Assessing the Endometrial Environment in Recurrent Pregnancy Loss and Unexplained Infertility

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1. PURPOSE OF THE STUDY

a. Brief Summary

Recurrent pregnancy loss and unexplained infertility are emotionaland difficult diagnoses for patients. Despite a thorough medicalinvestigation, most cases of recurrent pregnancy loss remainunexplained and unexplained infertility is by definition so.Understanding endometrial factors that may contribute to recurrentpregnancy loss and unexplained infertility are of increasinginterest. The purpose of this study was to determine if patientswith recurrent pregnancy loss or unexplained infertility havealtered gene expression in the uterus, or an altered microbiome,during the window of implantation and to assess whether lutealphase progesterone supplementation improves or alters geneexpression and to assess whether oral antibiotics followed by vaginal probioticsimproves an altered microbiome.

b. Objectives

The investigators hope to determine if endometrial gene expression is altered in recurrent pregnancy loss or unexplained infertility patients compared to healthy controls. Furthermore the investigators hope to learn if luteal phase progesterone support normalizes women with altered endometrial gene expression and if oral antibiotics and over the counter vaginal probiotics improve an altered uterine microbiome. This would give us better evidence to justify using progesterone support which is often done empirically without strong evidence for or against and suggest a uterine mechanism of an altered microbiome in infertility and recurrent pregnancy loss. This knowledge would provide a better means to counsel patients in these populations.

c. Rationale for Research in Humans

Human subjects must be used for this study as the purpose of the study is to understand human endometrial genetic expression and the human microbiome in an unexplained recurrent pregnancy loss and unexplained infertility patient populations. Furthermore this is a minimal risk study where patients are already undergoing endometrial biopsies as a

standard of care to rule out endometrial infection. For healthy controls this is a minimal risk study as the endometrial biopsy is a minimal procedure. The benefit to the healthy controls would be access to the information which may be relevant to them in their future reproductive attempts.

2. STUDY PROCEDURES

a. Procedures

Patients enrolled in the study will be those presenting to the clinic for evaluation and treatment of recurrent pregnancy loss and infertility. Healthy controls will be patients presenting for elective egg freezing and elective sex selection and recruited from the general population by way of flier.

Recurrent pregnancy loss and infertility patients will undergo the routine clinical standard evaluation including a uterine cavity evaluation, evaluation for endometritis, ovarian reserve testing, and for patients with recurrent pregnancy loss also parental chromosome analysis, antiphospholipid antibody blood testing and thyroid screening. Egg freezing patients will undergo routine clinical evaluation which includes uterine ultrasound evaluation and ovarian reserve testing and elective sex selection patients will undergo a routine evaluation which includes a uterine cavity evaluation, ovarian reserve testing and blood testing.

For new patients presenting with no prior evaluation we will perform the uterine cavity evaluation as per standard of care in the follicular phase of the menstrual cycle. The endometrial biopsy is typically done during this same appointment however the endometrial biopsy will be deferred for the purposes of the study in order to perform the endometrial biopsy during the window of implantation after ovulation. This endometrial biopsy will be sent to pathology to rule out endometritis as per clinical standard of care and will allow us to evaluate endometrial gene expression during the window of implantation. The endometrial sample will be sent to Ivigen for an endometrial receptivity array (ERA)and microbiome analysis at no extra cost to the patient. The ERA provides information on the receptivity of a patient's endometrium to the implantation of an embryo. It is hypothesized that unexplained recurrent pregnancy loss and unexplained infertility patients are more likely to have a nonreceptive endometrium then healthy historic controls.

For return patients with prior standard evaluation they will be invited to participate in the study. These patients will undergo an endometrial biopsy as above. The endometrial sample will be sent to Ivigen to our collaborators (Dr. Carlos Simon) for an endometrial receptivity array (ERA)and microbiome analysis at no extra cost to the patient. Dr. Simon's group will perform the analysis of the sample.

For patients whose ERA shows a non receptive endometrium in the subsequent menstrual cycle, vaginal progesterone medication which is often prescribed empirically for recurrent pregnancy loss will be prescribed to start 3 days after ovulation detected by home urine LH kits. A second endometrial biopsy will be sent for a repeat ERA at not

extra cost to the patient. The patient will still be able to participate in the study if they decline this second biopsy.

For patients who microbiome is altered, a 2 week course of oral doxycycline will be prescribed followed by 2 weeks of over the counter vaginal probiotics. A second endometrial biopsy will be sent for a repeat microbiome at not extra cost to the patient. The patient will still be able to participate in the study if they decline this second biopsy.

If on the first biopsy there is endometritis the patient will first be treated based on standard of care practice with routine use of oral antibiotics prior to undergoing a second endometrial biopsy which is also routine practice. If the microbiome or ERA is also abnormal (It would be for example EXPECTED that the microbiome will be altered in all endometritis patients) for an abnormal ERA- vaginal progesterone would be added prior to rebiopsy and/or vaginal probiotics would be added.

The patients' follow up visits and treatment regimens will be determined by the treating physician according to the standard of care treatment of recurrent pregnancy loss, unexplained infertility or other medical comorbidities.

These patients are not required to consent to any additional testing or drug treatments outside of that which is standard treatment of their condition(s). They will consent to follow up communication (phone call or email) for up to 5 years after enrollment in the study to allow for completion of data collection and pregnancy outcomes except for the healthy controls who will be followed for 1 year.

The only procedures which would be considered entirely research would be sending the endometrial biopsy tissue for ERA testing (currently endometrial biopsy is a standard of care for recurrent pregnancy loss and unexplained infertility) however this is not sent for ERA testing.

Emperic use of vaginal progesterone is often used in the setting of recurrent pregnancy loss and unexplained fertility however doing a repeat biopsy in this setting would be considered research- not standard of care.

Use of antibiotics is often used in the setting of non-specific findings for endometritis in recurrent pregnancy and unexplained fertility however using this in the setting of an abnormal microbiome in combination with over the counter vaginal probiotics would be considered research.

b. Procedure Risks

New patients will not be exposed to any additional researchprocedures outside the standard of care. For return patients theywill undergo a simple uterine biopsy that they will have theknowledge ahead of time about as they have already undergone thesame procedure previously. For the healthy controls they will undergo one minorprocedure any may benefit from the testing in the future. The endometrial biopsy ingeneral is a low risk procedure with a risk of complications of less than 1 in1,000 with the most common side

effects of short lived (lasting afew minutes at most)of mild cramping and mild spotting**Use of Deception in the Study**

Deception will not be used.

d. Use of Audio and Video Recordings

There will be no audio or video recording.

e. Alternative Procedures or Courses of Treatment

For unexplained recurrent pregnancy loss and unexplained infertility patients there is no standard of care treatment. Empirical treatment includes supplementing with progesterone in a subsequent pregnancy started in the luteal phase or with the first pregnancy test however there is little evidence based medicine behind these options. At most this would be a 1-2 month delay of starting this empiric treatment for patients who fall in the abnormal group who had the option for treatment with progesterone or antibiotics/probiotics and subsequent biopsy.

Another option we review with our recurrent pregnancy loss patients is in vitro fertilization with PGS (pre-implantation genetic screening) however this has not been shown to improve time to live birth and is very expensive. Again if patients decided to have this treatment the study would only again delay this by 1-2 months and have no effect on the patient's success rate of the treatment itself given a minor time delay. Furthermore it may allow us to better time an embryo transfer for these patients as ERA has been used to identify the best window of implantation after IVF.

For unexplained infertility treatments in include oral fertility medications (clomid) plus insemination cycles or in-vitro fertilization cycles. Again this would be at most a 1-2 month delay which we do not anticipate would change their overall prognosis or outcome for pregnancy. Again if patients proceed to IVF and embryo transfer the knowledge from the study could be used to better time an embryo transfer.

For the healthy controls there is at least a 1-2 month set up to proceed with egg harvesting. The biopsy would be done during this set up time and would not cause a clinically significant delay.

f. Will it be possible to continue the more (most) appropriate therapy for the participant(s) after the conclusion of the study?

As there is no standard treatment of unexplained recurrent pregnancy loss, all therapy as described about will be possible for these patients. For unexplained infertility the standard appropriate therapy will resumed after the conclusion of the study.

g. Study Endpoint(s)

For patients who are found to have a receptive endometrium, the study end point will be after the standard medical evaluation as described. For patients who are found to have a non receptive endometrium or abnormal microbiome the study end point will be after the second endometrium biopsy. Participants will be allowed to continue any standard

medically indicated treatments throughout the study. For healthy controls the study end point will be after the first endometrial biopsy and we will not plan a repeat biopsy. The study will end once the anticipated total number of participants is met to complete an adequate power analysis.

3. BACKGROUND

a. Past Experimental and/or Clinical Findings

Recurrent pregnancy loss is a difficult clinical diagnoses defined as when women have >2 unexplained miscarriages (not accounted for by genetically abnormal fetal tissue) <10 weeks. After a full evaluation for genetic, endocrine, anatomic, immunologic, infectious and autoimmune conditions, the majority of cases remain unexplained. In unexplained infertility normal sperm, normal ovulatory function and patent fallopian tubes are documented and the reason for infertility remains unexplained. The role of endometrial factors in pregnancy loss and unexplained infertility is becoming of increasing interest. Studies have identified differences in the endometrium in protein expression and endometrial glandular development in women with unexplained recurrent pregnancy loss. Some studies have suggested that luteal phase progesterone may change the endometrial environment. Therefore the purpose of this study was to look at a more comprehensive genetic expression profile and the microbiome atmosphere during the window of implantation and to evaluate if luteal phase progesterone support normalizes this genetic expression profile for RPL patients who are found to have non- receptive endometrial biopsies. Understanding the genetic expression profile of the endometrium and the microbiome atmosphere during the window of implantation for unexplained infertility may help identify a previously unknown uterine factor in unexplained infertility. Prior studies have shown that a non receptive ERA and an abnormal microbiome (lactobacilli non dominant) is associated with decreased implantation rate, pregnancy rate and live birth rates in women undergoing IVF.

b. Findings from Past Animal Experiments

None

4. DEVICES USED IN THE STUDY

a. IDE-Exempt Devices

IND-Exempt Device 1	
Name:	ERA: Endometrial receptivity array (IVIGEN)
Description:	The ERA is a diagnostic tool used to evaluate the endometrial receptivity of a patient that determines whether or not an embryo is capable of implanting in the uterus.
IND-Exempt Device 2	
Name:	Vaginal progesterone (endometrin) (Ferring Pharmaceuticals Inc.)
Description:	Vaginal progesterone supplementation
IND-Exempt Device 3	
Name:	Doxycyline (Generic)
Description:	Doxycyline

5. DRUGS, BIOLOGICS, REAGENTS, OR CHEMICALS USED IN THE STUDY

a. Commercial Drugs, Biologics, Reagents, or Chemicals

Commercial Product 1	
Name:	PurFem priobiotic (Bifodan A/S)
Dosage:	Dispense 1 capsule per vagina daily x 10 days
New and different use? (Y/N)	No
Commercial Product 2	
Name:	Probaclac Vaginal Probiotic (Nicar Laboratories)
Dosage:	apply 1 vaginal capsule oer vagina daily x 14 days
New and different use? (Y/N)	No
Commercial Product 3	
Name:	Lactogyn (JADRAN-GALENSKI LABORATORIJ d.d. JGL d.d)
Dosage:	1 application to vagina per day
New and different use? (Y/N)	No

6. DISINFECTION PROCEDURES FOR MEDICAL EQUIPMENT USED ON BOTH HUMANS AND ANIMALS

N/A

7. PARTICIPANT POPULATION

a. Planned Enrollment

The number of participate expected to be enrolled at Stanford Medicine Fertility and Reproductive Health Clinic is 100 patients per patient population (100 recurrent pregnancy loss patients, 100 unexplained infertility patients and 100 health controls). Any patients eligible for the study but desiring no intervention will be offered the alternative of being included as an observational group only without intervention in order to compare pregnancy outcomes without intervention. These patients will be recurrent pregnancy loss and unexplained infertility patients seeking treatment at our facility. healthy controls will be patients presenting for elective egg freezing and sex selection.

b. Age, Gender, and Ethnic Background

All participants enrolled in this study will be female and of reproductive age 18-45 years old that present to our clinic for routine evaluation and treatment. Enrollment will be open to women of all ethnic backgrounds.

c. Vulnerable Populations

N/A

d. Rationale for Exclusion of Certain Populations

Children will not be included as recurrent pregnancy loss and infertility does not occur in children.

e. Stanford Populations

None

f. Healthy Volunteers

None

g. Recruitment Details

The participants will be recruited by their treating physician if they meet the inclusion criteria for the study. The study be open to the public and women who are already presenting to Stanford Medicine Fertility and Reproductive Health clinic will be invited to participate. Participants will be treated the same whether or not they agree to enrollment in the study. Those consenting to study participation will be willing to allow physician follow up for up to 5 years for thorough data collection.

h. Eligibility Criteria

i. Inclusion Criteria

Subjects must fulfill the following inclusion criteria to be eligible to participate in the study:
1. Have a diagnosis of recurrent pregnancy loss as defined as two or more unexplained pregnancy losses at less than 10 weeks (miscarriages with known chromosomal errors excluded) OR have a diagnosis of unexplained infertility defined as normal ovarian reserve testing, normal ovulatory function, normal semen analysis defined why WHO criteria and evidence of at least one patent fallopian tube.
2. Agree to the standard of care testing for evaluation of recurrent pregnancy loss OR unexplained infertility OR routine testing for preparation for oocyte freezing or elective sex selection.
3. Be at least 18 years old and less than 46 years old.
4. Have menstrual cycles that occur in 25-35 day intervals
5. Agree to follow up for 5 years from the date of enrollment

ii. Exclusion Criteria

1. Irregular menstrual cycles
2. Presence of untreated submucosal fibroids or polyps
3. an unligated hydrosalpinx
4. non-ovulatory progesterone level on the day of endometrial biopsy
5. Women <21 or >45 years old.

i. Screening Procedures

Study staff will invite women presenting for recurrent pregnancy loss or unexplained infertility or healthy controls to participate in this study. The staff will explain the purpose of the study to interested patients and the patients will then sign an informed consent for their enrollment. Patients will receive a thorough evaluation of their condition upon presentation. It will be emphasized that their participation is entirely voluntary and that they will receive the standard of care even if they choose not to enroll in the study or discontinue participation at any time.

j. Participation in Multiple Protocols

Patients will be asked if they are participating in any other studies on their consent forms. They may be enrolled in other studies concurrently with this study as long as it does not impact the outcome of this study.

k. Payments to Participants

No payment will be provided. Patients are receiving standard of care evaluation and management of recurrent pregnancy loss and unexplained infertility.

l. Costs to Participants

Standard recurrent pregnancy loss and unexplained infertility evaluation and treatment costs will be charged to the patient or her insurance. Similarly standard evaluation prior to oocyte freezing or sex selection cycles will be charted to the patient or her insurance.

m. Planned Duration of the Study

The probable duration of the study will be approximately 3-5 years to complete the patient's treatment of recurrent pregnancy loss and unexplained infertility and follow up the outcome of their pregnancies (if applicable) for up to 5 years.

8. RISKS

a. Potential Risks

i. Investigational devices

The ERA and microbiome tests pose no risks or injury to the subject. Participants will receive standard of care at the study site which includes an endometrial biopsy. A portion of this specimen will be sent free of charge for the endometrial receptivity array and microbiome evaluation.

ii. Investigational drugs

N/A

iii. Commercially available drugs, biologics, reagents or chemicals

Vaginal progesterone supplementation is often used empirically as an available treatment for patients with recurrent pregnancy loss. This will be offered to all patients who desire this empirical treatment if their ERA shows a non-receptive endometrium. These patients will be offered a repeat ERA biopsy to determine whether progesterone improves their endometrial receptivity.
Vaginal progesterone risks are considered minimal but patient's have reported in clinical trials to report symptoms such as abdominal pain (12%) nausea (8%) abdominal bloating (4%), constipation (2-3%) vomiting (3%), fatigue (2%), UTIs (2%) headache 4%, vaginal bleeding of 3%. Vaginal progesterone may also have similar side effects compared to other drugs containing progesterone including breast tenderness, bloating, mood swings, irritability, and drowsiness. If patients experience bothersome side effects that are not easily treated (for example a headache that resolves with a dose of Tylenol) we would stop vaginal progesterone treatment as is our current clinical practice.

Oral doxycycline is considered minimal risk but patients have reported in clinical trials to report symptoms such as nausea, decreased appetite, vomiting, diarrhea, hives and anemia. If these symptoms developed and didn't resolved with minimal intervention (taking food with medicine) the treatment would be stopped as is our current clinical practice.

iv. Procedures

Minimal risk to patients with non-invasive procedures. Patients will only receive standard of care diagnostic testing during their duration of treatment in our office. This will include ultrasounds, blood tests and a uterine cavity evaluation. A patient may experience mild discomfort at the venipuncture site and some lower abdominal cramping and light bleeding (spotting) associated with a uterine cavity evaluation. There are extremely low risks of infection or other complications associated with these minimally invasive procedures.

v. Radioisotopes/radiation-producing machines

N/A

vi. Physical well-being

Minimal risks to a patient's physical well-being. Patients are receiving standard medical treatment. Repeat biopsies will be offered to patients whose initial testing is not receptive. The risk of the repeat biopsy is again minimal physical risk and patients will be allowed to decline the repeat testing.

vii. Psychological well-being

Minimal risks to a patient's psychological well-being. Potential improvement to psychological well being as the information from the ERA may help explain a previously unexplained diagnosis of recurrent pregnancy loss or unexplained infertility.

viii. Economic well-being

Participants will not be responsible for any costs associated with this study. The are receiving standard medical care and if they agree to the endometrial biopsy, the ERA and microbiome testing will be done for no additional cost.

ix. Social well-being

x. Participation in this study should not effect a patient's social well-being. Overall evaluation of risk

Low

b. International Research Risk Procedures

N/A

c. Procedures to Minimize Risk

Clinical study staff will explain all procedures thoroughly to consented subjects and why each diagnostic test is being done during their evaluation. There are no anticipated

additional risks to a patient who is involved in this study as compared to a nonparticipating patient in the same clinical setting. Subject identity will be kept confidential.

d. Study Conclusion

The study will terminate once the desired number of participants has been reached and each patient has been followed for 5 years from the date of enrollment.

e. Data Safety Monitoring Plan (DSMC)

i. Data and/or events subject to review

Since we will be performing endometrial biopsies in patients enrolled in this study, there is a small risk of bleeding and infection that can occur as result of this intervention (<1%) We will keep track of all patients that experience complications as a result of this procedure.

For patients who use vaginal progesterone there is a small risk of side effects from systemic absorption of the progesterone: Bloating, stomach/abdominal pain, nausea, breast tenderness, headache, drowsiness, mood swings, irritability, or vaginal discomfort. In our experience we have found that <1% of our patients have bothersome side effects with vaginal progesterone which we routinely use for many infertility and recurrent pregnancy loss patients. We will keep track of all patients that experience side effects on this medication.

For patients using oral doxycycline there is a small risk of side effects including decreased appetite, nausea, vomiting, diarrhea, allergic reaction and anemia. In our experiences we have found that <1% of our patients have bothersome side effects with oral doxycycline which we routinely use for many of these patients. . We will keep track of all patients that experience side effects on this medication.

Vaginal probiotics is an over the counter supplement that is not expected to have side effects other then difficulty with insertion or change in typical vaginal discharge. None the less we will keep track if any patient reports any bothersome side effect and discontinue its use if there are any.

ii. Person(s) responsible for Data and Safety Monitoring

The protocol director will be monitoring all unanticipated problems and adverse events.

iii. Frequency of DSMB meetings

N/A

iv. Specific triggers or stopping rules

The risk of complications with an endometrial biopsy in the general population is approximately 1%. If we experience a higher complication rate then this we will terminate the procedure.

If any patient reports persistent side effects while on vaginal progesterone this will be stopped for this patient as it would be based on standard of care practice.

v. DSMB Reporting

N/A

vi. Will the Protocol Director be the only monitoring entity? (Y/N)

Yes

vii. Will a board, committee, or safety monitor be responsible for study monitoring? (Y/N)

No

f. Risks to Special Populations

N/A

9. BENEFITS

Participants will benefit by receiving a thorough evaluation of theirrecurrent pregnancy loss and unexplained infertility or baseline fertility testing for thoseundergoing egg freezing or sex selection. Participantswill be informed of their endometrial receptivity array andmicrobiome results which may lead to improvements in their futurecare. For example many of our patients do go on to have in vitrofertilization with subsequent embryo transfers (also the sex selection patients or thosepatients who froze eggs who are coming back to use them). During these embryotransfers the ERA is used to personalize and improve the embryotransfer success rate. The ERA has been shown to be consistent intime across menstrual cycles over many years. Knowledge of the ERAwould help patients as they would not have to go through or pay forthis testing at a later time.

10. PRIVACY AND CONFIDENTIALITY

All participant information and specimens are handled in compliance with the Health Insurance Portability and Accountability Act (HIPAA) and privacy policies of Stanford University, Stanford Health Care, and Stanford Children's Health.