Title: Randomized controlled trial of Combined Letrozole and Clomid (CLC II) versus Letrozole alone for women with anovulation

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Draft or Version Number: 1.3

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Protocol Version History

Protocol Version	Version Date	Brief description of protocol modification/actions requested, if any
1.0	11/16/2020	
1.1	7/27/2021	Addition of GAD-7, PHQ-9, FertiQoL, and Perceived Stress Scale Questionnaires. Specification of BMI cut-off. Addition of UI Jordan Creek site.
1.2	9/21/2021	Addition of UW site specific information.
1.3	8/2/2023	Clarification of eligibility criteria, provision of direction for additional potential results at midluteal visit, grammatical edits

STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and local IRB regulations. All personnel involved in the conduct of this study have completed human subjects protection training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator or Clinical Site Investigator:

Signed: _____ Date: _____

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TABLE OF CONTENTS

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LIST OF ABBREVIATIONS

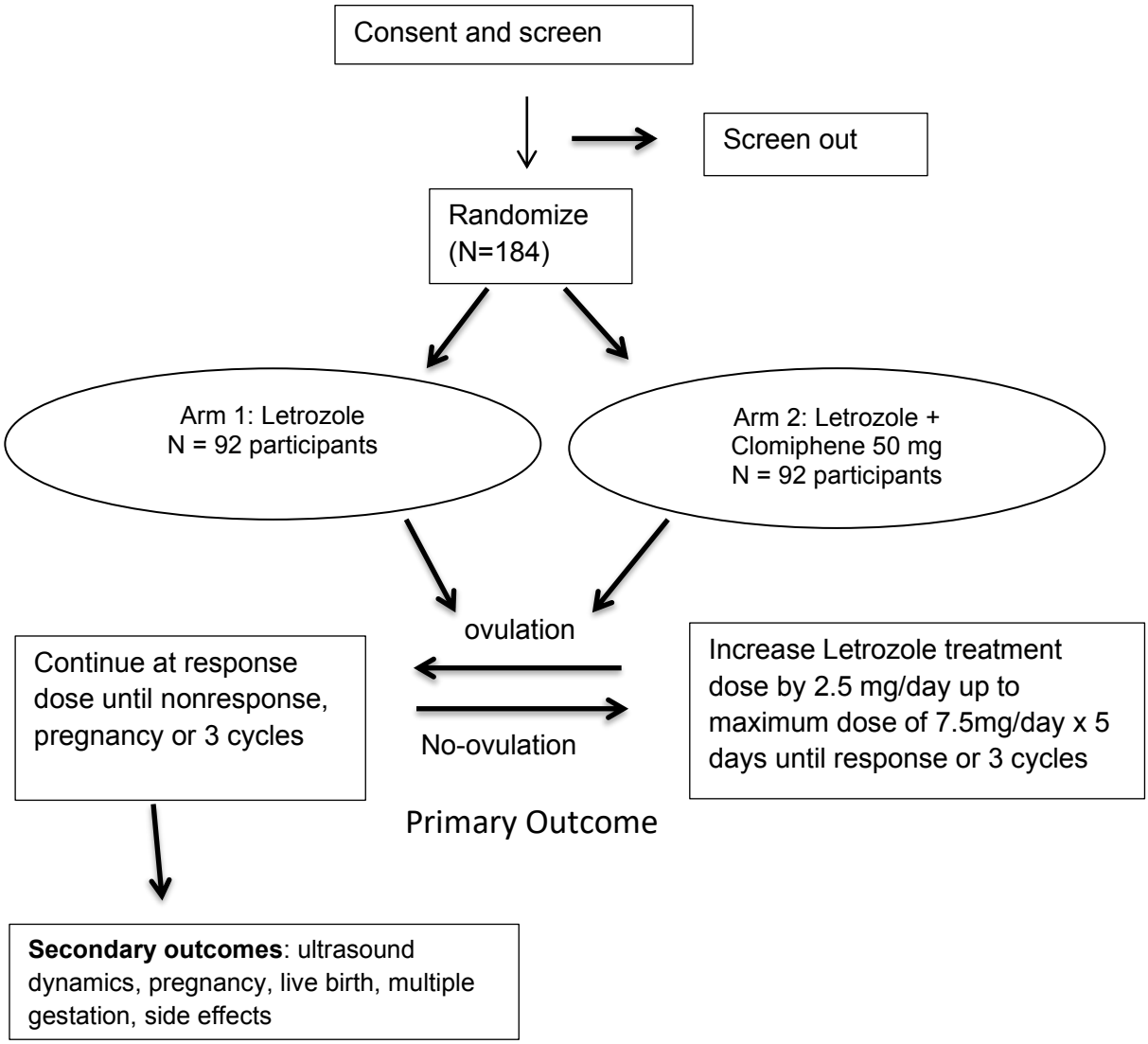
AE	Adverse Event/Adverse Experience
AMH	Anti-Müllerian Hormone
BMI	Body Mass Index
CD	Cycle Day
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DHEAS	Dehydroepiandrosterone Sulfate
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
HbgA1c	Hemoglobin A1C
hCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
IUP	Intrauterine Pregnancy
LH	Luteinizing Hormone
MedDRA®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NIH	National Institutes of Health
P4	Progesterone
PPCOS II	Pregnancy in Polycystic Ovary Syndrome Study II
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
REDCap	Research Electric Data Capture
SAE	Serious Adverse Event/Serious Adverse Experience
SHBG	Sex Hormone Binding Globulin
TSH	Thyroid Stimulating Hormone
UIHC	University of Iowa Hospitals and Clinics
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	Randomized controlled trial of Combined Letrozole and Clomiphene (CLC II) versus letrozole alone for women with anovulation
Précis:	This is a multi-center, randomized controlled open-label clinical trial of letrozole alone versus combination of letrozole and clomiphene citrate (CC) for up to 3 treatment cycles. Letrozole dosing will be increased in a stair-step fashion based on ovulation results. Participants will return for a mid-luteal ultrasound and progesterone lab to determine ovulatory status. Women will be randomized in a 1:1 ratio and the randomization scheme will be coordinated through the UIHC. Randomization will be stratified by site, age and BMI. The primary analysis will use an intent-to-treat approach to evaluate ovulatory rate in the two treatment arms.
Objectives:	To determine efficacy and safety of the combination letrozole and CC compared to letrozole alone in achieving ovulation in infertile women with anovulation. **Primary Outcome**: Ovulation will be the primary outcome determined by a mid-luteal progesterone level $\geq$ 3 and cumulative ovulation rate will be the primary efficacy parameter. **Secondary Outcome**: Secondary efficacy parameters will include pregnancy rate (conception and clinical pregnancy), live birth rate, multiple gestation live birth rate, pregnancy loss rate (biochemical, miscarriage, ectopic) and time to pregnancy. Side effect profile and complication rate will be monitored and reported. Obstetric outcomes will include pregnancy complication rate, birth weight and sex, neonatal complication rate. Ultrasound findings will be reported and compared between the two groups including: number of corpora lutea and endometrial thickness.
ClinicalTrials.gov Identifier	201906826
Population:	184 healthy women infertile women with polycystic ovary syndrome or normogonadotropic normoestrogenic anovulation or oligo-ovulation, age $\geq$ 18 to $\leq$40. Additionally, the couple will have no other major infertility factor.
Phase:	IV
Number of Sites:	4 sites

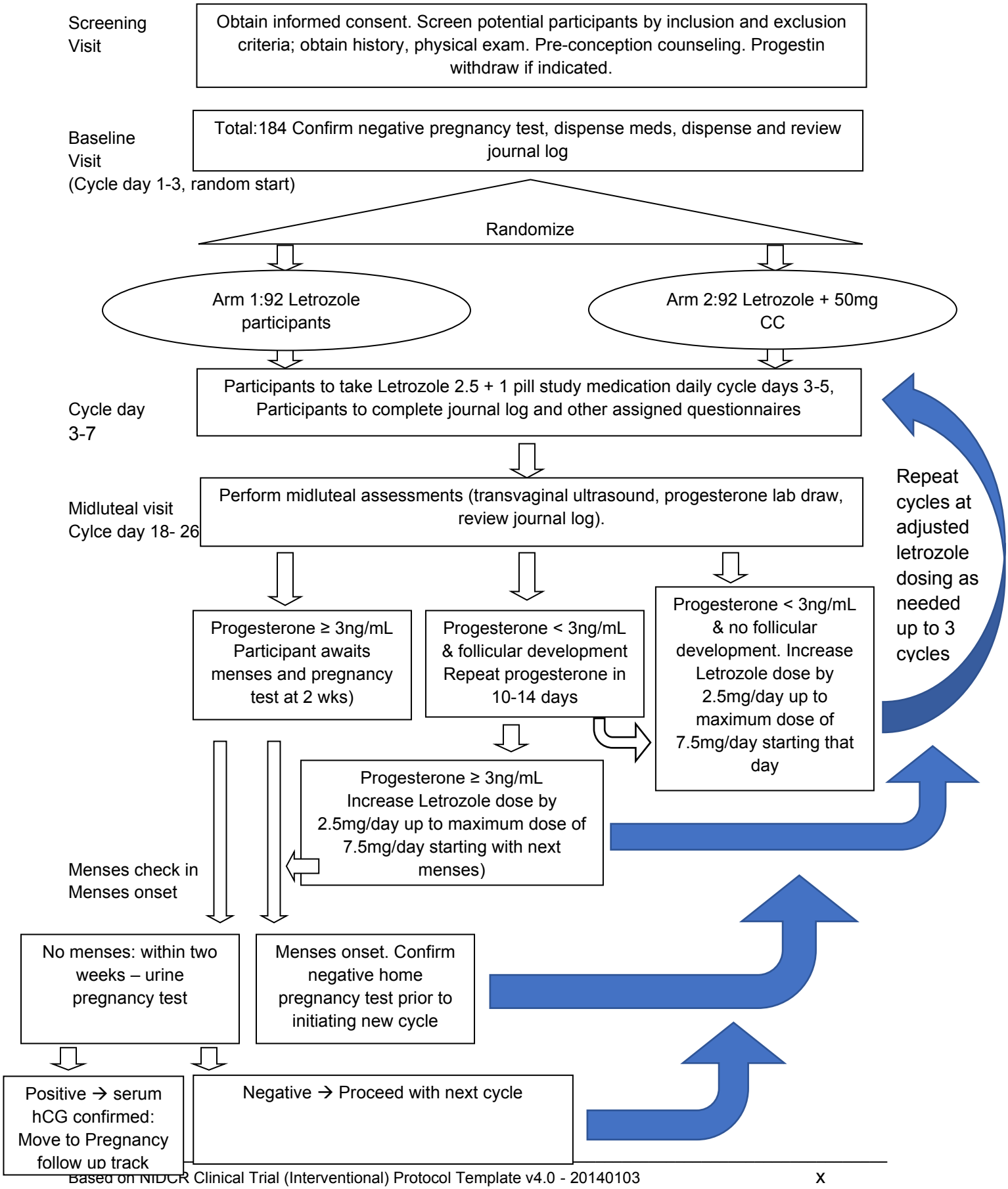
	University of Iowa Hospitals and Clinics Iowa City, IA with 2 ancillary sites - Quad Cities Clinic, Davenport, IA - Jordan Creek Clinic, Des Moines, IA University of Wisconsin, Generations Clinic, Middleton, WI
Description of Intervention:	184 women will be equally randomized to two different treatment arms at time of menses (spontaneous or induced with progestin withdrawal) or random start: A) letrozole 2.5 mg every day for 5 days (days 3-7 of cycle), or B) letrozole 2.5 mg and clomiphene citrate 50 mg every day for 5 days (days 3-7 of cycle), for a total of up to 3 treatment cycles. Letrozole dose will be increased in subsequent cycles in both groups based on ovulatory response in stairstep fashion (details within protocol). Maximum letrozole dose will be 7.5 mg a day for 5 days.
Study Duration:	2 years
Subject Participation Duration:	Approximately 15 weeks of active participation time. Clinical chart abstraction and remote follow up will be continued until 6-8 weeks post-delivery in pregnant women.
Estimated Time to Complete Enrollment:	20 months with 3 months follow up for final enrollees. 21 months by enrolling ~ 10 women per month across the 4 sites.

Schematic of Study Design:



Revised from PPCOS II Trial protocol study diagram

Detailed Schematic of Study Design:


Screening Visit
Obtain informed consent. Screen potential participants by inclusion and exclusion criteria; obtain history, physical exam. Pre-conception counseling. Progestin withdraw if indicated.
Baseline Visit
(Cycle day 1-3, random start)
Total:184 Confirm negative pregnancy test, dispense meds, dispense and review journal log
Randomize
Arm 1:92 Letrozole participants
Arm 2:92 Letrozole + 50mg CC
Cycle day 3-7
Participants to take Letrozole 2.5 + 1 pill study medication daily cycle days 3-5, Participants to complete journal log and other assigned questionnaires
Midluteal visit
Cylce day 18- 26
Perform midluteal assessments (transvaginal ultrasound, progesterone lab draw, review journal log).
Progesterone ≥ 3ng/mL Participant awaits menses and pregnancy test at 2 wks)
Progesterone < 3ng/mL & follicular development Repeat progesterone in 10-14 days
Progesterone < 3ng/mL & no follicular development. Increase Letrozole dose by 2.5mg/day up to maximum dose of 7.5mg/day starting that day
Repeat cycles at adjusted letrozole dosing as needed up to 3 cycles
Progesterone ≥ 3ng/mL Increase Letrozole dose by 2.5mg/day up to maximum dose of 7.5mg/day starting with next menses)
Menses check in
Menses onset
No menses: within two weeks – urine pregnancy test
Menses onset. Confirm negative home pregnancy test prior to initiating new cycle
Positive → serum hCG confirmed: Move to Pregnancy follow up track
Negative → Proceed with next cycle

1 KEY ROLES AND CONTACT INFORMATION

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Infertility in the US: The CDC National Survey of Family Growth (2011-2015) estimated that 7.3 million American women of reproductive age have used infertility services(1). Ovulatory disorders are the cause of approximately 30% of infertility(2). The most common ovulatory disorder, polycystic ovary syndrome (PCOS), comprises approximately 70% of anovulatory infertility cases(2). Based on these estimates, over 1.4 million American women have sought care for infertility related to PCOS. In this proposal, we focus on evaluating a novel cost- effective treatment strategy to improve fertility in women with PCOS.

Current treatments for infertility and their limitations: Anovulatory disorders, specifically PCOS have been extensively studied, including significant research on oral ovulation induction agents by the NICHD Reproductive Medicine Network. This work has established letrozole as the first-line treatment for anovulation, due to superior delivery rates compared to clomiphene citrate (CC) (3). Although Letrozole is the preferred initial treatment, ovulation and delivery rates remain poor. For example, the PPCOS II trial demonstrated an ovulation rate over 5 treatment cycles of 61.7% with letrozole relative to 48.3% with CC, with live birth rates of 27.5% and 19.1%, respectively (4). Thus, over 70% of women will not achieve a live birth and will require more aggressive therapies.

More aggressive therapies pose greater risks, particularly to the children conceived. The second-line treatment for women who fail to conceive with oral agents is gonadotropin ovulation induction (3), which carries a dramatically higher risk of multiple gestation as compared to oral ovulation induction agents. Eijkemans *et al* found a 13.6% multiple gestation rate among pregnancies conceived with gonadotropins in anovulatory women, with a 4.5% rate of higher order multiples (5). In contrast, in the PPCOS II trial, twins comprised 3.4% of pregnancies conceived with letrozole and 7.4% of pregnancies conceived with CC, with no higher order multiple gestations (4). Women who fail gonadotropin ovulation induction regimens will often proceed to in vitro fertilization (IVF). Multiple birth rates with IVF, although dropping, remain significantly higher than those seen with letrozole or CC, with twins comprising 13.7-17% of live births (6). Furthermore, pregnancy complications such as preterm delivery and low birth weight are increased in singleton IVF pregnancies (7, 8).

Not only are risks increased with second- and third-line therapies, costs are also considerably higher. Gonadotropin ovulation induction has been estimated to cost an additional €3615 ($4121) per live birth compared with CC (9). Costs of IVF are estimated to be more than *twenty times* the cost of ovulation induction medication ($19,234 versus $912) (10). These estimates do not account for the increased costs related to care for multiple gestations. More cost-effective therapies for ovulation induction in women with PCOS are urgently needed, due to the relatively poor outcomes of existing first line therapies, and the high expense and multiple gestation rates of second- and third-line therapies.

Mechanisms of drug action – potential for additive effect: CC is a non-steroidal selective estrogen receptor modulator that binds to the estrogen receptor in the hypothalamus. This central action affects the receptor recycling (11) and interferes with the normal feedback mechanism, resulting in increased

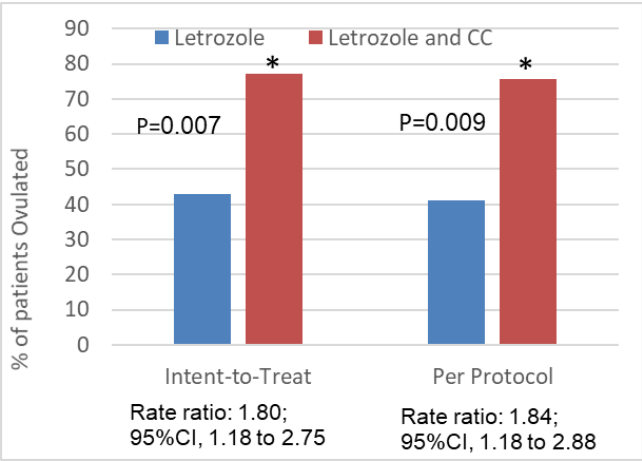
and prolonged FSH and LH secretion (12). These changes are more pronounced in the early follicular phase and are critical in initiating the development of follicles within the ovary. In a normo-ovulatory cycle, the rise in serum estrogen exerts a negative feedback in gonadotropin secretion.

Unlike CC, letrozole has a peripheral action whereby it blocks aromatase and the cytochrome P450 enzyme complex. This results in a peripheral hypo-estrogenic state, leading to increased FSH secretion. Letrozole also increases serum LH, androstenedione and testosterone levels (12). The rise in androgen levels increases follicular sensitivity to FSH by synergistic action with insulin-like growth factor (13-15) and FSH gene amplification (15, 16). The subsequent rise in serum estradiol provides a negative feedback on FSH, limiting multi-follicular recruitment and allowing endometrial proliferation (12).

CC has an antiestrogenic effect on endometrial development, which has not been demonstrated with letrozole. It is important to evaluate and confirm that by combining these two medications we do not see thinning of the endometrium demonstrated by ultrasound.
Herein we propose that the combination of letrozole and CC has an additive effect whereby CC decreases pituitary sensitivity to estrogen negative feedback while letrozole directly decreases serum estradiol, further promoting FSH secretion. We speculate that these alternate actions increase rates of ovulation and pregnancy in women with PCOS.

Preliminary Data

Addition of CC to letrozole improves ovulation rate: We investigated the impact of combining CC with letrozole as compared to letrozole monotherapy in 70 women with PCOS. We found that the combination of letrozole 2.5 mg and CC 50 mg **nearly doubled the ovulation rate** as compared to use of letrozole monotherapy (77% vs. 43%, P=0.007; rate ratio for ovulation (95% CI) 1.80 (1.18 to 2.75) (17). **These results were achieved in a single treatment cycle, without any multiple gestations in our study cohort**. In concordance with our findings, a prospective observational trial of combination letrozole and CC in patients who had previously failed CC and letrozole monotherapies found evidence of improved ovulation, with development of a dominant follicle in 82.9% of cycles (18). While it is possible that the difference between the two arms in our preliminary study could be explained by a dosing effect, the ovulation rate in our combination treatment group was higher than the 61.7% cumulative ovulation rate across five cycles of letrozole monotherapy with increasing doses up to 7.5 mg (4). Our finding of a similar ovulatory rate within the letrozole monotherapy arm to that previously reported suggests an additive effect of the combination of these medications.



Addition of CC to letrozole does not negatively impact ultrasound parameters: Data from our pilot study suggest among the subgroup who ovulated, endometrial thickness and number of follicles were similar across treatment arms (Table 1). This is reassuring in that CC is not having a negative impact on the endometrium and multi follicular development appears limited.

Table 1. Ultrasound characteristics among patients who ovulated

Cycle Characteristics	**Letrozole Group** n=14	**Letrozole + CC Group** n=25	*p* value
# of follicles > 15 mm	0.5 (0-1)	1 (0-1.5)	0.081
Largest follicle size (mm)	15.5 (10.0-18.3)	17.0 (13.5-19.5)	0.228
Endometrial lining (mm)	7.4 ± 2.3	8.6 ± 3.9	0.286

*Data are presented as mean ± SD, or median (interquartile range)

Both a commentary (19), and Fertility and Sterility Live Global Journal Club praised our pilot study, but **emphasized the need for additional studies to evaluate escalating dosages, multiple cycles, live birth, and multiple gestation with this novel combination treatment**. Given our promising initial data, the goal is to expand on our pilot and conduct a trial with escalating doses of letrozole across three cycles of either letrozole monotherapy or combination therapy in 184 women. This will provide not only additional data on ovulation rates but provide essential information on side effects, multiple gestation rate and live birth rate which will better situate us completing a multicenter study evaluating live birth rate.

Significance and Innovation

An improvement in the proportion of women with anovulatory infertility who conceive with oral ovulation induction agents is a public health imperative, as this increase can significantly reduce both the costs and complications of fertility treatments. We propose to study a **novel method** of oral ovulation induction with the combination of letrozole and CC. The mechanisms of ovulation induction differ when comparing letrozole (peripheral action at the ovary) and CC (central action in the hypothalamic-pituitary axis); therefore, it is reasonable to hypothesize that the two drugs combined have an additive effect. We have conducted the only randomized trial evaluating ovulation rates for this combination therapy compared to letrozole monotherapy. Our preliminary data demonstrate a nearly two-fold increase in ovulation rate with the combination of letrozole and CC, without evidence of an increase in the rate of multiple gestation (17). However, data comparing ovulation rates across multiple cycles with escalating Letrozole dosage is **critically needed** to plan a multi-center clinical trial to evaluate live birth rate.

2.2 Potential Risks and Benefits

2.2.1 Potential Risks

Both letrozole and CC are used for the treatment of infertility. Below is a table listing all tests or procedures involved in this research study and their related discomforts and risks.

Test or Procedure	Discomfort and Risks
Standard venipuncture for blood work	Slight pinch or discomfort, bruising at the site of puncture, small blood clot, infection or bleeding at the site, and fainting during the procedure
Transvaginal ultrasound	Abdominal or pelvic discomfort
Clomiphene Citrate	Hot flashes, visual changes (blurring of vision, double vision, floaters), abdominal pain, nausea, vomiting, constipation, mood changes, headache, fatigue, multiple pregnancies, formation of ovarian cyst, breast discomfort, abnormal uterine bleeding, bloating, formation of ovarian cysts
Letrozole	Fatigue, dizziness, nausea, hot flashes, discomfort in your joints, back pain, increased cholesterol levels, formation of ovarian cysts, multiple pregnancies

** Table is modified from PPCOS II trial*

It is expected that patients will not experience all of these side effects. Side effects associated with medications are temporary and manageable.

Although the goal of treatment is to achieve pregnancy, there is no guarantee that treatment will result in pregnancy or a live birth. CC has been associated with a 5-8% multiple pregnancy rate (7.4% twin pregnancy rate in PPCOS II) and Letrozole is associated with a 3.4% twin pregnancy rate in women with PCOS (PPCOS II data). The multiple pregnancy rate of the combination of these treatments is unknown. Multiple gestations are associated with preterm labor and delivery as well as most pregnancy complications including diabetes and high blood pressure.

If pregnancy occurs it is possible that the pregnancy results in a non-viable pregnancy or tubal (ectopic) pregnancy. In either of these cases occur, further medial or surgical treatment may be necessary.

The incidence of congenital anomalies in the general population is 3% to 5%. In the PPCOS II trial comparing letrozole and CC there were a total of 5 congenital anomalies (1/66 in CC group – 1.5%, 4/102 in letrozole group 3.9%) (4).

2.2.2 Potential Benefits

Possible benefits to participants: The potential benefit to the participants is that they will receive a treatment (letrozole) that is known to improve ovulatory and pregnancy rates in women with infertility secondary to anovulation compared to no treatment at all or CC alone. The participant may receive a treatment (letrozole + CC) that may prove to be more effective than letrozole alone. Treatments may result in pregnancy although this cannot be guaranteed.

With ultrasound and hormone testing the participant may receive additional information regarding ovulation status.

Possible benefits to others: The study will provide important information on the treatment for women with infertility secondary to anovulation. This will provide knowledge on effective ovulation treatments for this patient population. The knowledge gained from this research may help discover an effective and safe way to achieve ovulation and pregnancy.

3 OBJECTIVES

3.1 Primary Research Hypothesis

The primary research hypothesis is that ovulation induction with the combination of letrozole and clomiphene citrate is more likely to result in ovulation (increased ovulation rate) than ovulation induction with letrozole alone in infertile women with anovulation (PCOS and/or normogonadtropic normoestrogenic anovulation or oligo-ovulation).

3.1.1 Primary Outcome Measure

The primary outcome measure is the occurrence of ovulation during the study period. Ovulation is defined as a mid-luteal progesterone level $\geq$ 3 ng/mL. This will be tested 6-8 days following the patient reporting of an LH surge. If no LH surge is detected the lab will be drawn on cycle day 21-24. We will also analyze the ovulation rate of the follow up progesterone level.

We will utilize an intent-to-treat approach for the primary analysis of ovulation rate (utilizing the first progesterone level of each cycle) within the two treatment arms. If it is determined that timing of the initial progesterone level is inaccurate based on LH testing or patient symptoms, the progesterone test that is most clinically in line with accurate timing (6-8 days post LH surge) will be used. Ovulation rate will also be reported in those with a delayed response to treatment (needed repeat progesterone level due to anovulatory mid-luteal P4 level and follicle $\geq$ 14 mm). Patients will be analyzed according to the treatment group to which they are assigned, even if they did not receive the intended treatment or received only a portion of it. We will also perform a per protocol analysis to include only those patients who initiate treatment. Thus, those that are randomized but do not take the treatment due ineligibility will be excluded from the per protocol analysis. This per protocol analysis will provide data that is helpful to clinicians as it will include only those who initiated treatment.

3.2 Secondary Research Hypothesis and outcome measures

We will analyze secondary outcomes however due to sample size will not be able to power differences in pregnancy rate and live birth rate. Descriptive secondary outcomes will include positive pregnancy test, clinical pregnancy, and ongoing pregnancy. We will also evaluate number of corpora lutea determined by ultrasound, endometrial thickness and pattern by ultrasound. We will also monitor adverse events during treatment and side effect profile of both treatment arms.

We will evaluate pregnancy outcomes and report any fetal anomalies.

1. The combination treatment will result in higher live birth rate

    a. Outcome measurement: live birth rate

2. The shortest time to pregnancy will be with the combination treatment.

    a. Outcome measurement: time to pregnancy

3. Treatment with the combination will have a similar singleton pregnancy rate compared to treatment with letrozole.

    a. Outcome measurement: Singleton pregnancy and singleton pregnancy rate; singleton pregnancy is defined as presence of single intrauterine gestational sac with a single fetal pole and observable heart motion. Multiple pregnancy rate will also be compared.

4. Treatment with the combination will have a similar pregnancy loss rate compared to treatment with letrozole.

    a. Outcome measurements: Pregnancy loss (defined as spontaneous abortion occurring before 13 weeks gestation), biochemical (positive hCG test with spontaneously dropping hCG levels), ectopic pregnancy or pregnancy of unknown location

5. Side effect profile will be similar between both treatment groups.

    a. Outcome measurements: # of days of reported side effects and acceptability of side effects.

6. Endometrial thickness and corpora lutea number will be similar between the two groups.

    a. Outcome measurement: Endometrial thickness and corpora luteal number on CD21-23 transvaginal ultrasound or approximately 6-8 days following detection of LH surge. These outcomes will also be evaluated based on dosing.

4 STUDY DESIGN

4.1 Treatment Design

This will be a multicenter, open trial of oral letrozole versus combination of oral letrozole and clomiphene citrate for the treatment of infertility in patients with oligo-ovulation or anovulation. Patients will be randomized to one of two treatment arms, to receive either an initial dose of letrozole 2.5 mg alone or clomiphene citrate 50 mg and letrozole 2.5 mg for 5 days during their menstrual cycle. Letrozole dosing will be escalated in a stair-step fashion based on ovulation results. Max dose of letrozole will not exceed 7.5 mg.

Participation in active treatment will last for up to 3 treatment cycles, approximately up to 15 weeks. Participants will be monitored at monthly intervals during expected mid-luteal phase to evaluate response to medication by evaluating ultrasound and progesterone parameters.

The dose will be adjusted according to response at mid-luteal visit.

- Anovulatory progesterone level < 3 and no evidence of follicular development and corpora lutea→ increase letrozole dose on that day. This would be considered start of new treatment cycle (CD3).
- Anovulatory progesterone level < 3 with evidence of follicular development (follicle ≥14 mm) → repeat progesterone level based on LH testing (6-8 days following positive) or at 2 weeks if no positive → increase dose at menses visit (2^{nd} progesterone is ovulatory) or increase dose at time of second progesterone if level < 3.
- Progesterone level ≥3 → maintain same dose for next treatment cycle

Anytime ovulation induction medication is started, pregnancy test to be performed prior to initiation of new treatment cycle.

Randomization will be stratified by site, age (< 35 versus ≥ 35) and BMI (< 30 and ≥ 30) to ensure similar comparison groups in these characteristics that highly impact outcomes. Randomization will be via a computer based central randomization to ensure adequate allocation concealment.

Enrollment will take approximately 20 months if ~ 10 subjects are enrolled per month. Active subject participation will be through completion of 3 cycles. We will follow up with patient regarding pregnancy and delivery outcomes.

As previously mentioned, this is an open trial. Patient will be aware of the treatment intervention they are receiving. Currently, letrozole is considered the current standard of care/first line agent for WHO class II anovulation (women with PCOS). Both groups will be receiving at minimum the current standard of care treatment.

4.1.1 Rationale for dosing approach

The dosing approach has been designed to escalate the letrozole dosing in subsequent cycle if participants do not ovulate. Since aromatase inhibitors are the first line agent for women with PCOS we elected to first study the increase in this compared to the combination of letrozole and CC. Consideration was given to varying doses of CC as well, however, results would be challenging to interpret with two varying doses and would not be clear if it was the increase of one medication or another that impacted ovulatory rate. By keeping CC dose constant, we can assess the standard treatment of letrozole compared to the standard treatment with CC addition at constant dose.

Stair step approach was also selected since we will be assessing progesterone and follicular status on ultrasound during luteal phase. Stair step approach will keep patient treatment progressing rather than waiting for menses or inducing menses with progestin which, both which delay the next treatment cycle.

4.2 Study Summary

Pre-screen: If patient is interested in hearing about the study, initial pre-screen will be performed to confirm basic eligibility criteria. If general eligibility criteria is met, screening visit will be scheduled or initiated. This may be part of the Screening visit/period.

Screening visit/period:

1. Study staff review pre-screening questionnaire
2. Review and obtain informed consent
3. Confirm eligibility criteria – Inclusion/Exclusion criteria (RedCap Form)
4. Confirm documentation/abstract from medical record (RedCap form provided):
    a. Demographics, medical history
    b. Physical exam: vital signs, height, weight and BMI, hirsutism assessment
    c. Transvaginal ultrasound
    d. Assessment of other infertility factors: tubal and uterine factor, male factor, severe endometriosis
    e. Inclusion laboratory testing
5. Pre-conception counseling: weight loss (BMI >30), tobacco cessation, folic acid use, rubella/varicella documentation, genetic carrier screening options
6. If progestin withdrawal bleed necessary, progestin prescription provided and will be instructed when to take it.
    a. Progestin withdrawal bleed left to discretion of the provider
    b. Progestin withdrawal induced if no menses greater than 3 months

Baseline visit/Randomization visit:

Baseline visit will be scheduled during cycle day 1-3 (can go up to cycle day 5) following spontaneous menses or following progestin withdraw bleed. Visit can also be a random start if anovulatory progesterone and no follicular development on ultrasound.

1. Urine pregnancy test
2. Randomization once negative pregnancy test (no follicular development and anovulatory progesterone in cases for random start)
3. Dispense study medication
4. Review medication directions (handout provided) and journal log instructions (encourage online, paper is an option)
5. Completion of baseline questionnaires (GAD-7, PHQ9, Ferti-QoL, and Perceived Stress Scale) or instructions provided on how to complete online
6. Confirm completion and obtain information if needed for RedCap Baseline Abstraction form

Monthly Midluteal visit (18 to 26 days following initiation of medication), up to 3 mid-luteal visits. Timing will be based on LH testing, ideally around 7-8 days post-positive test and if no positive test, CD 20-24.

1. Transvaginal ultrasound
2. Progesterone lab draw
3. Dispense home urine pregnancy test
4. Collect and review journal logs (side effects, menstrual logs, intercourse log, concomitant medications, completion or missing entry)
5. Count medication and confirm contact method for informing participant of plan based on lab and pregnancy test at end of cycle.
6. Medication dispensed for next cycle or patient may return on CD1-3 (preferable if able).

Instructions based on progesterone level and ultrasound results:

1. Progesterone level $\geq$ 3 ng/mL → instruct participant to await menses and perform pregnancy test with menses or if no menses, perform pregnancy test 2 weeks after midluteal visit.
2. Anovulatory progesterone level < 3 ng/mL and with evidence of follicular development (mean follicle size $\geq$ 14 mm → repeat progesterone level in 10-14 days.
    a. If repeat (2^{nd} P4) is ovulatory progesterone level $\geq$ 3 ng/mL → increase treatment dose next cycle with menses after negative pregnancy test
    b. If repeat (2^{nd} P4) is anovulatory progesterone level < 3 ng/mL → increase treatment dose and initiate next cycle after negative pregnancy test.
3. Anovulatory progesterone level < 3 ng/mL and no evidence of follicular development or corpora lutea → increase ovulation medication on that that day after negative pregnancy test. This is considered the start of a new cycle. Next mid-luteal clinic visit would be scheduled for 7-8 days following positive LH test or 18 to 26 days following initiation of medication.

Menses check in appointment (via RedCap)

- This check in is necessary in order to initiate next cycle. At onset of menses, each participant will take a home pregnancy test and complete the RedCap form indicating "Negative Pregnancy test". If no menses within 2 weeks after the midluteal visit, participants will take a pregnancy test.

  - If positive urine pregnancy test, lab visit scheduled for serum hCG level and repeat in 48-72 hours.
  - If negative urine pregnancy test, start medication on Cycle day 3 of menses
- Submit journal logs via email or in person

Pregnancy lab Visits (part of standard of care)

- If positive urine pregnancy test, lab visit scheduled for serum quantitative hCG level and repeat in 48-72 hours.
- Appropriate rise:
  - 2 day rise – multiply level x 1.7
  - 3 day rise – multiple level x 2.3
  - 4 day rise – multiply level x 2.89
- If appropriate rise, schedule Transvaginal ultrasound at 6 to 7 weeks and visit with provider.
- If inappropriate rise, schedule early ultrasound (no earlier than 5 4/7 weeks) to confirm IUP and then follow up ultrasound based on initial ultrasound results. Timing of scan can be based on local protocol.
  - At UW, a 5 week ultrasound will be scheduled to confirm IUP and then follow up ultrasound based on initial ultrasound results

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Study Population

184 women with WHO class II anovulation/PCOS actively seeking pregnancy (or 92 per each treatment arm) aged ≥ 18 to ≤ 40 years will be randomized. The overall goal of enrollment criteria is to identify a population of healthy women with anovulatory or oligo-ovulation (WHO Class II anovulation) and infertility not specified by another cause. Infertility will be determined by clinical history. PCOS will be determined by including two of three findings: oligo- or anovulation, hyperandrogenism or polycystic ovaries on ultrasound. Definitions are from the 2018 Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary management(3).

- **Oligo-ovulation**: cycles less than 21 days or greater than 35 days or less than 8 cycles per year
    - **Ovulatory dysfunction/Anovulation:** women with regular cycles with anovulatory progesterone or with amenorrhea
- **Polycystic ovarian morphology (PCOM) on ultrasonography**: follicle number (measuring 2-9 mm) per ovary of ≥ 20 and/or ovarian volume >10 ml on either ovary, ensuring no corpora lutea, cysts or dominant follicles are present if using volume only.
- **Hyperandrogenism**: determined by evidence of clinical or biochemical hyperandrogenism
    - **Clinical**: symptoms and signs of hirsutism, acne or alopecia
    - **Biochemical**: elevations in calculated free testosterone or free androgen index (Total Testosterone level divided by SHBG level and multiply by 100). Levels should not have been assessed while on combined hormonal contraceptive
- **If PCOS criteria is not met, normogonadotropic anovulation should be met with confirmation of anovulation/olig-ovulation and normal FSH and estradiol levels.**
- **Infertility:** Inability of couple to achieve successful pregnancy after 12 months of regular timed unprotected intercourse in women less than 35 years of age; and after 6 months of regular intercourse without use of contraception in women 35 years and older(20). If in the clinical case of anovulation/oligo-ovulation, 12 month period of time “infertility” is not necessary.

5.2 Subject Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Diagnosis of infertility (described above)
2. Normogonadotropic normoestrogenic (WHO Group II) anovulation or oligo-ovulation:
    a. Diagnosis of PCOS as described above
    OR
    b. Self-reported menstrual cycle length < 21 or > 35 days or < 8 cycles per year. For women with variable cycle length not consistently <21 days or >35 days, a serum progesterone between day 21 and 35, <3 ng/ml will be considered evidence of oligo-ovulation AND normal estrogen level and FSH level
3. Age 18-40
4. BMI < 50
5. Normal semen analysis in past two years according to World Health Organization cut off points (concentration and motility) or a total motile sperm count greater than 10 million. Morphology results will not affect eligibility. If male partner has fathered a child, SFA is not necessary.
6. Desire to achieve a pregnancy
7. Willing to comply with study procedures for the duration of the study. Ability to have intercourse at least twice weekly and follow study protocol

5.3 Subject Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Current pregnancy
2. Current use of oral contraceptives, injectable contraceptives, implanted hormonal contraceptives, or oral, vaginal or transdermal estrogens or progestins. 1-month washout required, 3 months for injectable/implanted progestin contraceptives.
3. Patients with other known cause of infertility: endometriosis, tubal factor, diminished ovarian reserve (defined as AMH < 1 or AFC < 10), uterine abnormalities.
    a. Patients with unilateral tubal patency can be enrolled, provided at least one tube is open.
    b. Patients with mild endometriosis can be enrolled
4. Uncontrolled thyroid disease (normal TSH in past 3 years is adequate).

   a. Patients with uncorrected thyroid disease defined based on their local lab or at UIHC (0.27 to 4.20 µIU/mL). Once corrected and within this range, they may be enrolled if the other inclusion and exclusion criteria are met. A normal lab within the last 3 years is adequate for entry
5. Hyperprolactinemia (prolactin > 30 ng/dl x 2). A normal prolactin within the past year at local lab or at UIHC (4.8 to 23.3 ng/mL) is adequate for entry if history of hyperprolactinemia.
6. Medical conditions in which we recommend avoiding pregnancy until under improved control
   a. Patients with poorly controlled Type 1 or Type 2 diabetes (defined as a hemoglobin A1c > 6.5-7.0%)
   b. Uncontrolled hypertension (medicated or unmedicated, BP >160/100 x 2)
   c. Bariatric surgery within the past year
   d. Untreated endometrial hyperplasia
   e. Current alcohol use > 14 drinks per week
7. Patients with contraindications to clomiphene citrate: hypersensitivity to CC or any of its components, history of liver disease or known liver disease (LFT's are not necessary prior CC use and enrollment into the study), unknown cause of abnormal uterine bleeding, intracranial lesion.
8. Patients with contraindications to letrozole: hypersensitivity to letrozole or any of its components.
9. Patients taking medications known to affect reproductive function or metabolism or that are an absolute contraindication during pregnancy.
   a. Patients taking Metformin can be enrolled, provided they have documented impaired glucose tolerance or insulin resistance. Patients who are not taking Metformin for these indications will be excluded.
10. If patients are suspected based on clinical findings for other etiologies that mimic PCOS, work up must be completed to exclude other etiologies prior to enrollment (i.e. Cushing's syndrome, androgen-secreting tumor).
11. Prior diagnosis of congenital adrenal hyperplasia or Cushing's Disease or Syndrome

5.4 Strategies for Recruitment and Retention

Strategies for Recruitment

Potential study participants will be recruited from couples seeking fertility evaluation and/or ovulation induction at participating fertility clinics and other referring practices providing reproductive care. Study team members will review clinic schedules for their

center and will be approached regarding the study by their clinician, a study coordinator, or another member of the research team while at the clinic for their initial consult or clinical visit. Couples will be given information about the study, offered pre-screening to assess eligibility, and given the opportunity to schedule an enrollment visit.

Study information materials and contact information will be provided to outside and community clinics that commonly refer patients to participating study centers. A study coordinator or another member of the research team will review study information and assess eligibility with couples referred to the study from external clinics, clinicaltrials.gov, word of mouth, or other external sources.

> Recruitment at UW- Recruitment will occur in the clinical setting through posters, brochures, digital posters displayed at UW Health Clinics, and referral in both the Department of Obstetrics and Gynecology and the UW Health Generations Fertility Clinic through conferencing software. If a patient hears about the study from a referring provider or someone not on the research team, the referring provider will direct the patient to contact the research team if they are interested in participating or hearing more about the study. The referring provider will provide recruitment materials so that the patient can contact the team directly.

Recruitment strategies for individual sites have been approved by local IRBs.

Strategies for Retention

Study coverage of ovulation induction medication and ultrasounds are expected to be sufficient incentive for participation in the study. Additional measures to retain subjects for up to three cycles of ovulation induction will involve establishment of positive rapport between the study team and participants, appointment and scheduling reminders, and regular communication regarding ultrasound and lab results.

Participants will be given the opportunity to schedule future study visits during the preceding study visit or any time prior to the study visit. Participants will receive an appointment reminder within the week prior to their scheduled appointment. Study staff will utilize a system of reminder emails and phone calls to contact participants who need to schedule their next study visit of if a study visit is missed.

Retention strategies for pregnancy outcome data will involve obtaining consent to release information forms at participant enrollment visits, and study team confirmation of the participant's planned obstetric care provider when pregnancy is detected. In the event that an updated consent to release information form is not on file post-delivery,

study staff will utilize a system of reminder emails, phone calls, and mailed correspondence to obtain an up-to-date form. This system will involve an initial email and two mailed letters with request for completion of forms, with each email request and mailed letter request followed by two phone calls in the case of participant non-response.

Consent to obtain information forms for infants born during study follow up will be obtained following delivery.

> At the University of Iowa- Study staff will utilize a follow up system of reminder emails, phone calls, and mailed correspondence to obtain parental consent to release information for infants. This system will involve an initial email and two mailed letters with request for completion of forms, with each email request and mailed letter request followed by two phone calls in the case of participant non-response.
>
> At the University of Wisconsin- At the time of consent, the subject will sign a consent form that includes consent to obtain information from the newborn's medical record. A release of information form to obtain information for neonates born during study follow up will be obtained when the subject becomes pregnant. Study staff will have these forms explained and completed at a standard clinical visit or virtually

5.5 Treatment Assignment Procedures

Participants will be assigned to either combination letrozole and clomid or letrozole monotherapy in a 1:1 allocation ratio. Randomization will be blocked (with random sequences of block sizes 2, 4 and 6) and stratified by age (< 35 versus ≥ 35), body mass index (<30 and ≥30), and site to ensure that balance between the treatment groups within age, BMI, and site is maintained over the enrollment period.

5.5.1 Randomization Procedures

Participants will be randomized using randomization tables generated by a statistician and uploaded to the project in RedCap. The randomization scheme will not be accessible to study staff in order to maintain allocation concealment. Study staff will randomize subjects by entering BMI category (<30 or 30+), age category (<35 or 35+) and site on the RedCap Randomization page. Once these fields are entered and confirmed to be accurate, staff will click the "Randomize" button under treatment group to receive the participants assigned treatment."

5.6 Subject Withdrawal

Subjects may withdraw voluntarily from the study or the investigator may terminate a subject's participation.

5.6.1 Reasons for Withdrawal

Subjects are free to withdraw from participation in the study at any time upon request.

An investigator may terminate a study subject's participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- The study is terminated

6 STUDY INTERVENTION

6.1 Study Product Description

Study medications, letrozole and clomiphene citrate will be purchased through the University of Iowa Research pharmacy. Letrozole comes in 2.5 mg tablets and CC comes in 50 mg tablets.

6.1.1 Formulation, Packaging, and Labeling

Letrozole will be packaged based on dosage indicated per the study protocol. Letrozole 2.5 mg (5 total tablets) will be supplied for the first cycle for all participants. Letrozole dose will be increased if patient did not ovulate on the dose prescribed or had significantly delayed ovulation based on the protocol. If prescribed Letrozole 5.0 mg, 10 tablets will be prescribed and if prescribed Letrozole 7.5 mg, 15 tablets will be prescribed. The total dosage per cycle needed will be provided in one vial with a medication label.

If the patient is in the combined group, they will receive letrozole mediation as above and also receive CC 50 mg (5 tablets). This will be packaged in its own vial with a medication label.

All medications will be dispensed in containers which meets the requirements of the Poison Prevention Packaging Act of 1970, 15 U.S.C. ss. 1471-1476 (2001), unless otherwise requested by the subject, and of Section 502G of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. ss. 301 et seq. (2001).

Preprinted adhesive medication labels will be affixed to the container(s) in which the drug is dispensed. Labels will include: name, address, and phone of prescribing physician; name of patient; date dispensed; directions for administering the prescription medication; expiration date, and name and strength of the prescription drug in the container. Labels will be edited by each site to comply with local regulations. Example labels where patient name, dispense date and prescribing physician and location can be filled when dispensing are as follows:

Clomiphene 50mg:

Name: Date:
Take 1 tablet daily on cycle days 3 through 7. Clomiphene Citrate tablets 50 mg Total tabs #5
CLC II Study Dr:
UIHC-1360 N Dodge Iowa City, IA, 52242 319-384-5413

Letrozole 2.5mg:

Name: Date:

Take 1 tablet daily on cycle days 3 through 7.
Letrozole tablets 2.5 mg Total tabs #5

CLC II Study Dr:

UIHC-1360 N Dodge Iowa City, IA, 52242 319-384-5413

Letrozole 5.0mg:

Name: Date:

Take 2 tablets daily on cycle days 3 through 7.
Letrozole tablets 2.5 mg Total tabs #10

CLC II Study Dr:

UIHC-1360 N Dodge Iowa City, IA, 52242 319-384-5413

Letrozole 7.5mg:

Name: Date:

Take 3 tablets daily on cycle days 3 through 7.
Letrozole tablets 2.5 mg Total tabs #15

CLC II Study Dr:

UIHC-1360 N Dodge Iowa City, IA, 52242 319-384-5413

6.1.2 Product Storage and Stability

Study medications will be stored at each site in a locked room in a locked cabinet and be inaccessible to unauthorized personnel. Study medication will be stored at room temperature, with temperature tracked by a min/max thermometer with temperatures recorded weekly in a temperature log.

Letrozole should be stored at 20˚ to 25˚C (68˚ to 77˚ F) and Clomid at 15˚ to 30˚C (59˚ to 86˚ F).

Expiration date from the original vial will be included on the medication label. Prior to dispensing, dispenser will ensure expiration time has not elapsed prior to dispensing.

At University of Wisconsin storage of the drugs will be according to the UW Health Administrative (non-clinical) Investigational and Study Drug Control policy.

6.2 Administration of Study Product

University of Iowa Study Team will deliver medication to satellite recruiting clinics.

The medication will be dispensed to the patient by one of Study Team members. Verification of correct tablet number, correct packaging and expiration date will be performed prior to dispensing.

6.3 Accountability Procedures for the Study Product

All received and dispensed medications will be documented on medication logs maintained by each study site. Record logs must comply with applicable regulations and guidelines and should include:

- Drug name and strength, Manufacturer, and Lot Number
- Date and Quantity Received
- Date and Quantity Dispensed, Subject ID, Name of prescriber, and Dispenser's initials
- Date and Quantity Returned by Subject
- Date and Quantity Stock Destroyed
- Dates and Findings of Inventory Checks

6.4 Assessment of Participant Compliance with Study Product Administration

Participant compliance will be assessed with questionnaires and pill counts. Participants will be asked to report the number of pills taken per day on their journal log. Participants will also be asked to bring their pill bottles with any remaining medication to their mid-luteal and end-of-treatment visits. Study staff will perform pill counts at each study visit. Study staff will perform pill counts outside of the room where the participant is located when possible.

7 STUDY SCHEDULE

A diagram of the Schedule of Events is provided in Appendix A.

7.1 Screening

Screening Visit

- Obtain and document consent from potential subject on screening consent form.
- Review medical history to determine eligibility based on inclusion/exclusion criteria.
- Review medications history to determine eligibility based on inclusion/exclusion criteria.
- Review when to schedule study enrollment visits for individuals who are eligible and available for the duration of the study.
    - Cycle day 1-3 or
    - At random once pregnancy and recent/imminent ovulation and follicular development are excluded (no follicular development on ultrasound and anovulatory progesterone) (within a week of these studies)
- At University of Wisconsin, A consenting visit will be scheduled on a day when the potential subject has availability, after she has reviewed the consent form. This visit will be done by video conferencing so the coordinator can witness the subject signing the consent form.
    - During the remote visit, if eligibility has been confirmed, the coordinator may complete informed consent. The identity of the subject will be verified during the e-visit that will be set up for remote consenting. During this visit, conducted via video conference, the coordinator will ask the patient to say her name and DOB to identify herself. Confirmation of the subject's identity can be recorded in a memo in the patient's study record. The subject will electronically sign the consent form through DocuSign. Email permission will be obtained before sending the electronic consent form to the potential participant as there is an email permission comment in the phone script,
    - The date and time of the consent, along with the people who are present and the method of consent, will be documented in a memo for each subject. Upon receipt of the signed consent form, the coordinator will complete the consent by signing and dating both forms. At this point, the subject is enrolled. The coordinator will contact the clinical staff about the enrolled subject.

7.2 Enrollment/Baseline

The enrollment/baseline visit will be scheduled when participants are on Cycle day 1-3 of their cycle or when ready to begin taking medication after pregnancy and recent/imminent ovulation have been excluded. All participants must have a negative urine pregnancy test prior to randomization.

Enrollment/Baseline Visit (Cycle Day 1-3 or at random as noted above)

- Obtain and document consent from subject on study consent form.
- Verify inclusion/exclusion criteria.
- Obtain a urine pregnancy test and confirm negative result.
    - Participation will be concluded at this point for participants with a positive urine pregnancy test; however, if a miscarriage results, participants can continue participation
- Review medical history and record in participants record in RedCap
- Assign participant ID
    - Participants IDs are assigned sequentially by site, with a 5 digit ID comprised of two components:
        - The first 2 digits of the ID are assigned based on the participant's primary study site:
            - Iowa City: 01
            - Quad Cities: 02
            - University of Wisconsin: 03
            - Des Moines: 04
        - The final 3 digits of the ID are assigned based on where the participant falls sequentially in enrollment at that site.
            - Ex. The first participant at Iowa city would be 01001, the second at this site 01002, the third 01003 etc.
            - The next sequential number for the site can be identified by viewing the "Participants enrolled" report in RedCap and filtering on the participant's primary site. The assigned number should follow the final number on the report for that sites sequence.
- Randomize participant in RedCAP to determine treatment allocation

- Dispense study medication for initial cycle
- Review instructions on how to complete the calendar log and how to take study medication
    - Participants should complete the calendar log to document sexual activity, side effects, and LH surge beginning the first day they take the study medication. Calendar logs are available on RedCAP and weekly links for the calendar log will be sent to the participant's email after randomization. Subjects who do not wish to complete the calendar log electronically will have the option of completing on paper and returning to study staff at their next visit.
    - Participants should take the study medication on cycle day 3 through 7 for a total of 5 days. If participants are unable to begin the medication on cycle day 3, they may begin on cycle day 4 or 5, but should begin medication no later than cycle day 5. Participants who meet criteria for random start will take the medication for 5 days at random start, with the first day of medication considered cycle day 3.
- Have participant complete baseline questionnaires (GAD7, PHQ9, FertiQoL, and Perceived Stress Scale, or if participant prefers to complete online, provide instructions on completing forms after visit
- Obtain a consent to obtain medical information from the participant if medical records will need to be obtained from an external provider for either fertility treatment chart abstraction and/or for potential future pregnancy chart abstraction

7.3 Intermediate Visits

Participants will return for a midluteal study visit on cycle day 21-24 or 7-9 days following positive ovulation prediction test to determine if ovulation occurred. This visit will include an ultrasound and a progesterone lab.

If there is no ovulation, the daily dose of letrozole will be increased by 2.5 mg, to begin immediately. During the first cycle after letrozole dose increase, participants would receive 5mg daily for 5 days. If participants receive a second letrozole dose increase, they would receive 7.5 mg Letrozole daily for 5 days. The maximum dose for Letrozole in this study is 7.5 mg. CC 50 mg will be given on the same days as the letrozole in all cycles in the combination arm. Midluteal visits will be performed in all cycles. Each participant will complete a maximum of 3 cycles of medical therapy. On average we expect this to be about 4 months time, however, delayed ovulation, a biochemical pregnancy, or 1st trimester pregnancy loss in the first or second treatment cycle could delay the start of subsequent cycles, and therefore would extend active participation.

Cycle 1 Midluteal Visit

Timing will be based on LH testing, ideally around 7-8 days post-positive test or CD21-24 if no positive test

- Transvaginal ultrasound to assess for corpora lutea and endometrial thickness and pattern
- Serum progesterone assessment for ovulation
- Review subject calendar log and record adverse events as reported by subject or observed by investigator.
- Determine dosage for next cycle based on ultrasound and progesterone results and record in RedCap
    - If there is no ovulation, the daily dose of letrozole will be increased by 2.5 mg (5mg). The combination arm will continue to receive CC 50mg along with the increased letrozole dose.
    - If there is ovulation, treatment will be repeated in the next cycle with the same dose of letrozole. The combination arm will continue to receive CC 50mg along with letrozole 2.5mg.
- Schedule a follow up Progesterone lab if necessary
    - Participants with anovulatory progesterone levels (<3 ng/ml) and evidence of follicular development will return for a repeat progesterone level assessment in 10-14 days.
    - Participants with elevated anovulatory progesterone levels (1.5 < P4 < 3.0) and corpus luteum on ultrasound will return for a repeat progesterone level assessment in 2-3 days
- Dispense pregnancy test and medication for next cycle and review instructions for medication (dispense medication if unable to return to pick up meds, if can easily return, have participant return for med pick up)
    - Participants without ovulation nor evidence of follicular development are to complete pregnancy test and begin medication at new dose immediately following negative pregnancy test
    - Participants with ovulation are to wait until onset of menses, or two weeks after midluteal visit to complete pregnancy test. They can begin medication at same dose following negative pregnancy test. Participants with a positive pregnancy test will be moved to pregnancy track and will return dispensed medication at their next study visit.

- Collect any unused medication from current cycle and document participant compliance in RedCap
- Record results of ultrasound and serum progesterone in RedCap

Cycle 1 Follow-up Midluteal Visit*

Participants with anovulatory progesterone levels (<3 ng/ml) and evidence of follicular development at their midluteal visit will return for a repeat progesterone level assessment in 10-14 days. This can be performed at a local lab if the participant prefers.

- Serum progesterone assessment for ovulation
- Review subject calendar log and record adverse events as reported by subject or observed by investigator.
- Review instructions for previously dispensed medication/dispense medication if not yet dispensed
    - Participants without ovulation are to complete pregnancy test and begin medication at new dose following either a spontaneous menses or withdraw bleed and a negative pregnancy test. They can begin taking medication to induce a withdraw bleed on cycle day 35, following a negative pregnancy test.
    - Participants with ovulation are to wait until onset of menses, or two weeks after midluteal visit to complete pregnancy test. They can begin medication at new dose following negative pregnancy test (if ovulation did not occur on or before cycle day 21). Participants with a positive pregnancy test will be moved to pregnancy track and will return dispensed medication at their next study visit.
        - Participants who were initially anovulatory at a study visit prior to cycle day 21, who are ovulatory at a follow up visit by cycle day 21 will be maintained on the same dose of medication. This may occur if participant's study visit was moved up due to an early false positive ovulation prediction test.
- Record results of serum progesterone in RedCap

Cycle 1 Menses Check-In/Cycle 2 Start (menses from Cycle 1)*

Two weeks post Midleuteal visit if no menses before then

Participants with ovulation are to wait until onset of menses, or two weeks after midluteal visit to complete pregnancy test. They can begin medication at new dose following negative pregnancy test. This visit can be completed remotely with participant logging information in RedCap. If they don't have medication, they will come in to pick it up. Participants with a positive pregnancy test will be moved to pregnancy track and will return dispensed medication at their next study visit.

Cycle 2 Midluteal Visit

Timing will be based on LH testing, ideally around 7-8 days post-positive test or CD21-24 if no positive test.

- Transvaginal ultrasound to assess for corpora lutea and endometrial thickness and pattern
- Serum progesterone assessment for ovulation
- Review subject calendar log and record adverse events as reported by subject or observed by investigator.
- Determine dosage for next cycle based on ultrasound and progesterone results and record in RedCap
    - If there is <u>no ovulation</u>, the daily dose of letrozole will be increased by 2.5 mg.
        - Participants on Letrozole 2.5 this cycle will be increased to Letrozole 5.0 in cycle 3.
        - Participants on Letrozole 5.0 this cycle will be increased to Letrozole 7.5 in cycle 3.
        - The combination arm will continue to receive CC 50mg along with the increased letrozole dose.
    - If there is ovulation, treatment will be repeated in the next cycle with the same dose of letrozole. The combination arm will continue to receive CC 50mg along with either Letrozole 2.5 or 5.0.
- Schedule a follow up progesterone lab if necessary
    - Participants with anovulatory progesterone levels (<3 ng/ml) and evidence of follicular development will return for a repeat progesterone level assessment in 10-14 days (lab can be done locally or at clinic site).

- Participants with elevated anovulatory progesterone levels (1.5 < P4 < 3.0) and corpus luteum on ultrasound will return for a repeat progesterone level assessment in 2-3 days

- Dispense pregnancy test and medication for next cycle and review instructions for medication
    - Participants without ovulation nor evidence of follicular development are to complete pregnancy test and begin medication at new dose immediately following negative pregnancy test
    - Participants with ovulation are to wait until onset of menses, or two weeks after midluteal visit to complete pregnancy test. They can begin medication at same dose following negative pregnancy test. Participants with a positive pregnancy test will be moved to pregnancy track and will return dispensed medication at their next study visit.
- Collect any unused medication from current (or previous) cycle and document participant compliance in RedCap
- Record results of ultrasound and serum progesterone in RedCap

Cycle 2 Follow-up Midluteal Visit*

Participants with anovulatory progesterone levels (<3 ng/ml) and evidence of follicular development at their midluteal visit will return for a repeat progesterone level assessment in 10-14 days. This can be performed at a local lab if the participant prefers.

- Serum progesterone assessment for ovulation
- Review subject calendar log and record adverse events as reported by subject or observed by investigator.
- Review instructions for previously dispensed medication
    - Participants without ovulation are to complete pregnancy test and begin medication at new dose immediately following negative pregnancy test
    - Participants with ovulation are to wait until onset of menses, or two weeks after midluteal visit to complete pregnancy test. They can begin medication at new dose (if ovulation did not occur on or before cycle day 21) following negative pregnancy test. Participants with a positive pregnancy test will be moved to pregnancy track and will return dispensed medication at their next study visit.

- Participants who were initially anovulatory at a study visit prior to cycle day 21, who are ovulatory at a follow up visit by cycle day 21 will be maintained on the same dose of medication. This may occur if participant's study visit was moved up due to an early false positive ovulation prediction test.

-

- Record results of serum progesterone in RedCap

Cycle 2 Menses Check-In/Cycle 3 Start (menses following Cycle 2)*

Two weeks post Midleuteal visit if no menses before then

Participants with ovulation are to wait until onset of menses, or two weeks after midluteal visit to complete pregnancy test. They can begin medication at new dose following negative pregnancy test. This visit can be completed remotely with participant logging information in RedCap. Participants with a positive pregnancy test will be moved to pregnancy track and will return dispensed medication at their next study visit.

Cycle 3 Midluteal Visit

Timing will be based on LH testing, ideally around 7-8 days post-positive test or CD21-24 if no positive test.

- Transvaginal ultrasound to assess for corpora lutea and endometrial thickness and pattern
- Serum progesterone assessment for ovulation
- Review subject calendar log and record adverse events as reported by subject or observed by investigator.
- Schedule a follow up ultrasound if necessary
    - Participants with anovulatory progesterone levels (<3 ng/ml) and evidence of follicular development will return for a repeat progesterone level assessment in 10-14 days.
- Dispense pregnancy test review instructions for study completion
    - Participants with ovulation are to wait until onset of menses, or two weeks after midluteal visit to complete pregnancy test. Participants with a positive pregnancy test will be moved to pregnancy track.

- Collect any unused medication from current or previous cycles and document participant compliance in RedCap
- Record results of ultrasound and serum progesterone in RedCap

Cycle 3 Follow-up Midluteal Visit*

Participants with anovulatory progesterone levels (<3 ng/ml) and evidence of follicular development at their midluteal visit will return for a repeat progesterone level assessment in 10-14 days. This can be performed at a local lab if the participant prefers.

- Serum progesterone assessment for ovulation
- Schedule a follow up Progesterone lab if necessary
    - Participants with anovulatory progesterone levels (<3 ng/ml) and evidence of follicular development will return for a repeat progesterone level assessment in 10-14 days.
    - Participants with elevated anovulatory progesterone levels (1.5 < P4 < 3.0) and corpus luteum on ultrasound will return for a repeat progesterone level assessment in 2-3 days
- Review subject calendar log and record adverse events as reported by subject or observed by investigator.
- Review instructions for study completion
    - Participants with ovulation are to wait until onset of menses, or two weeks after midluteal visit to complete pregnancy test. Participants with a positive pregnancy test will be moved to pregnancy track.

Record results of serum progesterone in RedCap

7.4 Pregnancy Track Visits (part of routine care obstetrical care)

Patients who report a positive urine pregnancy test will have a serum hCG to confirm pregnancy, with a follow up serum hCG 48-72 hours after initial hCG. Results from patients' viability ultrasound will be obtained through chart abstraction. Patients with ongoing pregnancies will be followed through postpartum with 3 remote visits. Patients who experience biochemical pregnancy or 1st trimester pregnancy loss in pregnancies resulting from their 1st or 2nd treatment cycle may return to the treatment track and begin their next treatment cycle after one normal menses and a negative pregnancy test.

Pregnancy Lab Visit

Participants with positive urine pregnancy test will have pregnancy confirmed with serum hCG assessment. This can be performed at a local lab if the participant prefers.

- Serum hCG assessment for pregnancy
- Review subject calendar log and record adverse events as reported by subject or observed by investigator.
- Review instructions for pregnancy follow up, return to treatment track, or end of study as necessary
- Review plans for pregnancy care and obtain consent for obtaining information if pregnancy provider records will need to be requested and consent to obtain medical information on file is not up to date
- Collect any unused medication from current or previous cycle(s) and document participant compliance in RedCap
- Record results in RedCap

Pregnancy Lab Visit 2

Pregnant participants will have a repeat serum hCG assessment 48-72 hours after initial hCG. This can be performed at a local lab if the participant prefers.

- Serum hCG assessment
- Review instructions for pregnancy follow up, return to treatment track, or end of study as necessary
- Review plans for pregnancy care and obtain consent for obtaining information if pregnancy provider records will need to be requested and consent to obtain medical information on file is not up to date
- Record results in RedCap

Ongoing Pregnancy Follow up

Approximately 3 weeks after positive pregnancy test.

- Obtain viability ultrasound and enter results in RedCap
- Review instructions for pregnancy follow up, return to treatment track, or end of study as necessary
- Review plans for pregnancy care and obtain consent for obtaining information if pregnancy provider records will need to be requested and consent to obtain medical information on file is not up to date

12-15 Week Pregnancy Follow up

- Review records (if available) to determine if participant has experienced a loss.
- Participant will complete 12-15 week pregnancy follow up questionnaire via online surveys or phone.
- Review instructions for pregnancy follow up, return to treatment track, or end of study as necessary
- Review plans for pregnancy care and obtain consent for obtaining information if pregnancy provider records will need to be requested and consent to obtain medical information on file is not up to date
- Record abstraction details or participant responses in RedCap

Postpartum Pregnancy Follow up

- Review records (if available) to determine outcome of pregnancy.
- Participant will complete postpartum pregnancy follow up questionnaire via online surveys or phone.
- Review instructions for return to treatment track or end of study as necessary
- Review pregnancy care received and delivery and obtain consent(s) for obtaining information for mother if pregnancy provider records will need to be requested and consent to obtain medical information on file does not include named providers
- Obtain consent(s) for obtaining information for infant(s) if delivery records will need to be requested
- Record infant date of birth, birth weight and length, and any birth defects noted by the pediatrician or mother in RedCap. This information can be obtained from mother the RedCap Postpartum survey.
- Have participant complete questionnaires (GAD7, PHQ9, FertiQoL, and Perceived Stress Scale, or if participant prefers to complete online, provide instructions on completing forms after visit

7.5 Final Study Visit/Cycle 3 Menses check in

An end of treatment visit or phone call will occur after menses occurs with a negative pregnancy test or negative pregnancy test in the case of an anovulatory treatment cycle or following a positive pregnancy test. This visit will be held within 1 week of outcome (negative or positive pregnancy test) Any unused study medication will be returned to the

study team at this visit. Participants who completed paper journal logs will submit any remaining journal logs at this visit, and the end of treatment questionnaire will be completed.

Final Study Visit

- Record adverse events as reported by subject or observed by investigator.
- Record results of home urine pregnancy test and subsequent serum hCG tests.
- Review subject calendar log and record adverse events as reported by subject or observed by investigator.
- Collect any unused study medication and document participant compliance in RedCap
- Complete end of treatment questionnaire with subjects if they have not completed online
- Complete post questionnaires (PHQ9, GAD7, and Perceived Stress Scale) with participant if they have not completed online
- Provide final instructions to subject

7.6 Missed Study visits

In the event a subject is unable to complete their midluteal ultrasound, progesterone lab, or follow up progesterone lab, ovulation will be assessed by presence and timing of spontaneous menses.

Subjects can postpone a cycle if they know that they will not be able to complete midluteal visit components within the projected window, taking a cycle off between study cycles. Timing of cycles will be captured in RedCap. However, in the event of unexpected inability to attend, as may occur if a subject were to contract a COVID-19 infection during their study cycle, menses can be used as an indication of ovulation in place of progesterone lab and ultrasound results.

8 STUDY PROCEDURES /EVALUATIONS

8.1 Study Procedures/Evaluations

- Medical history will be assessed by chart abstraction and confirmation with the patient at the Screening or Enrollment visit.
- Physical examination will include chart abstraction of height and weight and BMI and if not in chart will be performed at screening visit.
- Participants will be asked about hirsutism symptoms.
- Transvaginal ultrasound of uterus and ovaries will be performed to obtain the following measures: endometrial thickness and evaluation for presence of corpora lutea and number of corpora lutea.
- Urine pregnancy tests
- Participant Journal Logs will be used to track medication adherence, side effects, intercourse, and concomitant medications

Standard Clinical care:

- Transvaginal ultrasound will also be performed at the ongoing pregnancy visit to record the number of gestational sacs, measurement of gestational sac(s), yolk sac(s), crown rump length, presence of fetal heart motion and rate and any abnormalities.
- Assessment of tubal factor and uterine cavity by saline sonohystogram, hysterosalpingogram, or laparoscopy if recommended by provider however not required to participate in the study
- Semen analysis of male partner to rule out male factor

8.2 *Medical record review will be performed for labs, including: TSH, prolactin, HbgA1c, 17-OH Progesterone, AMH, Rubella, FSH and estradiol, Total testosterone and SHBG*; progesterone documenting anovulation. All of these labs are not required for participation but if they have been done should be documented. *Documentation of TSH, HbgA1c and Rubella status recommended for participation. These will be entered into RedCap.*

8.2.1 Clinical Laboratory Evaluations performed at local labs

- Progesterone will be assessed at the local lab to determine treatment plan for next cycle and ovulatory status
- Urine hCG will performed by the patient

- Serum hCG if indicated will be assessed at the local lab to confirm pregnancy status if positive urine hCG (standard clinical care)

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

9.1.1 Adverse Events

An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

9.1.2 Serious Adverse Events

SAEs are a subset of all AEs. A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

9.2 Time Period and Frequency for Event Assessment and Follow-Up

Unanticipated problems will be recorded in the data collection system throughout the study.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

9.3 Characteristics of an Adverse Event

9.3.1 Relationship to Study Intervention

The Medical Monitors and the Study PI will assess the relationship of all adverse events to study intervention or study participation as either related or not related. Evaluation of relatedness will consider etiologies such as natural history of the underlying disease, concurrent illness, concomitant therapy, study-related procedures, accidents, and other external factors.

To assess relationship of an event to study intervention, the following guidelines will be used:

1. Related (Possible, Probable, Definite)
    a. The event is known to occur with the study intervention.
    b. There is a temporal relationship between the intervention and event onset.
    c. The event abates when the intervention is discontinued.
    d. The event reappears upon a re-challenge with the intervention.
2. Not Related (Unlikely, Not Related)
    a. There is no temporal relationship between the intervention and event onset.
    b. An alternate etiology has been established.

9.3.2 Expectedness of SAEs

The Medical Monitors and the Study PI will be responsible for determining whether an SAE is expected or unexpected. An adverse event will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention.

9.3.3 Severity of Event

The following scale will be used to grade adverse events:

1. Mild: no intervention required; no impact on activities of daily living (ADL)
2. Moderate: minimal, local, or non-invasive intervention indicated; moderate impact on ADL
3. Severe: significant symptoms requiring invasive intervention; subject needs major assistance with ADL

9.4 Reporting Procedures

Sites will report unanticipated problems (to subjects or others) in either the subjects REDCap record or via direct communication with the University of Iowa PI and study coordinator through email or phone call as needed. Alerts will be set up in REDCap, so that the University of Iowa study team is notified when an unanticipated problem is recorded. Unanticipated problems will be reported to the IRB per institution reporting guidelines.

Adverse Events and Serious Adverse Events will be recorded in RedCAP, entry of Serious Adverse Events will trigger email notification to the PI and the study coordinator. Upon receipt of an adverse event alert, University of Iowa staff will follow up with the study site or subject who entered the data to trigger the alert to confirm accuracy. Data will be reported to the IRB of record and relying IRBs in accordance with institution policies. Additionally, reports of project safety data will be prepared for the safety monitors review twice annually.

10 STUDY OVERSIGHT

In addition to the PI's responsibility for oversight, study oversight will be under the direction of a safety monitors with expertise in Gynecology and in Reproductive Endocrinology and Infertility. The safety monitors will review safety data every 6 months. If safety concerns arise, more frequent reviews may be conducted.

Data reports will include side effect profiles and reports of any adverse events. We will also provide data on any multiple gestations.

11 CLINICAL SITE MONITORING

Throughout the study information and documents such as IRB approval memos, IRB approved materials, and study wide and site-specific amendments will be sent to relying sites via email and will be discussed with sites on monthly study conference calls. Information and documents from relying sites such as local context checklist, site delegation logs, changes in research personnel and information for continuing review will be sent to the University of Iowa study coordinator via email and may be discussed on monthly conference calls.

All study data will be entered in REDCap by participating site staff. REDCap data quality functionality of field validation and queries will be used to identify and resolve discrepancies in data in real time. The REDCap report feature will be utilized by University of Iowa team to track completeness of data entry, any identified issues will be communicated to participating sites for confirmation and completion. REDCap provides a secure data entry platform which allows external collaborators to be added to projects, with data access groups to limit access to site specific records, while allowing all data to be entered into a single central project.

Protocol deviations can be assessed through both cycle information entered in standard data entry fields in subjects REDCap files or in a specified field for protocol deviations. REDCap data quality features will be used to flag deviations from study protocol related to timeframe of study events, dosing of study medication, and missing expected protocol events. Site staff will be contacted regarding these deviations to confirm accuracy of data entry and to determine if a deviation occurred. In addition to these fields, staff will be able to enter additional information on protocol deviations in a specific field in the subjects REDCap file. Data entered in this field will generate an alert to University of Iowa Study staff to review the deviation.

Any concerns about noncompliance at participating sites will be discussed with that site’s PI. University of Iowa PI and study coordinator may request to review participating cites study records, including consent documents, in the event of concerns related to non-compliance. In the event non-compliance is found/or found to be a reasonable concern, the University of Iowa PI or designee will report the issue to the IRB of record. To resolve issues of non-compliance, staff training may be provided via memos or via conference call. Should retraining efforts not provide satisfactory resolution to noncompliance and/or violation of the contract/protocol, the site’s participation in the study may be terminated.

11.1

12 STATISTICAL CONSIDERATIONS/DATA ANALYSIS

12.1 Justification of Effect Size

12.1.1 Prior Studies

Strong data on the per cycle cumulative ovulation with letrozole is available from the PPCOS II study. Unfortunately, there are not comparative quality data from randomized trials of the combination of letrozole and clomiphene citrate (CC), as discussed in the background section. The cumulative live birth proportion for subjects randomized to receive letrozole was 0.275 for the five-treatment cycle study period.

Per Cycle Ovulation and Live Birth Results in Letrozole arm of the PPCOS II study

	Ovulations/subject
Pre-treatment cycle 1	4/14 (28.6%)
Treatment cycle 1	178/360 (49.4%)
Treatment cycle 2	204/318 (64.2%)
Treatment cycle 3	179/268 (66.8%)

12.1.2 Minimum Clinically important differences

Considering the cumulative ovulation rates over 3 cycles from PPCOS II, data from our initial pilot study, as well as a clinically relevant increase in ovulation, we designed the study with 80% power to detect a 20% increase in ovulation rate among women treated with letrozole combined with CC, compared to those treated with letrozole monotherapy. We believe this method balances the risk of avoiding a type 2 error and along with an attainable sample size.

12.1.3 Significance Testing

Primary statistical analyses will invoke the intent-to-treat approach. Primary efficacy analysis will be performed by comparing the treatment groups with respect to the primary outcome of ovulation using Pearson chi-square test. Additional analysis will be performed using logistic regression modeling to adjust for other factors if needed such as the randomization stratification of study site, prior exposure to study medications, age and BMI. Summary descriptive statistics including mean, standard deviation, number of observations and quartiles will be provided for continuous variables; while frequencies and percentages will be provided for categorical variables. All hypothesis tests will be two-sided and all analyses will be performed using SPSS or SAS statistical software.

12.1.4 Sample Size Calculations

Considering the cumulative ovulation rate over 3 cycles from PPCOS II, data from our initial pilot study, and a clinically relevant increase in ovulation, we designed the study with 80% power to detect a 20% absolute difference in ovulation rate among women treated with letrozole combined with CC, compared to those treated with letrozole monotherapy. Allowing 10% dropout following randomization requires 92 patients per arm, or 184 total. Allowing for an additional 15-20% screening failure rate requires 220 consented to reach our randomization goal.

12.2 Statistical Analysis

All analyses will utilize the intention-to-treat (ITT) principle. All statistical tests of hypotheses will be two-sided. Baseline characteristics will be summarized by treatment arm. The primary outcome, cumulative ovulation, will be analyzed by comparing proportions utilizing a chi square test with significance set at 0.05. Dichotomous secondary outcomes will be analyzed in the same manner as the primary outcome. Two-sided t-tests will be used to compare endometrial lining thickness. Wilcoxon-Mann-Whitney tests will be used to compare number of corpora lutea. Time to pregnancy will be analyzed as a continuous variable using Kaplan-Meier survival analysis. Per-protocol analyses will be conducted to provide additional insight.

12.3 Secondary Analyses

12.3.1 Descriptive Summary

We propose a number of secondary analyses, including assessing differences in conception and clinical pregnancy rates, live birth rates and multiple pregnancy rates, pregnancy loss rates (biochemical, miscarriage, ectopic), time to pregnancy, ultrasound measures (number of corpora lutea, endometrial thickness and pattern by ultrasound) and obstetric outcomes of pregnancy complication rate, birth weight, and neonatal complication rate. We will also evaluate side effect profile of both groups and compare % percentage of patients that would not take study treatment due to side effects.

12.3.2 Statistical Analyses

12.3.3 Other Secondary Analyses

Generalized estimating equations will be used as appropriate for secondary analyses of differences in means and binary outcomes where the main independent variables will be treatment group, time, and their interaction, in order to account for

within-subject variability in repeated observations over time. Potential covariates in the models include recruitment site, prior exposure to the medication, use of insulin sensitizers (metformin), baseline value of the outcome, and age.

Cox proportional hazards models and Kaplan-Meier method will be used to compare the time to pregnancy between groups.

In addition to the primary intent-to-treat analysis, a per protocol analysis will be performed as a secondary analysis.

12.3.4 Sub study Analyses

In our sub study analyses we will compare the differences in corpora luteal number and endometrial thickness between the two arms of the study. Previously reported ultrasound parameters for Letrozole are presented in the table below.

Mean and standard deviation ultrasound parameter assumptions for letrozole treatment group

Endometrial Thickness (mm)	6.2 ± 2.2 in Letrozole group 8.3 ± 3.6 in Combo group	Mejia et al, 2018	34 cycles of letrozole 2.5 and 33 cycles of combo (letrozole 2.5 + CC 50) in women with PCOS on cycle day 12-14
Number of follicles > 10 mm	0.4 ± 0.56 in Letrozole group 2.0 ± 2.13 in Combo group	Mejia et al, 2018	34 cycles of letrozole 2.5 and 33 cycles of combo (letrozole 2.5 + CC 50) in women with PCOS on cycle day 12-14
Largest follicle size (mm)	11.71 ± 4.50 in Letrozole group 15.61 ± 5.9 in Combo group	Mejia et al, 2018	34 cycles of letrozole 2.5 and 33 cycles of combo (letrozole 2.5 + CC 50) in women with PCOS on cycle day 12-14

An additional sub study analysis will report subsequent fertility treatments and outcomes in patients who do not have an ongoing pregnancy at the conclusion of participation. The follow up window for these participants will be 10 months.

12.3.5 Sample size calculation

Considering midluteal endometrial thickness previously reported in ovulatory letrozole treatment cycles and hypothesized ovulation rates, the planned sample of 184 patients will have 94% power to detect a .25mm difference in midluteal endometrial thickness between the two arms among patients who ovulate. Assuming follicles >15mm on CD 12-14 in our preliminary study are indicative of corpora lutea, the planned sample will have 90% power to detect a 0.3 difference in mean number of corpora lutea among patients who ovulate.

12.4 Missing Data

Every attempt will be made to obtain the full set of outcome values for each participant. Despite this effort, we expect there will be some missing data due to dropouts and missed visits. Missing data for binary outcomes will be calculated with both conservative (assumed negative (e.g. no ovulation) and liberal with assumed yes ovulation unless later data contradicts this. If necessary, imputation will be used.

12.5 Adverse Event Analysis

Adverse events will be categorized and frequency and percentage of patients experiencing adverse events and serious adverse events during the study period will be reported. Chi-square tests will be utilized to compare differences in proportion of total and categories of adverse events within each treatment arm.

12.6 Interim Analysis

We propose not to perform an interim analysis. Both the small sample size and relatively low risk of multiple gestation we do not see that an interim analysis is necessary.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6.

13.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the IRB for review and approval. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study and then the Protocol document will be updated accordingly.

The University of Iowa will serve as the single IRB for this multi-site trial.

13.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to subjects and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the subject. Consent forms will be IRB-approved, and the subject is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the subject and answer any questions that may arise. The subject will sign the informed consent document prior to any study-related assessments or procedures. Subjects will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The consent process will be documented in the clinical or research record. Different versions of the informed consent documents may be used across sites to comply with local IRB policies. Apart from policy required modifications, all sites will use informed consent documents with the same content.

13.4 Subject Confidentiality

Subject confidentiality is strictly held in trust by the investigators and study staff. This confidentiality is extended to cover testing of biological samples in addition to any study information relating to subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the PI.

The study monitor or other authorized representatives of the sponsor may inspect all study documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the study subjects. The clinical study site will permit access to such records.

13.5 Study Intervention Compliance

This section describes the strategy, responsibilities, and quality management activities in place to demonstrate that there is adequate monitoring of the clinical trial by the Principal Investigator (PI) to ensure:

- The trial is conducted according to the investigational plan, protocol and applicable laws, regulations, policies and guidance
- The rights, welfare, and safety of human subjects are protected
- Proper reporting of study data to the FDA and IRB
- The PI is providing adequate oversight of the clinical trial

The plan includes both internal quality and external, independent safety management processes used throughout the study, including but not limited to staff training, standardized procedures, methods for data collection, study and data monitoring, and routine team meetings to review the study progress and isolate any compliance issues and/or trends.

13.5.1 Study Team Training

Members of the study team are trained on the protocol and/or study procedures applicable to their roles and responsibilities. When the protocol and/or study procedures are updated, staff will be trained on the revisions prior to implementation, as applicable. Training is documented and maintained in the study files.

13.5.2 Investigator/Study Team Member Agreement

All members of the study team are informed of their responsibilities specific to their role(s) in this study, their obligation to follow the approved clinical research protocol, the applicable regulations, guidelines and institutional policies. Documentation of this agreement is maintained in the study files following site specific policies.

13.5.3 Financial Disclosure

Financial disclosure information is collected for all members of the study team that make a direct and significant contribution to the data. Financial disclosure documentation is maintained following site specific policies.

*13.5.4 **Routine Study Team Meetings***

Routine study team meetings ensure on-going supervision and oversight of the study and study personnel involved in the conduct of the study. In the meetings, the principal investigator (PI) and study team members discuss evaluations of study-related activities (as applicable): identification of deviations or noncompliance, review of adverse events, and overall study progress. Training on protocol, procedure and/or form updates may also be performed during routine meetings.

14 DATA HANDLING AND RECORD KEEPING

All study data will be entered in REDCap by participating site staff. REDCap data quality functionality of field validation and queries will be used to identify and resolve discrepancies in data in real time. The REDCap report feature will be utilized by University of Iowa team to track completeness of data entry, any identified issues will be communicated to participating sites for confirmation and completion. REDCap provides a secure data entry platform which allows external collaborators to be added to projects, with data access groups to limit access to site specific records, while allowing all data to be entered into a single central project.

While most data will be directly entered into RECap, sites are expected to retain subject informed consent documents and other source documents when they exist. Clinical data (including AEs, laboratory values, ultrasound results, and solicited events data) and clinical laboratory data will be abstracted from the patient's electronic medical record (EMR) and entered into REDCap. The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. The investigators will maintain adequate case histories of study subjects, including accurate case report forms (CRFs), and source documentation.

Study consent documents will be maintained for at least ten years from the date that the project is closed in the IRB, or longer as needed to comply with local IRB record retention policies. Other study records will be retained in RedCap through completion of collection and data cleaning of live birth and other delivery/infant outcomes for all participants in the project. After data are cleaned, de-identified data will be downloaded and stored for analyses and publications.

15 PUBLICATION/DATA SHARING POLICY

Publication and authorship procedures will be established and agreed upon by all investigators.

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APPENDICES

Appendix A: Schedule of Events

APPENDIX A: SCHEDULE OF EVENTS

			Treatment visits (Repeat for up to 3 treatment cycles)				Pregnancy Follow up			
	Screening Period	**Baseline Visit**	**Monthly Midlueteal visit**	**Follow up Midluteal visit***	**Menses check in appointment**	**End of Treatment follow-up**	**Pregnancy lab visit**	**Ongoing pregnancy follow up**	**12-15 week f/u**	**Postpartum f/u**
Assessments		Cycle day 1-3 (+2), random start	18 -26 days post medication start	10-14 days after midlueteal visit	Onset of menses or 2 weeks after midluteal visit	After completion of 3rd treatment cycle or following pregnancy	48-72 hrs after initial hCG	~3 weeks after positive pregnancy test		
Pre- Screening questionnaire	X[c]									
Informed consent	X [c]									
Confirm Inclusion/Exclusion criteria	X									
Abstract Demographics, medical history	X									
Abstract physical exam (Anthropometrics, hirsutisum assessment) Vital signs & weight	X									
Transvaginal ultrasound	(abstract)		X					(abstract)		
Randomization		X								
Inclusion laboratory testing[a]	(abstract)									
Dispense Meds		X	X		X					
Serum Progesterone[a]			X	X						
Serum hCG[a]							X			
Biospecimen collection, blood[b]:										
Urine pregnancy test		X (1st)			X[c]	X[c]				

Assessment of other infertility factors: tubal factor and uterine cavity, male factor	(abstract)									
Journal Log		Throughout Treatment Follow up[c]					X[c]	X[c]		
AE query			Throughout Follow up[c]							
Follow up questionnaire						x			X[c]	X[c]
Follow up chart abstraction									X	X

Screening can be done on the same day as the baseline visit.

*If Anovulatory progesterone level < 3 ng/mL and evidence of follicular development

[a]Testing to be performed at local lab

[b] Samples to be sent to central laboratory for biobanking and future testing; if consented for biobank

[c] Component may be completed via RedCap or phone, can be completed by Iowa staff

Tool Revision History:

Version Number	Version Date	Summary of Revisions Made:
		Original version with Tool Summary Sheet
		Revised text regarding Safety Oversight method, Final Statistical Analysis, Data Sharing, and Clinical Site Monitoring; clarified instructional text and added text placeholders