Assessment and Prevention of Pain During Ovarian Stimulation in Patients With Endometriosis

NCT04002141

Protocol & Statistical Analysis Plan

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CLINICAL STUDY PROTOCOL

ASSESSMENT AND PREVENTION OF PAIN DURING OVARIAN STIMULATION IN PATIENTS WITH ENDOMETRIOSIS (APPOSE TRIAL)

Study Number

18-26544

IND Number

Pending

Clinical Phase

Phase I

Protocol Version

February 20, 2019

version 3

UCSF

Clinical Research Protocol

Protocol Number:	18-26544
Version Date:	2/20/2019
Investigational Product:	Letrozole 5mg
IND Number:	Pending
Development Phase:	Phase 1
Sponsor:	Marcelle Cedars, MD 499 Illinois St, 6th floor San Francisco, CA 94158
Funding Organization:	UCSF Division of Reproductive Endocrinology & Infertility
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Approval:

_____ _____

PI or Sponsor Signature (Name and Title) *Date*

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: 18-26544

Protocol Title: Assessment and Prevention of Pain During Ovarian Stimulation in Patients with Endometriosis (APPOSE TRIAL)

Protocol Date: 2/20/2019

_____ _____

Investigator Signature *Date*

_____

Print Name and Title

Site # _____

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Synopsis

Primary Objective

To evaluate the impact of ovarian hyperstimulation on endometriosis-related symptoms.

Secondary Objectives (if applicable)

To evaluate the impact of letrozole use during ovarian hyperstimulation with respect to endometriosis-related symptoms, embryo/egg quality and quantity, and pregnancy rates.

Primary Outcome Variables

- To compare the mean endometriosis pain score at baseline and 6 and 12 weeks following egg retrieval.
- To evaluate how endometriosis pain scores change throughout controlled ovarian hyperstimulation.

Endometriosis associated symptoms will be evaluated using a survey modeled after the clinical survey developed by the World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonisation Project. The survey will use a visual analog scale to quantify pain and symptoms experienced by participants.

Secondary and Exploratory Outcome Variables (if applicable)

- To compare the mean endometriosis pain score at baseline, 6 and 12 weeks following egg retrieval in the placebo, letrozole and control groups.
- To compare how endometriosis pain scores change throughout controlled ovarian hyperstimulation in the placebo, letrozole and control groups.
- To compare egg/embryo quantity and quality in the placebo, Letrozole and control groups.
- To compare follicular fluid estradiol levels between placebo, letrozole and control groups.
- To compare pregnancy rates in the placebo, Letrozole and control groups.

Study Duration

The total study duration will be approximately 12months. The total duration per participant will be approximately 14 weeks. Participants will be asked to take letrozole or placebo throughout their ovarian stimulation and for 2 weeks following retrieval. Participants will be asked to complete surveys up to 12 weeks following retrieval.

Study Design

Randomized double blinded placebo-controlled trial. There will be a total of 60 participants, with 20 participants with endometriosis randomized to the placebo group, 20 participants with endometriosis randomized to the letrozole group and 20 control patients with no history of endometriosis. Letrozole and placebo medication will be started on the first day of gonadotropin injections and continued until the day of trigger shot. Medication will be restarted the night of egg retrieval and continued for 2 weeks post retrieval. Endometriosis associated symptoms will be evaluated using a survey modeled after the clinical survey developed by the World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonisation Project. Surveys will be administered at baseline ultrasound appointment for controlled ovarian hyperstimulation cycle, the day of trigger shot, 3 weeks following egg retrieval, 6 weeks following egg retrieval and 12 weeks following egg retrieval.
Study Population Reproductive age women with a prior diagnosis of endometriosis, who are undergoing controlled ovarian stimulation.
Number of Participants A total of 60 participants (20 in each of the primary comparison groups) as well as an additional 20 age matched controls without a history of endometriosis will be enrolled. Enrollment will occur over a 12-month period, from April 2019 to April 2020.
Number of Study Sites There is a single study site for this trial. All participants will be recruited, and all study related activities will be completed, at the University of California San Francisco Center for Reproductive Health.

Table of Contents

1 - Introduction

1.1 Introductory Statement

Endometriosis is an estrogen dependent disease present in 25-35% of infertile women. Given controlled ovarian hyperstimulation leads to a considerable rise in serum estradiol concentrations, patients and providers must consider the possible risk of disease and symptom progression related to controlled ovarian hyperstimulation. Aromatase inhibitors, such as Letrozole, markedly suppress plasma estrogen levels and are commonly used during controlled ovarian hyperstimulation in cancer patients with estrogen-sensitive cancers. It is therefore reasonable to investigate the benefit of using letrozole during controlled ovarian hyperstimulation in patients previously diagnosed with endometriosis.

2 - Background

2.1.1 Preclinical Experience

See Clinical Experience below in section 2.1.2 (below).

2.1.2 Clinical Experience

Aromatase inhibitors, such as letrozole, markedly suppress plasma estrogen levels by competitively inhibiting the activity of the aromatase enzyme and are effective in treating endometriosis related pain [1-4]. Letrozole is FDA approved for use in cancer patients with estrogen-sensitive cancer undergoing fertility preservation. The addition of letrozole has been shown to decrease serum estradiol levels closer to that observed in natural cycles (i.e., E2 <500 pg/mL) without affecting oocyte or embryo yield [5, 6]. It is therefore reasonable to consider the use of letrozole during controlled ovarian hyperstimulation in patients with endometriosis, with a goal of minimizing any impact on patient symptoms. Additionally, given improved pregnancy outcomes following suppression of endometriosis with GnRH agonists, letrozole suppression of endometriosis during controlled ovarian hyperstimulation may improve cycle outcomes in patients with endometriosis.

2.2 Background/prevalence of research topic

Endometriosis is an estrogen dependent disease present in 25-35% of infertile women. Given controlled ovarian hyperstimulation leads to development of multiple follicles and considerable rise in serum estradiol concentrations, patients and providers must consider the possible risk of disease and symptom progression related to controlled ovarian hyperstimulation. This may be especially true with repeated stimulations.

With respect to disease progression, data have been contradictory, but overall favor that there is no significant risk of disease progression with controlled ovarian hyperstimulation. The impact of controlled ovarian hyperstimulation on endometriosis related symptoms is less clear, with a single prospective cohort study of 64 patients with endometriosis, published in 2011, showing no marked worsening in symptoms [7]. Study limitations include lack of a control group, small sample size and a limited follow-up time of 3-6 months. Anecdotally, providers from our institution's Center for Reproductive Health and Center for Endometriosis do feel patients often report a flare in endometriosis related symptoms following controlled ovarian hyperstimulation. Overall, the question remains open and further investigation is warranted.

Additionally, the impact of endometriosis on in vitro fertilization outcomes has been a long-debated topic, with some studies showing poorer outcomes compared to controls and others showing no impact. Prolonged use of a GnRH agonist before in vitro fertilization with embryo transfer, in patients with endometriosis, has been associated with significantly higher ongoing pregnancy rates [8, 9].

3 - Rationale/Significance

3.1 Problem Statement

Endometriosis is an estrogen dependent disease commonly present in infertile women. Controlled ovarian hyperstimulation is a common treatment for infertility, however results in a considerable rise in serum estradiol concentrations. Given this, it is important for patients and providers to consider and minimize the possible risk of disease and symptom progression related to controlled ovarian hyperstimulation.

3.2 Purpose of Study/Potential Impact

Given the high prevalence of endometriosis among infertile women, optimizing the management of symptoms during controlled ovarian hyperstimulation in this patient population is a challenge faced by all clinical reproductive endocrinologists. Currently there are no evidence-based recommendations to improve patient experience or ovarian simulation outcomes in this patient population.

Letrozole is an aromatase inhibitor that markedly suppresses plasma estrogen levels by competitively inhibiting the activity of the aromatase enzyme. It has previously been shown to be an effective treatment for endometriosis related pain outside of controlled ovarian hyperstimulation. Letrozole, is regularly used in cancer patients with estrogen-sensitive cancer undergoing controlled ovarian hyperstimulation for fertility preservation.

This trial could provide evidence for the use of Letrozole in patients with endometriosis. This would be a relatively low cost, minimally invasive, intervention for this patient population. Further, it would be the first evidence-based recommendation to improve patient experience and/or ovarian simulation outcomes in this patient population.

3.3.1 Potential Risks

Risks and side effects related to the possible use of Letrozole include those which are:

<u>Likely</u>

• Flushing

• Mood changes

• Hot flushes

• Bloating

<u>Less Likely</u>

• Headache

• Fatigue

• Insomnia

<u>Rare but serious</u>

• Headache with vision changes

• Blood clot

Patients undergoing controlled ovarian hyperstimulation are monitored every 1-3 days by University of California Center for Reproductive Health providers. During this monitoring patients will be assessed for any possible concerns or side effects of the study medication. All concerns will be recorded and reviewed by the Data Safety and Monitoring Board on a monthly basis.

3.3.2 Potential Benefits

Taking part in this study may or may not make participants health better. If letrozole proves to be better than the placebo group, this group may benefit from participating in the study, but this cannot be guaranteed. Information gained from this study will help improve the IVF process for future patients, particularly patients with endometriosis.

In return for the time and effort participants put into completing the study surveys, they will be paid $60 in compensation. They will received $30 after the completion of survey two and an additional $30 at the completion of survey 5.

4 - Study Objectives

4.1 Hypothesis

Null hypotheses:

a) Ovarian hyperstimulation has no impact on endometriosis-related symptoms.

b) The use of letrozole during ovarian hyperstimulation has no impact on endometriosis-related symptoms.

c) The use of letrozole during ovarian hyperstimulation has no impact on egg/embryo quantity or quality.

d) The use of letrozole during ovarian hyperstimulation has no impact on pregnancy rates.

4.2 Primary Objective

To evaluate the impact of ovarian hyperstimulation on endometriosis-related symptoms.

4.3 Secondary Objectives (if applicable)

To evaluate the impact of letrozole use during ovarian hyperstimulation with respect to endometriosis-related symptoms, embryo/egg quality and quantity and pregnancy rates.

5 - Study Design

5.1 General Design Description

Randomized double blinded placebo-controlled trial.

5.1.1 Study Date Range and Duration

We anticipate it will take 12 months to enroll and complete this study. Enrollment is scheduled to begin in April of 2019 and conclude by April 2020.

The total duration per participant will be approximately 14 weeks. Participants will be asked to take letrozole or placebo throughout their ovarian stimulation and for 2 weeks following their retrieval. Participants will also be asked to complete surveys up to 12 weeks following their retrieval. Ovarian follicular fluid will be obtained at the time of egg retrieval for hormonal evaluation.

The study will involve long-term follow-up, including periodic review of participant medical records to evaluate future treatments received to treat their endometriosis and evaluate the use and outcomes of the eggs/embryos preserved during their ovarian stimulation cycle.

5.1.2 Number of Study Sites

There is a single study site for this trial. All participants will be recruited, and all study related activities will be completed, at the University of California San Francisco Center for Reproductive Health.

5.2.1 Primary Outcome Variables

- To compare the mean endometriosis pain score at baseline, 6 and 12 weeks following egg retrieval.
- To evaluate how endometriosis pain scores change throughout controlled ovarian hyperstimulation.

5.2.2 Secondary and Exploratory Outcome Variables (if applicable)

- To compare the mean endometriosis pain score at baseline, 6 and 12 weeks following egg retrieval in the placebo, letrozole and control groups.
- To compare how endometriosis pain scores change throughout controlled ovarian hyperstimulation in the placebo, letrozole and control groups.
- To compare egg/embryo quantity and quality in the placebo, letrozole and control groups. This will include evaluation of: total oocyte yield per starting antral follicle count, total mature oocyte yield per oocytes collected, embryo yield per starting antral follicle count, embryo yield per oocyte retrieved, fertilization rate per mature oocyte retrieved, ovarian follicular fluid hormone levels.
- To compare pregnancy rates per embryo transfer in the placebo, letrozole and control groups.

5.3 Study Population

Our target population is reproductive age women with endometriosis, who are undergoing controlled ovarian hyperstimulation. Our accessible population are those seen at the University of California San Francisco Center for Reproductive Health. Our sample population are those recruited from our clinic, who meet the study criteria, and agree to participate in the study. We will also recruit a group of age-matched controls, without a history of endometriosis.

5.3.1 Number of Participants

We will be aiming for a total of 40 participants with a prior diagnosis of endometriosis. Of those 40 participants, 20 participants will be randomized to the placebo group and 20 participants will be randomized to the letrozole group. We will also enroll 20 age-matched controls without a history of endometriosis. This will result in 60 study participants.

5.3.2 Eligibility Criteria/Vulnerable Populations

Inclusion Criteria:

- Age 18-42 years
- Planning to undergo controlled ovarian hyperstimulation
- Prior diagnosis of endometriosis by one of the following: (1) surgical diagnosis (2) endometrioma on pelvic ultrasound
- Planning to freeze all retrieved oocytes/embryos prior to transfer

Exclusion Criteria:

- Hypersensitivity to Letrozole
- Any significant concurrent disease, illness, or psychiatric disorder that would compromise patient safety or compliance, interfere with consent, study participation, follow-up, or interpretation of study results

6 - Methods

6.1.1 Identity of Investigational Product/New Drug

Letrozole 5mg encapsulated oral tablet versus placebo encapsulated oral tablet.

6.1.2 Dosage, Admin, Schedule (if applicable)

Whether placed into the intervention, placebo or control group, all participants will receive their usual gonadotropin injections and trigger shot for their controlled ovarian hyperstimulation cycle, as standardly prescribed by their primary provider at the University of California Center for Reproductive health. In addition:

- The intervention group will receive oral letrozole 5 mg per day, starting on the first day of gonadotropin injections and continued until the day of trigger shot. Oral letrozole 5mg per day will be then be restarted the night of egg retrieval and continued for 2 weeks post retrieval.
- The placebo group will receive oral placebo, starting on the first day of gonadotropin injections and continued until the day of trigger shot. Daily oral placebo will be restarted the night of egg retrieval and continued for 2 weeks post retrieval.

6.1.3 Method of Assignment/Randomization (if applicable)

Patients will be randomized into either the intervention or the control group at the onset of the study. Randomization will be completed by non-clinical personnel using a computer-generated randomizer.

Safeway Palo Alto Compounding Pharmacy will be compounding and providing the encapsulated letrozole and placebo capsules. Letrozole capsules will be placed in pre-aliquoted bottles labeled A. Placebo capsules will be in pre-aliquoted bottles labeled B. Medications will be mailed to the study coordinator in batches at the start of the study. Medications will be stored at room temperature 68-77 degrees Fahrenheit and away from heat, moisture and light. The shelf life of stored medication is 12 months.

Study participants will be blinded to the content of the medication and the placebo and letrozole tablets will both be encapsulated and identical in appearance. Due to the suppressive effect of letrozole on serum estrogen levels, both providers and participants will be blinded to estradiol levels during controlled ovarian hyperstimulation. Estradiol blood draws will be obtained with each scheduled ultrasound, as routinely done during ovarian stimulation. Study participants will be counseled about this at the time of study consent and enrollment. They will be advised that at UCSF providers routinely monitor estradiol levels to guide cycle management, however many IVF clinics rely solely on ultrasound findings and do not routinely use estradiol levels. Further, when letrozole is used, estradiol levels can no longer be used for clinical management. Given letrozole is routinely used in patients with estrogen sensitive cancers undergoing ovarian stimulation without any negative impact on their cycles outcomes, we do not anticipate any negative impact from the blinding of serum estrogen levels. The cost of estrogen blood draws will be removed from the patient's IVF costs.

6.1.4 Blinding and Procedures for Unblinding (if applicable)

Patients and providers will be randomized into either the intervention or placebo group at study onset as discussed above and instructed to take medication labeled either A or B. (A) and (B) correspond to either the active ingredient medication or placebo. At completion of the study by all participants and statistical analyses of the data (based on A vs. B medication), the study coordinator will reveal patient allocation to interventional or placebo groups.

6.1.5 Packaging/Labelling

The study pharmacist at Safeway Palo Alto Compounding Pharmacy will make identical appearing encapsulated letrozole versus placebo pills available in a pre-aliquoted bottles. Twenty-six doses will be in each bottle and 1 bottle will be provided to the patient at the time of randomization.

The bottles will be labeled "letrozole (A)" vs. "letrozole (B)." (A) and (B) correspond to either the active ingredient medication or placebo. The study participants and providers will be blinded to the content of the medication - whether A is active or placebo medication.

6.1.6 Storage Conditions

Medications will be stored at room temperature 68-77 degrees Fahrenheit and away from heat, moisture and light. The shelf life of stored medication is 12 months. The pharmacist will send medications in batches such that medication will be available at the time of patients starting the study but not stored for so long that the shelf life expires.

Upon returning for routing post in vitro fertilization visit, all used and unused bottles will be returned to the study personnel to assess for patient compliance. The amount of study drug dispensed and returned by the subject will be recorded. The study monitor will verify these documents throughout the course of the study.

6.1.7 Concomitant therapy

As discussed above, aside from study medication (placebo versus letrozole) and blinding of providers to estradiol levels, all patients will receive the standard ovarian hyperstimulation protocol prescribed by their primary provider at the University of California Center for Reproductive Health.

6.1.8 Restrictions

See exclusion criteria and risks above.

6.2 Assessments

Endometriosis associated symptoms will be evaluated using a survey modeled after the clinical survey developed by the World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonisation Project [10]. The survey will use a visual analog scale (VAS) to quantify pain and symptoms experienced by participants. Participants will complete this survey at their baseline ultrasound appointment for their controlled ovarian hyperstimulation cycle, the day of trigger shot, 3 weeks following egg retrieval, 6 weeks following egg retrieval and 12 weeks following egg retrieval if not pregnant. The first survey will include questions regarding any recent or prior medical or surgical treatment of their

endometriosis. It will also assess baseline use of pain medications for their endometriosis-associated pain. Study participants will be allowed to take acetaminophen when needed (Tylenol, 650 mg tablet) for pain control, but asked to record the number of tablets used.

Secondary outcomes looking at egg and embryo quality and quantity will be assessed using standard embryologists evaluation and grading of all eggs and embryos collected during controlled ovarian hyperstimulation at the University of California Center for Reproductive Health. Embryologist will be blinded to the study participants' assignments to either placebo or control group.

6.2.1 Efficacy

Letrazole 5mg oral daily has been routinely used in women with estrogen sensitive cancer undergoing controlled ovarian hyperstimulation. It has also been used off-label for many years as a standard treatment for pain related to endometriosis.

6.2.2 Safety/Pregnancy-related policy

Letrazole is contraindicated during female pregnancy because of probable adverse effects on the fetus (FDA pregnancy risk category X). Pregnancy is ruled out in all women undergoing controlled ovarian hyperstimulation prior to starting stimulation medications.

6.2.2.1 Adverse Events Definition and Reporting

Risks and side effects related to the possible use of letrozole include those which are:

<u>Likely</u>

• Flushing

• Mood changes

• Hot flushes

• Bloating

<u>Less Likely</u>

• Headache

• Fatigue

• Insomnia

<u>Rare but serious</u>

• Headache with vision changes

• Blood clot

Patients undergoing controlled ovarian hyperstimulation are monitored every 1-3 days by University of California Center for Reproductive Health providers. During this monitoring patients will be assessed for any possible concerns or side effects of the study medication. All concerns will be recorded and reviewed by the Data Safety and Monitoring Board on a quarterly basis.

6.2.3 Pharmacokinetics (if applicable)

Not applicable.

6.2.4 Biomarkers (if applicable)

Not applicable.

6.3 Study Procedures

Management of participants' ovarian stimulation will be done by their primary doctor based on their knowledge, expertise and established standard of care guidelines. Participants will come to the clinic for transvaginal ultrasound visits to track follicle development, and blood draws will be completed per routine. Serum estradiol levels will be run on all participant blood samples at the end of the study to assure blinding of patients and providers, as discussed in section 6.1.3 above. When follicles are an adequate size, participants will be instructed to take a trigger shot, an injectable hormone medicine that helps to prepare eggs for retrieval. On the day of the egg retrieval procedure, the treating physician will use standard of care transvaginal aspiration to drain all of the visible follicles in order to retrieve eggs. After the egg has been isolated, the excess fluid and cells are usually discarded. We will collect the follicular fluid from the first follicle aspirated in each ovary to perform hormone assays. Throughout the ovarian stimulation process, participants will be asked to complete electronic surveys about symptoms they are experiencing related to their endometriosis.

6.3.1 Study Schedule

M.D. Visit	• M.D. to identify patient as potential study participant • M.D. discusses study with patient • M.D. to notify study team if patient interested
Meet Study Team	• Discuss study with patient and answer questions • Subject signs study and HIPPA consents • Randomization completed, patient provided medication
Baseline Ultrasound	• Completion of survey #1
Stimulation Monitoring	• Daily letrozole/placebo starting with first day of stimulation medications • Routine uterine and ovarian ultrasound monitoring every 1-3 days at UCSF CRH • Routine blood draws for hormonal assessment every 1-3 days at UCSF CRH (patients and providers blinded to estradiol levels)
Trigger Shot	• Based on routine trigger shot parameters, including mean diameter of largest follicles • Completion of survey #2 • Discontinuation of letrozole/placebo
Oocyte Retrieval	• Retrieve all visible follicles • Follicular fluid obtained for hormonal evaluation • Restart letrozole/placebo evening of retrieval and continue daily for 2 weeks
Embryology Lab	• Count total number of retrieved oocytes • Count mature oocytes and fertilization rate (if making embryos) • Assess embryo quality using UCSF CRH standard grading criteria

3 Weeks Post Retrieval	• Completion of survey #3
6 Weeks Post Retrieval	• Completion of survey #4
12 Weeks Post Retrieval	• Evaluation of pregnancy status • Completion of survey #5
Long-term Follow-up	• Medial record review for pregnancy outcomes of transferred embryos and additional endometriosis treatment.

6.3.2 Informed Consent

Once an individual is found to qualify for the study, details and all questions will be discussed during the informed consent process. Signed informed consent from the individual will be obtained. The informed consent/permission/assent form covers information about the overall purpose of the study, what the study entails, potential risks, potential benefits to participating individuals and society, the confidentiality of data, and contact information for the Principal Investigator and the IRB.

Once informed consent/permission/assent has been obtained, the research staff will have the form reviewed by a fellow research team member, who will confirm that it is fully and accurately completed before it is filed in a secure location under double-lock when not in use and with restricted access during work hours and/or when unattended.

Consent/Assent/Permission must be obtained within 30 days prior to study entry. If more than 30 days has elapsed since consent/assent/permission was obtained, consent/assent /permission must be verbally reaffirmed on the day of study entry prior to implementing any study activities.

All investigators obtaining consent will be HIPAA and CITI certified in addition to having prior experience in other studies obtaining consent.

6.3.3 Screening

Potential participants will be receiving services at the University of California San Francisco Center for Reproductive Health. Medical providers will identify potential participants and notify the research team. The research team will then recruit participants for the study by speaking with patients face-to-face or by telephone. Information regarding the study will be provided and interest in participation will be assessed.

6.3.4 Recruitment, Enrollment and Retention

Each potential participant identified by Center for Reproductive Health providers will have their medical records reviewed to assess potential eligibility. They will meet with research staff to further discuss the details of the study. If enrollment is desired, consents will be signed, randomization completed and medications dispensed. This will be scheduled to coincide with other necessary clinic visits in preparation for their planned controlled ovarian hyperstimulation cycle.

Patients consented for study participation will have the following information entered on the Study Recruitment Log, which will be maintained in a secure area at the site: name or initials, date of birth, age, and enrollment status. Individuals who were assessed as ineligible for enrollment will have the reasons for ineligibility recorded. Individuals who are approached, but do not consent to participate will also be recorded

Information collected on enrolled participants in the Study Recruitment Log, excluding names or initials and dates of birth, will be entered in the study-specific database.

When study accrual ends, project staff will obliterate all information on the Study Recruitment Log belonging to individuals who did not consent to participate in the study.

6.3.5 On Study Visits

Enrollment:

As described above, participants will be identified by their primary provider as a possible study candidate. The research team will be notified, review the patient's chart for eligibility and meet with the patient to further discuss the study. If the patient desires enrollment, consents will be signed, randomization will be completed and medication will be dispensed to the patient.

Baseline Ultrasound:

Participants will present for their routine baseline ultrasound as part of their controlled ovarian hyperstimulation protocol. This is typically done on cycle day 2, prior to the initiation of gonadotropin medications and study medication. Participants will also complete survey #1 within 24 hours of this visit. The survey can be completed on a clinic provided electronic device at the time of their visit or at home electronically via a secure RedCap portal.

Stimulation Monitoring:

Patients will take their study drug and gonadotropins daily as advised by their primary team. They will present for monitoring every 1-3 days at the University of San Francisco Center for Reproductive Health. At each visit, patient symptoms will be evaluated and as previously discussed, any concern for side effects from the study drug will be reported to the research team.

Trigger Shot:

Per routine trigger shot parameters, including mean diameter of largest follicles, the participant will be instructed when to administer their trigger shot. Participants will be instructed to complete survey #2 within 24 hours of this visit. The survey can be completed on a clinic provided electronic devise at the time of their visit or at home electronically via a secure RedCap portal. Participants will also be advised to discontinue their study medication until the night of their retrieval.

Oocyte Retrieval:

Participants will undergo a standard egg retrieval procedure at the University of California Center for Reproductive Health procedure suite. Follicular fluid from the first follicle aspirated in each ovary will be collected to perform hormone assays. The embryology lab

will complete their routine evaluation of the retrieved oocytes as well as the fertilization rate and embryo quality. On the day of retrieval, participants will be reminded to restart their study medication and continue to take it daily for two weeks.

Post Retrieval Surveys:

At 3- 6- and 12-weeks post retrieval, participants will be contacted by phone and reminded to complete survey number 3, 4 and 5 respectively. These surveys will be completed at home electronically via a secure RedCap portal.

Long Term Follow Up:

After completion of survey 5 participants will not be contacted again about the study. Their chart will be periodically reviewed to assess pregnancy outcomes from the controlled ovarian hyperstimulation cycle. Treatments they receive related to their diagnosis of endometriosis will also be reviewed.

6.3.6 End of Study and Follow-up

Regardless of whether participants have completed ovarian stimulation with letrozole or placebo, they will be scheduled for a post-IVF visit with their treating physician to discuss the outcome. This visit is a standard of care procedures for all IVF patients. The participant's nurse will schedule this visit.

6.3.7 Removal of subjects

Participants will be prematurely discontinued from the study if any of the following occurs:

- The participant withdraws consent
- The participant is lost to follow-up
- The participant experiences an untoward event that warrants discontinuation form the study
- The participant develops a health problem and needs treatment that would affect the results of this study
- The study is cancelled for other administrative reasons
- Death of the participant

At the time of premature discontinuation, an off- study form will be completed and no further data collection will occur. Data through the time of enrollment and premature discontinuation will still be used for study purposes.

6.4.1 Statistical Design

An intention-to-treat analysis of the results will be used, meaning results of the study will be based on the initial treatment assignment and not the treatment eventually received. The primary outcome will be assessed by changes in pain survey scores before, during and following controlled ovarian hyperstimulation.

6.4.2 Sample Size Considerations

Primary Outcome: change in endometriosis-related pain score pre and post controlled ovarian hyperstimulation.

Test: two sample test.

1:1 randomization powered at 0.9 to detect a 3-point difference on the VAS pain scale.

A standard deviation of 2.5 was used, based on previous literature [11].

The effect size of 3 was based on review of the literature which supports the most accurate evaluation of endometriosis related pain using a visual acuity scale. Using this scale, a change in 3 points typically results in change in pain category (mild, moderate, severe) and therefore represents a clinically significant reduction or increase in symptoms.

Sample Size Estimate: A total of 40 participants (20 in each of the primary comparison groups) would provide 90% power to detect a change in pain score of 3 between the two groups. This accounts for an estimated study participant dropout rate of 20%.

6.4.3.1 Primary Analyses

Statistical analysis will be performed using Stata versions 14 (Stata Corp., College Station, TX) and under the guidance of a statistician. A univariate analysis will be performed to compare patient cycle characteristics between the patient groups. Cycle characteristics of interest will include: patient age, number of prior stimulations, most recent treatment for endometriosis, presence of an endometrioma, number of stimulation days, number of follicles 13mm+ and 10-12mm, number of retrieved oocytes, number of mature oocytes.

For continuous data, including evaluation of pain scores, a two-sample t-test or Wilcoxon rank sum will be used as appropriate. All tests will be performed at the 0.05 level of significance.

6.4.3.2 Secondary Objectives Analyses

For our secondary outcomes, we will be evaluating the outcomes of the placebo and letrozole groups. Specifically, this will include:

- Comparison of the mean endometriosis pain score at baseline, 6 and 12 weeks following egg retrieval in the placebo, letrozole and control groups.
- Comparison of how endometriosis pain scores change throughout controlled ovarian hyperstimulation in the placebo, letrozole and control groups.
- Comparison of egg/embryo quantity and quality in the placebo, letrozole and control groups. This will include evaluation of: total oocyte yield per starting antral follicle count, total mature oocyte yield per oocytes collected, embryo yield per starting antral follicle count, embryo yield per oocyte retrieved, fertilization rate per mature oocyte retrieved, follicular fluid hormone levels.
- Comparison of pregnancy rates per embryo transfer in the placebo, letrozole and control groups.

6.4.3.3 Safety/Pregnancy-related policy

As discussed above in section 6.2.2.

Pregnancy will be ruled out in all participants prior to starting controlled ovarian hyperstimulation and study medications.

6.4.3.4 Analysis of Subject Characteristics

As discussed in above sections.

6.4.3.5 Interim Analysis (if applicable)

Interim analysis will not be performed. Only upon completion of all 60 participants will results be analyzed and study personnel unblinded to use of letrozole versus placebo.

6.4.3.6 Health economic evaluation

Taking part in the study will not represent any additional cost to participants.

The costs of all visits, treatments, and tests aside from the study medication are a routine part of controlled ovarian hyperstimulation and will be billed to participants' insurance carrier. Insurance companies and other carriers sometimes refuse to pay the costs of treatment for ovarian stimulation. All participants are aware of this and understand in this setting they will be billed for the care their insurance will not cover. Financial counselors are available through the hospital accounting department to discuss this with all patients undergoing controlled ovarian hyperstimulation.

The letrozole and placebo medication will be will be provided to the patient free-of-charge and insurance will not be charged for this medication.

6.4.3.7 Other

Not applicable

6.4.4 Subsets and Covariates

The following covariates will be collected and considered:

- Age in years
- Baseline antral follicle count
- Body mass index

6.4.5 Handling of Missing Data

We will conduct missing data analyses in order to differentiate between data that are missing at random (MAR) and data that are missing related to letrozole use. If missing data can be regarded as MAR, multiple imputations may be used. If the MAR assumption is not plausible, sensitivity analyses will be conducted to evaluate the impact of MAR violations on analyses by specifying models for non-ignorable missing data mechanisms.

7 - Trial Administration

7.1 Ethical Considerations: Informed Consent/Assent and HIPAA Authorization

Consent for participation will occur in-person by trained providers. The purpose of the study will be explained in detail. In addition, the study process, benefits, risk and compensation will be reviewed in detail. Written consent will be obtained using an IRB approved APPOSE Trial and HIPPA consent form.

7.2 Institutional Review Board (IRB) Review

Pending review.

7.3 Subject Confidentiality

We will use REDCap, a browser-based, metadata-driven, Health Insurance Portability and Accountability compliant, electronic data capture software solution and workflow methodology for designing clinical and translational research databases, to collect our data. All pain surveys will also be completed electronically by patients through REDCap. We will use the REDCap installation which is maintained by the San Francisco Coordinating Center (SFCC) and will store the REDCap data on SFCC servers. These servers are maintained behind a firewall in a secure server room; all servers are backed up regularly off-site.

All consent forms will be filed and stored separate from the raw data in a secured location under double-lock when not in use and with restricted access during work hours and/or when unattended.

All research staff involved in the study are certified by UCSF to conduct research on human participants.

7.4 Deviations/Unanticipated Problems

All deviations from the protocol will be submitted to the Institutional Review Board to determine next steps to resolve the unanticipated problems or deviations.

7.5 Data Collection

As described above in section 7.3.

7.6 Data Quality Assurance

Investigators must protect research participants and produce reliable study information. The study physician will work with site staff to facilitate the receipt of data, provide technical assistance as necessary, and meet regularly via telephone or email with the staff person responsible for site-specific quality assurance. Data edits through range checks and field inconsistencies will be built into the database to enable real time correction of key entries and completion errors.

7.7 Study Records

Maintained on RedCap as discussed in section in 7.3.

7.8 Access to Source

Only IRB-approved individuals will have access to stored RedCap data.

7.9 Data or Specimen Storage/Security

Each individual participant who has their medical records reviewed to assess potential eligibility, is approached for recruitment, and/or consented for study participation will have the following information entered on the Study Recruitment Log, which will be maintained in a secure area at the site: name or initials, date of birth, age, sex assigned at birth, race, ethnicity and enrollment status. Individuals that were assessed as ineligible for enrollment will have the reasons for ineligibility recorded. Information collected on the Study Recruitment Log for enrolled participants, excluding names or initials and dates of birth, will be entered in the study-specific database.

For those individuals who were not eligible or did not consent to participate in the study, will have their information recorded only on the Study Recruitment Log. When study accrual ends, project staff will obliterate all names or initials and dates of birth belonging to individuals who did not consent to participate in the study.

Participants will complete all 5 planned surveys directly through RedCap. The data will be secured on the RedCap website with only research staff allowed access to the questionnaire results.

All consent forms will be filed and stored separate from the raw data, in a secured location under double-lock when not in use and with restricted access during work hours and/or when unattended.

7.10 Retention of Records

Refer to section 7.9 above.

7.11 Study Monitoring

We will follow IRB procedure for reporting and managing untoward events. Project staff will record any untoward events experienced by the participants.

7.12 Data Safety Monitoring Plan

There will be a Data Safety Monitoring Board (DSMB) to ensure the safety of the participants and the validity and integrity of the data. The board will meet monthly to review any safety concerns raised by the study. These will include adverse events, breaches of confidentiality, unanticipated problems involving risk to participants or others.

The Data and Safety Monitoring Board, comprised of Heather Huddleston (Reproductive Endocrinologist) and Victor Fujimoto (Reproductive Endocrinologist), will meet monthly during the study and review any safety violations or concerns generated by patients and study personnel completing the study protocols.

An adverse event (AE) is any untoward medical occurrence in a subject during participation in the clinical study or with use of the experimental agent being studied. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.), or any combination of these.

A serious adverse event (SAE) is any adverse event that results in one or more of the following outcomes:

- Death
- A life-threatening event
- Inpatient hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly or birth defect
- An important medical event based upon appropriate medical judgment

AEs will be labeled according to severity, which is based on their impact on the patient. An AE will be termed "mild" if it does not have a major impact on the patient, "moderate" if it causes the patient some minor inconvenience, and "severe" if it causes a substantial disruption to the patient's well-being

AEs will be categorized according to the likelihood that they are related to the study intervention. Specifically, they will be labeled definitely unrelated, definitely related, probably related, or possibly related to the study intervention. These will be reported to the IRB on an annual basis.

Results on the progress of the study will be reported annually to the IRB and also upon the study completion. The research team will adhere to the UCSF IRB reporting requirements. No interim analysis will be completed as it would decrease the overall power of the study.

This study will be stopped prior to its completion if: (1) the intervention is associated with adverse effects that call into question the safety of the intervention; (2) difficulty in study recruitment or retention will significantly impact the ability to evaluate the study endpoints; (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) other situations occur that might warrant stopping the trial.

A participant can leave a research study at any time. When withdrawing from the study, the participant should let the research team know that they wish to withdraw. A participant may provide the research team with the reason(s) for leaving the study, but this is not required. The research team may need to have the participant return so that he/she can be monitored for any future adverse effects from the study treatments, procedures or interventions.

7.13 Study Modification

All study modifications will be reported to the IRB for approval prior to proceeding.

7.14 Study Discontinuation

This study may be discontinued at any time by the Data and Safety Monitoring Board.

7.15 Study Completion

Study completion is expected to occur in 6 months upon complete accrual of 60 participants. This allows for 12 weeks of follow from the enrollment of the final participant and completion of statistical analyses.

7.16 Conflict of Interest Policy

No investigators or providers involved in this study have any conflicts of interest.

7.17 Funding Source

This study is funded by a Society for Reproductive Investigation and Bayer Discovery/Innovation Grant and the UCSF Center for Reproductive Health departmental funds. The Society for Reproductive Investigation, Bayer and UCSF Center for Reproductive Health have no financial interests in this study.

7.18 Publication Plan

Data will be made available to other investigators after a reasonable time period that includes enough opportunity to prepare and have submitted for publication the manuscript presenting the basic outcomes of the project.

8 – References

1. Agarwal, S.K. and W.G. Foster, *Reduction in Endometrioma Size with Three Months of Aromatase Inhibition and Progestin Add-Back.* Biomed Res Int, 2015. **2015**: p. 878517.
2. Almassinokiani, F., et al., *Effect of Letrozole on endometriosis-related pelvic pain.* Med J Islam Repub Iran, 2014. **28**: p. 107.
3. Ferrero, S., et al., *Letrozole and norethisterone acetate versus letrozole and triptorelin in the treatment of endometriosis related pain symptoms: a randomized controlled trial.* Reprod Biol Endocrinol, 2011. **9**: p. 88.
4. Smith, I.E. and M. Dowsett, *Aromatase inhibitors in breast cancer.* N Engl J Med, 2003. **348**(24): p. 2431-42.
5. Oktay, K., et al., *Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy.* J Clin Endocrinol Metab, 2006. **91**(10): p. 3885-90.
6. Testart, J., et al., *Steroids and gonadotropins during the last pre-ovulatory phase of the menstrual cycle. Time relationships between plasma hormones levels and luteinizing hormone surge onset.* J Steroid Biochem, 1982. **17**(6): p. 675-82.
7. Benaglia, L., et al., *IVF and endometriosis-related symptom progression: insights from a prospective study.* Hum Reprod, 2011. **26**(9): p. 2368-72.
8. Sallam, H.N., et al., *Long-term pituitary down-regulation before in vitro fertilization (IVF) for women with endometriosis.* Cochrane Database Syst Rev, 2006(1): p. CD004635.
9. Surrey, E.S., et al., *Effect of prolonged gonadotropin-releasing hormone agonist therapy on the outcome of in vitro fertilization-embryo transfer in patients with endometriosis.* Fertil Steril, 2002. **78**(4): p. 699-704.
10. Vitonis, A.F., et al., *World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonization Project: II. Clinical and covariate phenotype data collection in endometriosis research.* Fertil Steril, 2014. **102**(5): p. 1223-32.
11. Abushahin, F., et al., *Aromatase inhibition for refractory endometriosis-related chronic pelvic pain.* Fertil Steril, 2011. **96**(4): p. 939-42.