (ME) from ROSE study participants since 2014, we have had our share of interesting conversations over the use of a menstrual cup as well as the external sponge. Having to use an interpreter for these conversations in our experience was unsuccessful and quite awkward and embarrassing for the participants.

24. WAIVER OR ALTERATION OF THE CONSENT PROCESS X☐ N/A

Complete this section if you are seeking an alteration or complete waiver of the consent process.

- *Describe the possible risks of harm to the subjects involved in this study and explain why the study involves no more than minimal risk to the subject:*
- *Explain why the waiver/ alteration will not adversely affect the rights and welfare of subjects*
- *Explain why it is impracticable to conduct this research if informed consent is required*
- *Explain why it is not possible to conduct this research without using the information or biospecimens in an identifiable form*
- *If appropriate, explain how the subjects will be provided with additional pertinent information after participation. If not appropriate to do so, explain why.*

N/A

Complete this section if you are obtaining informed consent but you are requesting a waiver of the documentation of consent (i.e., verbal consent will be obtained). *To proceed with a waiver based on these criteria, each subject must be asked whether they wish to have documentation linking them to this study.* ***Only complete subsection 1 <u>OR</u> subsection 2.***

SUBSECTION 1

- *Explain how the only record linking the subject to the research would be the consent document.*
- *Explain how the principal risk of this study would be the potential harm resulting from a breach in the confidentiality*
- *Indicate whether or not subjects will be provided with a written statement regarding the research.*

SUBSECTION 2

- *Describe the possible risks of harm to the subjects involved in this study and explain why the study involves no more than minimal risk.*
- *Confirm that the research only involves procedure for which consent is not normally required outside the research context.*
- *Indicate whether or not subjects will be provided with a written statement regarding the research.*

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25. WAIVER OF HIPAA AUTHORIZATION X☐ N/A

Complete this section if you seek to obtain a full waiver of HIPAA authorization to use and/or disclose protected health information.

- *Describe the risks to privacy involved in this study and explain why the study involves no more than minimal risk to privacy:*
- *Describe your plan to protect identifiers from improper use or disclosure and to destroy them at the earliest time.*
- *Indicate why it is not possible to seek subjects' authorization for use or disclosure of PHI.*
- *Indicate why it is not possible to conduct this research without use or disclosure of the PHI.*
- *Indicate if PHI will be disclosed outside NSLIJ Health System, and if so, to whom. Note: PHI disclosed outside NSLIJ Health System, without HIPAA authorization needs to be tracked. Please see guidance at www.nslij.com/irb for information about tracking disclosures.*

N/A

Complete this section if you seek to obtain a partial waiver of the patient's authorization for screening/recruitment purposes *(i.e., the researcher does not have access to patient records as s/he is not part of the covered entity)*

Note: Information collected through a partial waiver for recruitment cannot be shared or disclosed to any other person or entity.

- *Describe how data will be collected and used:*
- *Indicate why you need the PHI (e.g.PHI is required to determine eligibility, identifiers are necessary to contact the individual to discuss participation, other)*
- *Indicate why the research cannot practicably be conducted without the partial waiver (e.g. no access to medical records or contact information of the targeted population, no treating clinician to assist in recruitment of the study population, other)*

As interested participants answer the screen-in form on REDCap, they will either move forward if their answer is an inclusion criteria or their survey will end, with an option to circle back to correct their answer if entered in error. The amount of information obtained on REDCap will depend on how far into the questionnaire the interested participant proceeds. For this study, we need to definitively understand the participants positive symptoms (affected) or lack of symptoms (control) to fit the parameters for enrollment. Identifiers will not be collected for those deemed ineligible. We are requesting a partial waiver of HIPAA authorization for screening and recruitment purposes for those who meet inclusion criteria and provide their contact information. PHI will be stored in REDCap for all participants who meet inclusion criteria including those who do not continue on to sign the consent form and participate on the study. The enrollment criteria require specific health status questions to ensure eligibility and identifiers including contact information are needed to contact individuals to discuss their participation. PHI will also be stored for those eligible that do not enroll by signing the consent form. We need this

information to provide enrollment data (inclusive of positive enrollment and perceived declination of enrollment) to our sponsors. We have had many participants in previous studies who completed their enrollment months after completion of the initial interest form. If their record IDs from REDCap are deleted this will remove access for these patients as they may perceive the study as "closed" when they attempt to enroll at a later date. This is a potential loss of enrollment. This information is vital in understanding recruitment.

26. VULNERABLE POPULATIONS:

Indicate whether you will include any of these vulnerable populations. If indicated, submit the appropriate appendix to the IRB for review:

- ☐ *Children or viable neonate*
- ☐ *Cognitively impaired*
- ☐ *Pregnant Women, Fetuses or neonates of uncertain viability or nonviable*
- ☐ *Prisoners*
- ☐ *NSLIJ Employees, residents, fellows, etc*
- ☐ *poor/uninsured*
- ☐ *Students*
- ☐ *Minorities*
- ☐ *Elderly*
- X☐ *Healthy Controls*

If any of these populations are included in the study, describe additional safeguards that will be used to protect their rights and welfare.

The healthy controls in this study do not fall under vulnerable population as they are not being asked to consume a medication or utilize a device. They are being asked to [provide a sample of their menstrual effluent (ME). Healthy controls are recruited from the IRB-approved ROSE study (IRB#13-376A).

27. MULTI-SITE HUMAN RESEARCH (COORDINATING CENTER)

If this is a multi-site study where you are the lead investigator, describe the management of information (e.g. results, new information, unanticipated problems involving risks to subjects or others, or protocol modifications) among sites to protect subjects.

N/A

28. REFERENCES/BIBIOGRAPHY

Provide a reasonable list of references directly related to the study. Any diagrams for new medical devices or brief reprints from journals might also prove useful.

References

Aghajanova, L., & Giudice, L. C. (2011). Molecular evidence for differences in endometrium in severe versus mild endometriosis. Reprod.Sci., 18(3), 229-251.

Ballard, K., Lowton, K., & Wright, J. (2006). What's the delay? A qualitative study of women's experiences of reaching a diagnosis of endometriosis. Fertil Steril, 86(5), 1296-1301. doi:10.1016/j.fertnstert.2006.04.054

Brosens, I., Brosens, J. J., & Benagiano, G. (2012). The eutopic endometrium in endometriosis: are the changes of clinical significance? Reprod Biomed Online, 24(5), 496-502. doi:10.1016/j.rbmo.2012.01.022

Bulun, S. E., Cheng, Y. H., Yin, P., Imir, G., Utsunomiya, H., Attar, E., . . . Julie Kim, J. (2006). Progesterone resistance in endometriosis: link to failure to metabolize estradiol. Mol Cell Endocrinol, 248(1-2), 94-103. doi:10.1016/j.mce.2005.11.041

Chehna-Patel, N., Sachdeva, G., Gajbhiye, R., Warty, N., & Khole, V. (2010). "Spot"-ting differences between the ectopic and eutopic endometrium of endometriosis patients. Fertil Steril, 94(6), 1964-1971, 1971 e1961. doi:10.1016/j.fertnstert.2010.01.048

Drury, J. A., Parkin, K. L., Coyne, L., Giuliani, E., Fazleabas, A. T., & Hapangama, D. K. (2018). The dynamic changes in the number of uterine natural killer cells are specific to the eutopic but not to the ectopic endometrium in women and in a baboon model of endometriosis. Reprod Biol Endocrinol, 16(1), 67. doi:10.1186/s12958-018-0385-3

Dunselman, G. A., Vermeulen, N., Becker, C., Calhaz-Jorge, C., D'Hooghe, T., De Bie, B., . . . Embryology. (2014). ESHRE guideline: management of women with endometriosis. Hum Reprod, 29(3), 400-412. doi:10.1093/humrep/det457

Johnston, J. L., Reid, H., & Hunter, D. (2015). Diagnosing endometriosis in primary care: clinical update. Br J Gen Pract, 65(631), 101-102. doi:10.3399/bjgp15X683665
Liu, H., & Lang, J. H. (2011). Is abnormal eutopic endometrium the cause of endometriosis? The role of eutopic endometrium in pathogenesis of endometriosis. Med Sci Monit, 17(4), RA92-99. doi:10.12659/msm.881707

Nayyar A, Saleem MI, Yilmaz M, De Franco M, Klein G, Elmaliki KM, Kowalsky E, Chatterjee PK, Xue X, Viswanathan R, Shih A, Gregersen PK, Metz CN. (2020) Menstrual Effluent Provides a Novel Diagnostic Window on the Pathogenesis of Endometriosis. *Frontiers in Reproductive Health,* Jul 22;2:3. PMID: 36304708 PMCID: PMC9580670. doi.org/10.3389/frph.2020.00003.

Shih AS, Adelson RP, Vashistha H, Khalili H, Nayyar A, Puran R, Herrera R, Chatterjee PK, Lee AT, Truskinovsky AM, Elmaliki K, DeFranco M, Metz CN, and PK Gregersen (2022) Single cell analysis of menstrual endometrial tissues defines phenotypes associated with endometriosis.*BMC Medicine* 20(1):315. PMID: 36104692 PMCID: PMC9476391 . doi.org/10.1101/2022.02.10.22270810.

Tomassetti et al: International Working Group of Aagl, E. E., Wes, Tomassetti, C., Johnson, N. P., Petrozza, J., Abrao, M. S., . . . De Wilde, R. L. (2021). An international terminology for endometriosis, 2021(). Hum Reprod Open, 2021(4), hoab029. doi:10.1093/hropen/hoab029

Warren, L. A., Shih, A., Renteira, S. M., Seckin, T., Blau, B., Simpfendorfer, K., . . . Metz, CNGregersen, P. K. (2018). Analysis of menstrual effluent: diagnostic potential for endometriosis. Mol Med, 24(1), 1. doi:10.1186/s10020-018-0009-6