

**Combined Letrozole and Clomid in Polycystic
Ovary Syndrome: A randomized control trial of
combination of letrozole and clomiphene citrate
or letrozole alone for the treatment of infertility in
women with polycystic ovary syndrome**

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
BMI	Body Mass Index
CC	Clomiphene Citrate
DHEA-S	Dihydroepiandrosterone sulfate
DHHS	Department of Health and Human Services
FDA	Food and Drug Administration
IRB	Institutional Review Board
IRL	Iowa River Landing
PCOS	Polycystic Ovary Syndrome
PPCOS II	Pregnancy in Polycystic Ovary Syndrome Study II
P4	Progesterone
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
UIHC	University of Iowa Hospitals and Clinics
US	United States

PROTOCOL SUMMARY

- Title:** Combined Letrozole and Clomid in Polycystic Ovary Syndrome: A randomized control trial of combination of letrozole and clomiphene citrate or letrozole alone for the treatment of infertility in women with polycystic ovary syndrome
- Précis:** This will be a single center, randomized controlled open trial of letrozole versus letrozole and clomiphene citrate (CC) for one cycle. Women will be randomized in a 1:1 ratio to receive letrozole 2.5 mg or combination of letrozole 2.5 mg and clomiphene 50 mg for 5 days on days 3-7 of menstrual cycle. We will stratify the randomization by age (< 35 versus >35) and BMI (<30, > 30) to try to ensure equal groups to study. The women and their partners will be instructed to have regular intercourse with the intent to conceive during the cycle. Patients will have an ultrasound on between cycle day 12 -14 and will evaluate number of follicles (>15 mm), follicle size, endometrial thickness and pattern. Patients will have mid-luteal phase progesterone level drawn. The primary analysis will use an intent-to-treat approach to evaluate differences in ovulatory rate in the two treatment arms.
- Objectives:**
- Primary:** Cumulative ovulation rate determined by a mid-luteal (day 21) phase progesterone level. Progesterone level \geq 3 ng/mL will indicate ovulation.
- Secondary:** Size and number of developing follicles, endometrial thickness and pattern all determined by a mid-cycle ultrasound. Positive pregnancy test, clinical pregnancy and ongoing pregnancy. We will also report live birth rate, miscarriage rate, ectopic pregnancy rate and multiple pregnancy rate. We will also monitor complication rate and side effect profile of both treatment arms.
- Population:** 70 women with a diagnosis of polycystic ovary syndrome and infertility desiring to conceive. Participants will be 18-40 years of age.
- Phase:** IV
- Number of Sites:** 1 – University of Iowa Hospitals and Clinics, Women’s Health Clinic and Quad Cities clinic
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Description of Intervention:

70 women will be equally randomized to two different treatment arms: A) letrozole 2.5 mg every day for 5 days (day 3-7 of cycle) or B) letrozole 2.5 mg and clomiphene citrate 50 mg every day for 5 days (3-7 of cycle), for one cycle.

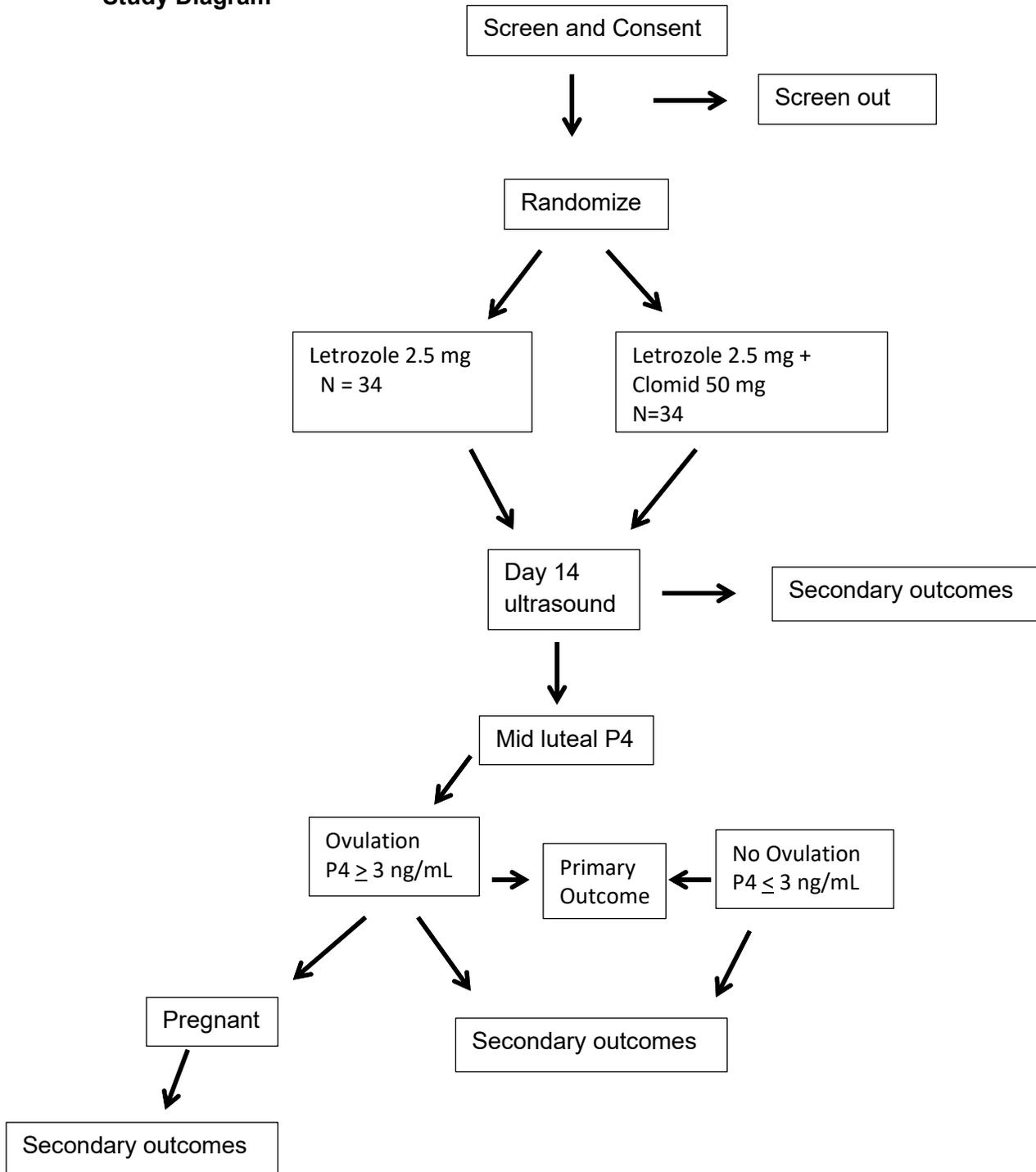
Subject Participation Duration:

Approximately 5 weeks of active participation time. Clinical chart abstraction and remote follow up will be continued for 10 months following participation.

Estimated Time to Complete Enrollment:

18 months by enrolling ~3.89women per month

Study Diagram



1 KEY ROLES AND CONTACT INFORMATION

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive age women and constitutes the most common cause of anovulatory infertility.¹ The treatment of infertility in patients with PCOS focuses on ovulation induction. Multiple treatment regimens have been used and all with varying success in regards to both ovulation and pregnancy rates.

Clomiphene citrate (CC) is a commonly used pharmacologic agent to induce ovulation in women with PCOS. It works as a selective estrogen receptor modulator (SERM) by competitively attaching to nuclear estrogen receptors. As the negative feedback of estrogen is lowered it in turn activates increased secretion of gonadotropin hormones which subsequently causes ovarian follicular growth. CC also has an antiestrogenic effect on endometrial development and cervical mucus production. This has been implicated to contribute to the low pregnancy rate despite a relatively high ovulation rate.^{2,3}

Letrozole has a different mechanism of action and works as a highly selective aromatase inhibitor thus preventing androgen-to-estrogen conversion. The negative feedback in the hypothalamus is decreased by the suppressed estrogen production which subsequently increases the concentration of follicle stimulating hormone (FSH). An additional mechanism of aromatase inhibitors may result from increase follicular sensitivity to FSH resulting from temporary increased intraovarian androgens.⁴ Studies in primates have shown that testosterone augments follicular FSH receptor expression which supports the idea that androgens promote follicular growth.⁵⁻⁷ Additionally, Garcia-Velasco et al demonstrated an increase in intraovarian androgen concentrations with the use of letrozole in low responder patients undergoing IVF.⁸ Letrozole offers a benefit over CC for ovulation induction since it does not deplete estrogen receptors in both central and peripheral target tissues and normal central feedback mechanisms remain intact. Recently, a randomized control trial comparing letrozole to clomiphene demonstrated that letrozole was associated with higher live-birth rate (27.5% vs 19.1%, P=0.007, 95% CI 1.10 to 1.87) and cumulative ovulation rate (61.7% vs. 48.3%, P<0.001) among women with polycystic ovary syndrome.⁹ Despite the superior ovulatory and live birth rates with letrozole compared to CC the question remains if there are medical alternatives without proceeding to gonadotropins or IVF both of which are associated with increased costs and risks.

Since letrozole and CC have different mechanisms of actions we postulate that the combination of these medications may offer a synergistic effect and improve the ovulatory rate. One study has prospectively reviewed treatment outcomes using a combination of letrozole and clomiphene who had previously failed CC for 6 cycles and letrozole for 4 cycles. This study enrolled 100 patients and showed an ovulatory rate based on development of dominant follicle of 82.9% of cycles (213/257) with the combination treatment.¹⁰ However, this study was not randomized and used a limited population of women with PCOS that were resistant to both clomiphene and letrozole alone. Additionally, patients received Gonal-f on day 11 if a dominant follicle was present and then received hCG trigger when the follicle was ≥ 18 mm in size

followed by IUI 36-38 hours later. Despite the prospective observational nature of the study it does point to a potential improved ovulatory rate with this combination treatment.

2.2 Rationale

Letrozole has been shown to have both higher live-birth and ovulation rates compared to CC among infertile PCOS women demonstrated by the PPCOS II trial. Despite the superior ovulatory and live birth rates with letrozole compared to CC the question remains if there are medical alternatives without proceeding to gonadotropins or IVF both of which are associated with increased costs and risks. One study has shown a potential benefit in combining letrozole and CC however a double blind trial is needed to test if this combination improves outcomes over letrozole alone.

Mechanistically, the combination may have a synergistic action with letrozole having a local effect at the level of the ovary to block estrogen synthesis and with clomiphene having a central effect by antagonizing the negative feedback of estrogen at the hypothalamus by depleting estrogen receptors. These different mechanisms of actions may provide a synergistic effect to provide improved ovulation rates over use of letrozole alone. Since the combination of letrozole and clomiphene has not yet been studied in a prospective randomized fashion and it is unclear if this will offer an advantage over letrozole alone in terms of both ovulation and pregnancy rate, we have designed a pilot study to characterize the ovulatory rate with this combination treatment. It is necessary to first determine if the combination treatment is at least equivalent and possibly better than the standard efficacy of letrozole. Prior to embarking on a larger trial to look at the most important outcome of live birth rate it is important to establish that this combination treatment does offer a superior ovulatory rate. The aim of this study is to test the hypothesis that combined therapy of letrozole and CC is an effective and superior method to letrozole alone to achieve ovulation in women with PCOS.

2.3 Potential Risks and Benefits

2.3.1 Potential Discomforts & Risks

Both letrozole and CC have 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
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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 and Letrozole is associated with a 3.4% multiple pregnancy rate in women with PCOS. 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 medical 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%).⁹

2.3.2 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 and PCOS 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 the mid-cycle ultrasound and hormone testing the participant may receive additional information and understanding related to their cause of infertility.

Possible benefits to others: The study will provide important information on the treatment for women with infertility and PCOS. 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 hypotheses

Treatment with the combination of letrozole and clomiphene is more likely to result in ovulation (increased ovulation rate) compared to treatment with letrozole alone in women with infertility and PCOS.

3.1.1 Primary Outcome measures

The primary outcome measure is the occurrence of ovulation defined as a mid-luteal progesterone level > 3 ng/mL. This will be tested 7 days following the patient reporting of an LH surge. If no LH surge is detected the lab will be drawn on cycle day 21 or 22. The primary analysis of ovulation rate within the two treatment conditions will employ an intent-to-treat approach.

3.2 Secondary outcomes

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 and size of developing follicles 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.

4 STUDY DESIGN

Treatment Design: This will be a single center, open trial of oral letrozole vs. combination of oral letrozole and clomiphene citrate in the treatment of infertility in patients with polycystic ovary syndrome. Patients will be randomized to receive either a dose of 2.5 mg of letrozole or 2.5mg of letrozole and 50 mg of CC for 5 days on cycle days 3 through 7 of the menstrual cycle. Patients will be monitored during the cycle with a mid-cycle ultrasound (cycle days 12-14) and a mid-luteal progesterone level (cycle day 21).

The study will last for -1 cycles or approximately -5 weeks.

Randomization will be stratified by age (< 35 versus \geq 35) and BMI (< 30 and \geq 30) to ensure equal groups to study. Randomization will be via a computer based central randomization to ensure adequate allocation concealment.

Enrollment will take approximately 18 months if ~ 3.89subjects are enrolled per month. Active subject participation will be through completion of -1 cycle. We will follow up with patient regarding pregnancy, delivery outcomes, and if information is not available via chart abstraction.

4.1 Study Summary

Initial visit:

1. Study staff review pre-screening questionnaire and confirm eligibility criteria
 2. Obtain signed informed consent
 3. Confirm documentation of vital signs, height, weight and BMI.
 4. Record and review medical history
 5. Request subject to sign medical release form to obtain information regarding ongoing infertility treatment, pregnancy and delivery information.
 6. Complete urine pregnancy test or dispense urine pregnancy test to complete at a later date if participant plans on delaying treatment.
 7. Randomization to treatment group once negative pregnancy test.
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- Orient and dispense calendar log which includes documentation of intercourse, menstrual log and side effect assessment

- Dispense study medications

Mid-cycle Visit (Visit #2):

1. Collect journal logs
2. Transvaginal ultrasound exam
3. Return medication containers and study medications you did not take
4. Dispense urine pregnancy test

Mid-luteal lab draw (visit #3)

1. Blood draw (P4)
2. Schedule End of treatment visit

End of Treatment Visit:

1. Collect Calendar log
2. If subject had a positive urine pregnancy test: blood draw will be performed and then repeated approximately 48 hours after the first one.
3. Complete End of Treatment questionnaire
4. Arrange for follow up and confirm we have a release of records for pregnancy and neonatal records.

4.2 Study Population:

70 women with PCOS actively seeking pregnancy (or 35 per each treatment arm) aged ≥ 18 to ≤ 40 years will be enrolled. The overall goal of enrollment criteria is to identify a population of healthy women with PCOS and infertility not specified by another cause. Infertility will be determined by clinical history. PCOS will be determined by Rotterdam criteria which includes two of three findings: oligo- or anovulation, hyperandrogenism or polycystic ovaries on ultrasound.

- Hyperandrogenism will be determined by evidence of hirsutism on exam or by biochemical elevations in total testosterone.
 - Ultrasound findings of polycystic ovaries including ≥ 12 follicles of 2-9 mm diameter or increased ovarian volume ($>10 \text{ cm}^3$) in either ovary.
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5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Subject Inclusion Criteria

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

1. Provide signed and dated informed consent.
 2. Willing to comply with all study procedure and be available for the duration of the study. Ability to have regular intercourse during the ovulation induction phase of the study.
 3. Diagnosis of 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.¹¹ If in the clinical case of anovulation, 12 month period of time “infertility” is not necessary.
 4. Diagnosis of polycystic ovary syndrome: based on Revised Rotterdam criteria which includes two of the three following findings¹² and the definitions and criteria are modified from the PPCOS II trial⁹:
 1. Oligomenorrhea or chronic anovulation: spontaneous intermenstrual periods of ≥ 45 days or a total of ≤ 8 menses per year. In women with more regular bleeding patterns but suspected anovulatory bleeding, a mid-luteal serum progesterone level < 3 ng/mL is consistent with chronic anovulation. For women with a history of ≤ 8 menses per year however now have regular menses due to initiation of insulin sensitizing agents or active weight loss efforts within the last year prior to the study would qualify as evidence of oligomenorrhea.
 2. Hyperandrogenism (clinical or biochemical): clinical hyperandrogenism demonstrated by hirsutism and biochemical hyperandrogenism demonstrated by hyperandrogenemia. Hyperandrogenemia defined as an elevated total testosterone based on the laboratory criteria. Labs could be local or from UIHC.
 3. Polycystic ovaries on ultrasound: defined as either an ovary that contains 12 or more follicles measuring 2-9 mm in diameter, or an increased ovarian volume (>10 cm³) on one ovary.
 5. Normal sperm concentration of 15 million/mL and with normal motility of $> 40\%$ according to World Health Organization cutoff points, in at least one ejaculate during the previous year.¹³ Morphology results will not affect eligibility. If male partner has fathered a child, SFA is not necessary.
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5.2 Subject Exclusion Criteria

1. Current pregnancy. Patient will take a urine pregnancy test prior to starting treatment medication.

2. Patients currently taking hormonal contraception. A 1 month washout for any type of combined contraceptive or oral progestins will be required. If use of hormonal implants or depo progestins a longer washout of 3 months will be required.

3. Patients with other known cause of infertility: endometriosis, tubal factor, uterine abnormalities.

4. Patients with uncorrected thyroid disease defined based on their local lab or at UIHC (0.27 to 4.20 μ IU/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 year is adequate for entry.

5. Patients with untreated hyperprolactinemia. A normal prolactin within the past year at local lab or at UIHC (4.8 to 23.3 ng/mL) is adequate for entry.

6. Medical conditions in which we recommend avoiding pregnancy until under improved control:

-Patients with poorly controlled Type 1 or Type 2 diabetes (defined as a hemoglobin A1c > 6.5%)

- Patients with poorly controlled hypertension (defined as systolic blood pressure \geq 160 mm Hg or diastolic \geq 100 mm Hg on two measures at least 60 minutes apart)

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.

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).

6 STATISTICAL CONSIDERATIONS

6.1 Justification of effect size

6.1.1 Prior Studies

The primary outcome is ovulation, defined by a mid-luteal progesterone level > 3 ng/mL. There is data demonstrating the cumulative live birth rate with clomiphene and letrozole from the PPCOS II trial. This trial compared letrozole to clomiphene demonstrated that letrozole was associated with a higher cumulative ovulation rate (61.7% vs. 48.3%, $P < 0.001$) among women with polycystic ovary syndrome and the rate of ovulation after one treatment cycle with letrozole 2.5 mg was 50%.⁹ Unfortunately, there is no comparative quality data from randomized trials of the combination of letrozole and CC. There is one study that prospectively reviewed treatment outcomes using a combination of letrozole and clomiphene who had previously failed CC for 6 cycles and letrozole for 4 cycles. This study enrolled 100 patients and showed an ovulatory rate (defined by development of dominant follicle) of 82.9% of cycles (213/257) with the combination treatment.¹⁰ However, this study was not randomized and used a limited population of women with PCOS that were resistant to both clomiphene and letrozole alone. Additionally, patients received Gonal-f on day 11 if a dominant follicle was present and then received hCG trigger when the follicle was ≥ 18 mm in size followed by IUI 36-38 hours later.

6.1.2 Minimum Clinically Important Difference

Since this is a pilot study our goal is to balance the risk of avoiding a type 2 error and having an attainable sample size. We have set a benchmark of 30% difference between groups as a clinically meaningful.

6.1.3 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 model to adjust for other factors such as the randomization stratification of age and BMI and prior exposure to study medications. A per protocol analysis will also be performed.

6.1.4 Sample Size calculations

A sample size of 31 subjects in each arm of the randomization yields 80% statistical power to prospectively demonstrate a 0.30 absolute difference in ovulation rate between treatment arms (0.50 for Letrozole and 0.83 for combined letrozole and CC) using the Pearson's chi-square test having a two-sided significance level of 0.05. The sample size has been inflated to 35/arm to allow for a dropout rate of 10%. Dropout rate in the PPCOS II trial was 19.5% in the letrozole group however this was a longer study in

which they did 5 cycles and also a more involved study than the one we are proposing. Since there are fewer visits, fewer surveys and shorter enrollment period we anticipate a lower dropout rate in this trial.

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