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THE PREGNANCY IN POLYCYSTIC OVARY SYNDROME II (PPCOS II) TRIAL: RATIONALE AND DESIGN OF A DOUBLE-BLIND RANDOMIZED TRIAL OF CLOMIPHENE CITRATE AND LETROZOLE FOR THE TREATMENT OF INFERTILITY IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

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Abstract

Polycystic Ovary Syndrome (PCOS) is a common cause of female infertility and first line treatment is currently oral clomiphene citrate, a selective estrogen receptor modulator, which results in both a high nonresponse rate and multiple pregnancy rate. Aromatase inhibitors such as letrozole may have more favorable ovarian and endometrial effects. The goal of the Pregnancy in Polycystic Ovary Syndrome II (PPCOSII) study is to determine the safety and efficacy of

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clomiphene citrate (CC) compared to letrozole, in achieving live birth in infertile women with PCOS. The population will consist of 750 infertile women with PCOS. Additionally, the couple will have no other major infertility factor. This will be a multi-center, prospective, double-blind clinical trial of CC vs. letrozole for 5 treatment cycles (or approximately up to 25 weeks). The randomization scheme will be coordinated through the central data coordinating center (DCC) and the randomization is stratified by each participating site. After progestin withdrawal as needed, 750 women will be equally randomized to two different treatment arms: A) CC 50 mg every day for 5 days (day 3–7 of cycle), or B) letrozole 2.5 mg every day for 5 days (day 3–7 of cycle), for a total of 5 cycles or 25 weeks. The dose will be increased in subsequent cycles in both treatment groups for non-response or poor ovulatory response up to a maximum of 150 mg of CC a day ($\times 5$ days) or 7.5 mg of letrozole a day ($\times 5$ days). The primary analysis will use an intent-to-treat approach to examine differences in the live birth rate in the two treatment arms.

Keywords

Polycystic Ovary Syndrome; Infertility; Ovulation Induction; Hyperandrogenism; Clomiphene Citrate; Letrozole

1 INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common causes of female infertility (1). Anovulation (1), increased early pregnancy loss (2), and later pregnancy complications (3) all have been implicated in the poor fecundity of these women. The etiology of the syndrome is not fully understood (4) though insulin resistance and hyperandrogenism have been implicated. Further, the treatment of the syndrome is largely based on expert opinion (4, 5). There are insufficient adequately powered and designed clinical trials to guide the treatment of infertility in women with PCOS. Most of these trials have focused on surrogate outcomes such as circulating hormone levels or ovulation, while failing to assess the most meaningful outcome for an infertility trial, i.e. live birth (6, 7). The best treatment for achieving a live birth is unknown, though for decades clomiphene citrate (CC) has been the front line therapy to treat the disorder, and remains the most effective methodology, as observed in the previous Pregnancy in Polycystic Ovary Syndrome (PPCOS I) study conducted by the Reproductive Medicine Network (RMN) (8). In that study, CC was found to be three times more effective than metformin at achieving live birth, with no significant added benefit of the combination of metformin and CC.

However, the PPCOS I trial also highlighted the limitations of CC and the need for better ovulation induction agents. These limitations included a relatively low success rate (only 23% had a live birth in the CC group of the PPCOS trial and a further 25% never had a single documented ovulation during the study period), concern about multiple pregnancies (4–6% multiple pregnancy rate with one triplet gestation in the CC containing arms), and possible adverse effects of CC (including visual changes, ovarian hyperstimulation syndrome, and pelvic pain likely due to ovarian cyst formation). Other limitations include estrogen antagonism on target organs, such as potential thinning of the endometrium and decreased efficiency of embryo implantation, and concern about the long half-life and effects of accumulated CC metabolites on ovulation and pregnancy, including the possibility for fetal teratogenic effects. (9). Many women also experience vasomotor symptoms, including hot flashes, headaches, and mood changes during treatment with CC that limit its use (8).

Aromatase inhibitors (AIs), primarily letrozole, have been promoted as potent ovulation induction agents (10). These drugs were developed as adjunctive agents to treat breast

cancer and they work as selective aromatase inhibitors, thus preventing the conversion of androgen to estrogen. The resulting altered sex steroid ratio may release the hypothalamic pituitary axis from inappropriate and excessive estrogen feedback (which in PCOS results primarily from peripheral conversion of elevated circulating androgens) and increase serum FSH levels, which would in turn encourage healthy ovarian follicular development (11). Third generation AIs can be given orally and are well tolerated (main side effects are GI disturbances, asthenia, hot flushes and back pain). Their half-life is around 45 h, significantly shorter than CC. Studies have shown that AIs improve endometrial thickness compared to CC and while multiple follicles are induced, multiple gestations appear to be less likely with AIs (12, 13). These factors may lead to higher pregnancy rates and a greater likelihood of a singleton pregnancy.

The quality of the medical evidence supporting the use of AIs for ovulation indication prior to the development of our protocol was generally inadequate and based upon sparse data. Studies were limited by small sample size, lack of a reference treatment, vague details of randomization, inadequate blinding of study medication, poor allocation concealment, a focus on surrogate outcomes such as follicle number or endometrial thickness, and also by subject heterogeneity, including the inclusion of subjects with ovulatory disorders and unexplained infertility in the same trial (12–16). Further there are very limited data about the effects of AIs on the oocyte, embryo, or implantation, as well as concerns about potential teratogenic effects (9). The Pregnancy in Polycystic Ovary Syndrome II (PPCOS II) study protocol was conceived in order to avoid these prior limitations. We report herein the study design of our ongoing PPCOS II study.

2 MATERIALS AND METHODS

2.1 Primary and Secondary Hypotheses

Our primary research hypothesis is that ovulation induction with letrozole, an aromatase inhibitor, is more likely to result in live birth than ovulation induction with CC, a selective estrogen receptor modulator in infertile women with PCOS. Letrozole has been theorized to improve outcomes when used for anovulatory infertility compared to CC, including lower multiple follicular recruitment rate, lower multiple pregnancy rates, and higher pregnancy rates potentially due to more favorable endometrial development, with associated improved implantation. A safety hypothesis will also be incorporated into the primary research hypothesis, in which we hypothesize both treatments are equally safe for mother and child. The primary outcome measure is the occurrence of a live birth during the study period. The primary analysis of live birth rate within the two treatment conditions will employ an intent-to-treat approach. Hence, participants 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 have developed a number of secondary research hypotheses including: 1) Treatment with letrozole is more likely to result in a singleton pregnancy compared to treatment with CC. 2) Treatment with letrozole will less likely result in a first trimester intrauterine fetal demise than treatment with CC. 3) Treatment with letrozole is more likely to result in ovulation (increased ovulation rate) compared to treatment with CC. 4) The shortest time to pregnancy will be with letrozole. 5) Age, body mass index and serum sex hormone binding globulin (SHBG), testosterone, LH, Anti-Mullerian Hormone (AMH), and degree of hirsutism and acne will be significant predictors of ovulation and conception regardless of treatment. 6) Improvement during treatment in serum levels SHBG, testosterone, AMH, and LH levels will be significant predictors of ovulation and conception regardless of treatment assignment. 7) DNA polymorphisms in estrogen metabolism and target genes will predict response to study drug. 8) Quality of Life will be better on letrozole than CC. 9) Letrozole

will be more cost effective at achieving singleton pregnancies than CC. Our study will have limited power to address many of these secondary hypotheses.

2.2 Study Population

750 women with PCOS actively seeking pregnancy (or 375 per each treatment arm) aged ≥ 18 to ≤ 40 years will be enrolled at one of 11 participating sites. The overall goal of the inclusion and exclusion criteria is to identify a population of healthy women with PCOS who have anovulation or oligoovulation as the exclusive infertility factor. Anovulation will be obtained by history or anovulatory midluteal progesterone levels. In addition, all subjects will have evidence of either hyperandrogenism or polycystic ovaries on ultrasound. Hyperandrogenism will be determined by evidence of hirsutism on exam or by biochemical elevations in total testosterone or free androgen index. Thus in addition to oligomenorrhea, the presence of any one of the following: hirsutism, an elevated total testosterone level, an elevated free androgen index, or a polycystic ovary will qualify for the diagnosis of PCOS. The respective biochemical laboratory cutoffs for androgens may vary according to assay methodology, and each site will establish cutoffs prior to study initiation, as the androgen determinations that are used to diagnose PCOS will be performed at each site.

Key Inclusion Criteria for Female Subjects (Must have ovulatory dysfunction and either hyperandrogenism or PCO)

1. Chronic anovulation or oligomenorrhea: defined as spontaneous intermenstrual periods of ≥ 45 days or a total of ≤ 8 menses per year, or for women with suspected anovulatory bleeding, a midluteal serum progesterone level < 3 ng/mL will be taken as indicative of chronic anovulation. For women who have been on ovarian suppressive therapy or other confounding medication (i.e. insulin sensitizing agents) within the last year prior to the study, a history of ≤ 8 menses per year prior to the initiation of this therapy will qualify as evidence of oligomenorrhea. For women with more regular bleeding patterns, but who are suspected to be experiencing anovulatory bleeding, a midluteal progesterone level < 3 ng/mL will be evidence of ovulatory dysfunction and qualify as anovulation. Undiagnosed persistent vaginal bleeding should be diagnosed and treated prior to enrollment.
2. Hyperandrogenism (either Hirsutism or Hyperandrogenemia) or Polycystic Ovaries on Ultrasound:
  - a. Hirsutism is determined by a modified Ferriman-Gallwey Score > 8 at screening exam (17). Subjects who have hirsutism do not need local or core labs documenting elevated androgen levels.
  - b. Hyperandrogenemia can be determined from serum measurements performed at local labs. Local cutoffs will be pre-determined by each site prior to study initiation. Hyperandrogenemia will be defined as an elevated total testosterone, or free androgen index (FAI). The FAI is calculated from measurable values for total T and SHBG, as previously described (18), using the following equation: $(\text{FAI} = \text{Total testosterone in nmol/L} / \text{SHBG in nmol/L}) \times 100$. Outside lab values obtained within the last year documenting elevated T or FAI levels are sufficient to meet criteria of hyperandrogenemia.
  - c. Polycystic Ovaries on Ultrasound: For study eligibility, we will use the revised Rotterdam criteria for diagnosing polycystic ovaries (19). PCO will be defined as either an ovary that contains 12 or more follicles measuring 2–9 mm in diameter, or an increased ovarian volume ($> 10 \text{ cm}^3$) on one ovary for entry into the study. If there is a follicle > 10 mm in

diameter, the scan will be repeated at a time of ovarian quiescence in order to calculate volume and area if the subject does not otherwise qualify for the study. The presence of a single polycystic ovary, either by volume or morphology, is sufficient to provide the diagnosis.

We will exclude subjects with medical conditions that represent contraindications to CC, letrozole and/or pregnancy or who are unable to comply with the study procedures. We will exclude subjects with major medical morbidity, including poorly controlled Type I or Type II diabetes; undiagnosed liver disease or dysfunction (based on serum liver enzyme testing); renal disease or abnormal serum renal function; significant anemia; a history of deep venous thrombosis, pulmonary embolus, or cerebrovascular accident; uncontrolled hypertension, known symptomatic heart disease; history of or suspected cervical carcinoma, endometrial carcinoma, or breast carcinoma; undiagnosed vaginal bleeding, and use of other medications known to affect reproductive function or metabolism (e.g., oral contraceptive pills (OCPs), GnRH agonists and antagonists, anti-androgens, gonadotropins, anti-obesity drugs, somatostatin, diazoxide, ACE inhibitors, and calcium channel blockers). We will allow a 2-month washout period for subjects who desire to participate and discontinue exclusionary medications (most commonly OCPs, but also possibly metformin), and a period of observation or treatment for correctable conditions. Adjuvant therapy with other ovulation induction agents (GnRH agonists and antagonists, gonadotropins), triggering ovulation with hCG, or performing intrauterine or intracervical inseminations will not be allowed for study subjects in this protocol.

Couple Inclusion Criteria (because this is an infertility trial, we will insure that there are the following factors demonstrating reproductive competence present in the male and female prior to randomization.)

1. Sperm concentration of 14 million/mL in at least one ejaculate within the last year, with at least some motile sperm.
2. Ability and willingness to have regular intercourse during the ovulation induction phase of the study.
3. At least one patent tube and normal uterine cavity as determined by sonohysterogram, hysterosalpingogram, or hysteroscopy/laparoscopy within the last 3 years. An uncomplicated intrauterine non-IVF pregnancy and uncomplicated delivery and postpartum course resulting in live birth within the last three years will also serve as sufficient evidence of a patent tube and normal uterine cavity as long as the subject did not have, during the pregnancy or subsequently, risk factors for Asherman's syndrome or tubal disease or other disorder leading to an increased suspicion for intrauterine abnormality or tubal occlusion.
4. No previous sterilization procedures (vasectomy, tubal ligation) that have been reversed. The prior procedure(s) may affect study outcomes.

2.3 Study Design

This will be a multicenter, prospective, double-blind trial of oral CC vs. letrozole in the treatment of infertility in patients with polycystic ovary syndrome, for up to 5 treatment cycles with study medication. Participants will be randomized to receive either an initial oral dose of 50 mg of CC or 2.5 mg of letrozole for 5 days per menstrual cycle. They will be monitored at monthly intervals during the anticipated luteal phase for response to medication as measured by physical, ultrasound, and hormonal parameters.

The dose may be adjusted according to the response or maintained if adequate response is determined. The maximum dose of CC will not exceed 750 mg/cycle and the maximum dose

of letrozole will not exceed 37.5 mg/cycle. Treatment will continue for up to 5 cycles or approximately 25 weeks. With 5 treatment cycles, subjects who are resistant to study medication at the two lower doses will have up to 3 treatment cycles on the highest dose of study medication. The number of ovulations will not be a factor in determining treatment cycles, they may not exceed 5.

A double blind trial is needed to test our hypotheses and to establish the safety and efficacy of letrozole compared to the current gold standard, i.e. CC to induce ovulation in women with PCOS. In order to maintain the double-blind, CC and letrozole will be overencapsulated and packaged in identically appearing numbered study kits (using Almac Clinical Services, Durham NC) which will then be directly shipped to each clinical site. The RMN has used this approach successfully with CC in the PPCOS I study (8).

2.4 Randomization

Seven hundred fifty (750) women will be randomized to one of the two treatment conditions. Using a 1:1 treatment ratio, there will be approximately 375 women assigned to each treatment group. The scheme will be a stratified randomization with permuted blocking (the block size is randomly permuted among 2, 4, and 6) within each stratum. The only stratification variable will be by site. Almac statisticians will generate the randomization scheme for the study. Because this is a double-blind study, the randomization scheme (including block size) will be disclosed to the DCC data manager, but not to any RMN investigators or staff, including the Protocol Lead Investigator.

The site investigator will be provided a password protected account for WebEZ, which is a web-based secured randomization service. After the participant has signed an informed consent and all required baseline evaluation procedures have been completed, an Investigator or designee will log into WebEZ in order to randomize a participant into the trial. The WebEZ will query the site for participant eligibility information. If the participant is eligible, the site will be provided with a participant identifier and a study kit number. The Study Coordinator at each site will be responsible for storing, dispensing, and performing pill counts on the study medication.

2.5 Study Specific Visits and Procedures

An overview of the study visits is found in Table 1. After screening and baseline visits, a monthly cycle will consist of two visits. The first visit will be a monthly midluteal visit that includes questions, a brief exam, ultrasound, and blood work; and the second visit will be a menses visit at the time of menses or if menses are missed to obtain a serum pregnancy screen prior to starting the new dose of medication. This second visit may be performed off site to ease participant participation in the study.

Visit 1: Screening Visit—The goal of screening will be to establish a diagnosis of PCOS, to exclude major medical illnesses, and to verify that there are no other significant infertility factors in the couple. Height, weight, blood pressure, and waist and hip circumferences will be obtained. All participants who are 21 or older should have had a normal Pap smear as recommended by current American College of Obstetricians and Gynecologist (ACOG) guidelines. A hirsutism assessment will be made via the modified Ferriman-Gallwey hirsutism score by trained study personnel (17). An acne assessment will be made by our trained study coordinators using a standard acne lesion assessment (count) diagram and definitions.

Additionally we will measure facial sebum with a sebumeter (20–25). We are assessing acne and sebum as these are stigmata of peripheral hyperandrogenism that are easier to

characterize than hair distribution, and they may predict response to medication. An ultrasound exam will be performed with a transvaginal probe. The following measures will be obtained: ovarian size in three dimensions, the size of the largest ovarian follicle, antral follicle count, and ovarian morphology, uterine dimensions, leiomyoma presence and size, other uterine abnormalities, and endometrial thickness. Women will present to the initial screening visit after a 12 hour fast. Blood specimens will be sent as described above in the inclusion and exclusion criteria to identify appropriate study subjects, as well as for baseline parameters of interest (AMH, LH, FSH, Testosterone, Androstenedione, Progesterone, Insulin, Glucose, IGF-1), including DNA for our planned pharmacogenomic analyses.

Mood, quality of life, and sexual function will be assessed at baseline and at the end of the study visit. Mood and quality of life will be assessed by the Medical Outcomes Survey (Prime MD-PHQ), and Short Form 36 (SF-36) (26). Female sexual function will be assessed by the Female Sexual Function Inventory (FSFI) along with the Female Sexual Distress Scale (FSDS) (27). This measure is considered the “gold standard” paper and pencil assessment of sexual function and has excellent psychometric properties (27). The International Index of Erectile Function (IIEF) is a multidimensional scale for assessment of erectile dysfunction. The measure addresses the relevant domains of male sexual function (erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction) (28). We will assess quality of life relating to infertility with the FertiQol survey (29, 30). We will also obtain a validated PCOS Quality of Life (QOL) questionnaire (31, 32). Abnormal Sleep patterns (including sleep apnea) have been associated with insulin resistance, systemic inflammation, and increased cardiovascular risk in women with PCOS (33, 34). There may also be some impact on fertility and we will investigate this in our study. The Sleep Habits questionnaire, which was the standard measure used to collect data for the 10-year long multi-center NHLBI Sleep Heart Health Study, will be administered (35).

During the screening process, all participants will be provided with preconception counseling. At a minimum this will consist of offering to verify rubella immune status, offering to check Varicella status if there is no history of chicken pox, and offering HIV screening and referral for treatment as necessary. In addition, all participants will be given a prescription for prenatal vitamins containing a minimum of 400 micrograms of folate as recommended by the U.S. Public Health Service. All subjects who are obese (BMI > 30) will be counseled about the potential benefits of weight loss prior to conception and recommended to pursue this as a first line therapy. However, because anti-obesity therapy, whether it be lifestyle based or pharmacologic is frequently unsuccessful, and because there are no adequate studies demonstrating that weight loss improves outcomes in this population or any obese population, obesity *per se* will not be an exclusion criteria. Bariatric surgery for morbidly obese women with PCOS remains a treatment option, but because the role of bariatric surgery in improving reproductive outcomes is uncertain and because these procedures involve substantial risk and cost to study subjects, this will not be incorporated into the study protocol. Additionally, potential subjects who have undergone bariatric surgery (>12 months previously) must have reached a stable weight, be nutritionally replete, and meet all inclusion criteria to participate in the study.

Similarly, smokers will also be counseled about adverse effects and advised to stop smoking, though continued smoking is not a study exclusion. All participants will fill out the ACOG genetic risk factor questionnaire and if indicated, further counseling about genetic risks will be coordinated by the PI at each site. Participants who are rubella non-immune will be offered the rubella vaccine, and their entry into the study delayed for 1 month as per CDC recommendations.

All subjects will receive instruction and journal logs to track menstrual bleeding and intercourse during the study. All subjects (and couples where the male partner is present) will be instructed to have intercourse on a regular basis during the study period. The male partner, if not present, will be informed of this recommendation when informed consent for male participation in the study is obtained. The optimal frequency will be considered every 2–3 days. The monthly visit will include a query about dates of intercourse and review of the intercourse diary. The importance of regular intercourse will be mentioned if the frequency on any week is < 2 episodes/week. Referrals will be made to appropriate caregivers if there is evidence of sexual dysfunction.

Randomization will take place as soon as the eligibility requirements are met, although study drug will not be dispensed until the next visit (baseline visit).

Visit 2: Baseline Visit—The baseline visit will occur on Day 1–5 of the subject's cycle that either has occurred spontaneously or because she has experienced a withdrawal bleed after progestin challenge. Day 1 is defined as the first day of vaginal bleeding (spotting does not count). A study drug kit will contain 3 bottles of 5 pills each of either CC (50mg) or letrozole (2.5mg). At this time, the subjects will be dispensed home pregnancy tests to be used throughout the cycle if pregnancy is suspected, and will be given journal logs to record menstrual flow, drug administration, intercourse frequency and adverse events throughout the study. If the serum pregnancy test is negative, the subject will be instructed to begin her study medication.

Monthly Midluteal Visits on Study Drug—The monthly visit will take place in the anticipated luteal phase of the cycle, and initially will be scheduled 3 weeks after the initiation of medication, with a window of 4 days on either side of this day (i.e. Day 17–25, assuming a Day 3 medication start, or Day 19–27, assuming a Day 5 start). At the monthly visit, the subject will undergo the brief exam, the transvaginal ultrasound exam, and the fasting phlebotomy (for serum for the core lab) as described above in the eligibility visit and in the table of study visits. The brief exam at this visit and all subsequent visits will consist of weight, blood pressure, facial sebum measurement, urine pregnancy test, local serum progesterone level, medication dispensing and accounting, query for adverse events and concomitant medications, collection and review of journal logs. A serum pregnancy test may also be performed if the PI thinks it is necessary. The measures obtained from this visit that will help in the further management of the subject include a urine pregnancy test, a serum sample to the local lab to determine serum progesterone level, and the results of the ultrasound (including the endometrial thickness and echogenicity, as well as the number, size, and echogenic characteristics of follicles/cysts on the ovary (including an antral follicle count). Further management will be dependent on whether the subject has responded or not responded to the study medication based on the serum progesterone level.

There will be three possible scenarios at this visit: 1) the patient has an ovulatory progesterone level and will follow up for the monthly menses visit; 2) the patient has a progesterone level in the anovulatory range, and there is evidence of follicular development (i.e. a simple follicle with a mean diameter ≥ 12 mm is present on ultrasound), in which case a follow up visit to check a serum progesterone should be arranged within 2 weeks or a progesterone added to the monthly menses visit; or 3) the patient has a progesterone level in the anovulatory range and no evidence of follicular development, in which case the patient should be given either Progestin to induce a withdrawal bleed or study drug (at the next highest dose if applicable). In the presence of an ovulatory progesterone level at the midluteal ultrasound, the size and number of corpora lutea should not be over-interpreted, if the patient is without symptoms.

Responders—Responders will be identified on the basis of an elevated progesterone level consistent with ovulation (Progesterone > 3 ng/mL). An elevated level > 3 ng/mL is evidence of response (36). The rationale for this is that it may not be possible to determine a midluteal peak progesterone level, but that the study visit may overlap the peak, and be elevated, but not reach peak levels. While ultrasound may provide presumptive evidence of ovulation, serum levels of progesterone should guide further management.

Indeterminate Response—As noted above, the investigator will schedule a follow-up visit or phlebotomy based on the results of the ultrasound if there is evidence of follicular development, (i.e. a follicle with a mean diameter ≥ 12 mm), but the serum progesterone level is low or non-detectable. Such a finding may indicate that the subject is experiencing a longer than anticipated follicular phase but adequate follicular development. Subjects will be informed by telephone or email of the serum progesterone levels and their meaning, and the subsequent plan. After the menses visit with a documented serum pregnancy test, the patient will again take the study drug from Day 3–7 of the cycle. Subjects may start study medication up to day 5 of the cycle with appropriate adjustment of the midluteal visit. Responders will continue at the same response dose until pregnancy, or a total of five cycles (or approximately 25 weeks) is reached.

Non-Responders—Non-response will be defined as the lack of an elevated progesterone level at the midluteal or subsequent follow-up visit if one has been scheduled, or based on the clinical impression of the site PI. A subject who has not ovulated and does not have evidence of follicular development will, as noted above, either receive progestin or the next dose of study medication.

If the patient fails to respond to treatment after these visits, she will be instructed to increase her study drug by one tablet per day for five days. The start of the increased dose will be designated as Day 3 of her cycle if she has not experienced a withdrawal bleed. Withdrawal with progestin is not necessary, but if it is utilized by the site PI, instructions are found in the following section. This dose will continue at this level if adequate ovulatory response is achieved until the completion of a total of five treatment cycles or approximately 25 weeks is reached. The dose may be increased by the end of the second cycle to the maximum dose if further nonresponse is noted. This may involve more than one cycle with the maximum CC or letrozole dose, as there are a total of 5 cycles in the study. However study subjects will never take more than 3 tablets per day or 15 tablets total/per cycle of study drug.

Induction of Withdrawal Bleeds for Non-Responders—There will be no mandatory induction of withdrawal bleeds for non-responders during the course of the protocol, unless as dictated by the PI at the site. The benefits and/or risks of this for short amounts of time (i.e. ≤ 5 months as in this protocol) have not been well described in the literature and as in the initial PPCOS trial we have opted to dispense with mandatory interval progestin use. The use and indication for inducing a withdrawal bleed should be noted on the case report forms. During the induction of the withdrawal bleed with medroxyprogesterone acetate (5 mg $\times$ 10 days) or other comparable progestin, the patient will not re-initiate study drug until instructed by the site PI upon experiencing a withdrawal bleed and obtaining a negative serum pregnancy test and a negative serum progesterone test (Progesterone < 3 ng/mL). If there is no spontaneous withdrawal bleed after the use of medroxyprogesterone acetate, a negative serum pregnancy test and a negative serum progesterone test will be a precondition to further use of study drug.

Monthly Menses Visit—The purpose of this visit is to prevent exposure of a fetus to study drug during the critical early period of implantation and organogenesis. The monthly menses visit will be obtained at the time of menses (spontaneous after ovulation) or within 2

weeks after the midluteal visit in the case of no menses (after ovulation). In the case of a patient who had an ovulatory progesterone level at the midluteal visit, this visit will consist of a serum pregnancy screen to verify the subject's pregnancy status. In the case of progestin challenge, either with or without menses, the subject will have both a serum pregnancy screen AND a serum progesterone level. If the serum pregnancy test indicates pregnancy or the progesterone level is in the ovulatory range, then the subject should not take study drug. This visit (consisting of serum pregnancy screen and possibly a progesterone level) may be performed at an outside lab and the results faxed to the study investigator if that eases the burden of study participation for the study subject. The end of study visit may be performed at the menses visit at the end of the 5th cycle for subjects who do not conceive during the study.

Pregnancy Visits—A urine or serum pregnancy test will be done at each visit. The subject will also receive urinary pregnancy tests to use at home. Pregnancy will be confirmed, if suspected, by measurement of serum hCG. Pregnancies will be followed by the serial rise of serum hCG and when a threshold level is obtained (2,000–4,000 mIU/mL), ultrasound will be utilized to determine location of the pregnancy and number of implantation sites. Participants who conceive will be followed through the study until the pregnancy has advanced to the point of determining the number of gestational sacs, their location, and fetal viability as determined by visualization of fetal heart motion by ultrasonography. At this point, at approximately 6–8 weeks gestational age, women will be referred to their prior or referring practitioner, or to an appropriate health care provider for prenatal care.

End of Treatment Visit—An end of treatment visit will be performed at the end of the ovulation induction phase for women who do not conceive in the trial, or with pregnancy if women conceive. The end of treatment visit will be performed as early in pregnancy as possible. A brief exam with the addition of repeat assessments of acne with sebumeter and hirsutism will be performed (Table 1). Subjects will return remaining study drug, their journal logs, and a final assessment of adverse events and concomitant medications will be done. Baseline measures will be repeated in all subjects, including safety labs and QOL surveys, (FertiQol will not be repeated in pregnant women). A final collection of blood for the core lab will also be collected. For any women with a positive pregnancy test, an obstetrical ultrasound will be done to determine viability at 6 to 8 weeks. For those women who have an ongoing pregnancy, arrangements will be made to follow the outcome of the pregnancy at the end of first trimester and also after delivery or termination of gestation. All pregnancies (including multiples) will be followed to determine the abortion rate, complication rates, and to determine pregnancy outcomes. Participants will be instructed to notify study personnel of the outcome of the pregnancy and we will obtain release of record forms from their treating physicians to obtain copies of relevant medical records. Phone contacts will be initiated if the participant has not contacted study personnel by six weeks beyond the original estimated date of delivery. Delivery records will be requested to determine the birth weight, length of gestation, and any perinatal complication of mother or infant. This methodology proved very successful in PPCOS I for tracking pregnancy outcomes, and we will expand it in this study by obtaining more information about the neonatal course of the infant. At this visit, we will also obtain separate consent to follow infants after birth to determine neurodevelopmental and behavioral outcomes as discussed below.

Establishment of a Pregnancy Registry—As per the recommended guidelines by the FDA (<http://www.fda.gov/downloads/ScienceResearch/SpecialTopics/WomensHealthResearch/>

UCM133332.pdf), we intend to establish a pregnancy registry for this trial to evaluate outcomes of pregnancy. This will overlap with our existing protocol, but because it follows infants after birth, we will create a separate protocol and we will consent women who conceive individually to participate in this extended protocol.

We will track the outcomes of all randomized subjects who have a positive serum pregnancy screen during the course of this study. We will record biochemical pregnancies (defined as positive serum pregnancy screens without ultrasonographically detected pregnancies), ectopic pregnancies, and all intrauterine pregnancy losses both before and after 20 weeks including missed abortions, spontaneous abortions, elective abortions, fetal demises, and stillbirths. We will review pregnancy and birth records of the mother and of the fetus to establish neonatal morbidity and mortality and the presence of fetal anomalies. The infant will be examined at each site within 60 days of birth by a dysmorphologist for potential congenital anomalies. Fetal anomalies will be classified using the CDC birth defects code list (<http://www.cdc.gov/ncbddd/bd/documents/MACDPcode%200807.pdf>). We will extract from these records concomitant medical and obstetrical conditions, exposure information on all other medical products used, including prescription products, over-the-counter (OTC) products, dietary supplements, vaccines, and insertable or implantable medical devices.

We will file individual case reports for all congenital anomalies, which will be considered a serious adverse event. Additionally we will provide to the FDA a written annual status report of the pregnancy registry as specified in the guidelines above. As per our discussions with the FDA, we acknowledge that our study will have minimum power to detect congenital anomalies given the relatively low number of pregnancies; however, it is viewed as an important first step. We also intend at a minimum to perform an annual parent directed screening questionnaire to assess the infant's developmental milestones for the first three years after birth as recommended by the FDA and to review the child's CDC growth curves and medical records.

2.6 Data Analysis

The primary outcome is live birth, defined as delivery of any viable infant. There are good data about the per cycle cumulative live birth rate with CC from the PPCOS I trial (Table 2). Unfortunately, there are not comparative live birth outcome data from randomized trials of letrozole, given their differing designs and relatively small sample sizes as summarized in Table 3. The overall live birth proportion for subjects randomized to receive CC was 0.23 for the six month trial (0.20 for 5 months). Based on our experience in PPCOS I, we shortened the trial from 6 treatment cycles to 5 cycles. There are several reasons for this. First, the live birth rate appeared to drop in the final 2 cycles of the PPCOS I trial (Table 2). Second, we experienced greater dropout over time in the PPCOS I trial (exceeding 10% in all treatment arms which threatened the external validity of the trial) and by shortening the current trial we will decrease the absolute number of dropouts. Third, the change will reduce the cost of the trial while providing the same quality of scientific data. Lastly, it will allow a total of 3 attempts at the highest dose of clomiphene/letrozole, analogous to clinical practice. The problem with dropout in the PPCOS I trial appeared to be related to failure to respond (i.e. higher in the metformin group with a lower ovulatory rate), and it increased in all groups over time likely due to failure to ovulate and/or to conceive (8).

Minimum Clinically Important Difference—The PPCOS I trial was powered to detect a 15% absolute difference in pregnancy rates. Thus a benchmark of 15% difference between treatment groups was set as an important minimum for a clinically important difference. We will be more conservative in PPCOS II and choose a 10% absolute difference as clinically meaningful. We therefore hypothesize that letrozole will increase the live birth proportion

by an absolute difference of 0.10 (i.e., a live birth proportion of 0.30, and relative to the live birth rate with CC in PPCOS I (8), an approximate 50% increase).

Significance Testing—All primary statistical analyses will invoke an intent-to-treat paradigm, wherein all randomized subjects are included according to their treatment assignment, regardless of actual treatment received, protocol violations, etc. Primary efficacy analysis will be done by comparing the treatment groups with respect to the primary outcome of live birth using the Pearson chi-square test. As secondary, supportive analysis, we will fit a logistic regression model to compare the treatment arms with respect to the primary outcome of live birth, adjusting for other factors such as randomization, stratification of study site, and prior exposure to study medications. The data will be summarized using descriptive statistics for continuous variables (mean, standard deviation, number of observations, and quantiles) and frequency statistics (frequencies and percentages) for categorical variables. All hypothesis tests will be two-sided and all analyses will be performed using SAS software (SAS Institute, Inc., Cary, NC) or R (open source).

Sample Size Calculations—A sample size of 300 subjects in each arm of the randomization yields 81% statistical power to prospectively demonstrate a 0.10 absolute difference in live birth proportions between treatment arms (0.20 for CC and 0.30 for letrozole) using the Pearson's chi-square test with a two-sided significance level of 0.05. The sample size has been inflated to 375/arm to allow for a dropout rate of 20%. The dropout rate in the CC arm of the PPCOS I trial was 26.3%; however, that was a 6-month trial and involved weekly visits for progesterone levels, as ultrasound was not utilized. The monthly visits will be less burdensome to participants and will avoid the weekly disincentive of a negative progesterone level in poor responders, as we discovered in the PPCOS I design. We therefore anticipate a lower drop out rate in this trial.

We recognize that the magnitude and the direction of the effect of letrozole remains something of an educated guess. Therefore we provide in Table 4 varying scenarios for decreased and/or increased live birth proportions for letrozole and the accompanying power based on a fixed live birth ratio in the CC arm taken from the PPCOS I data.

Secondary Analyses—We propose a number of secondary analyses, including time to pregnancy, analyzing differences in ovulation, spontaneous abortion and multiple pregnancy rates, as well as developing baseline and treatment-related predictive models for response (incorporating DNA markers), and finally a cost effectiveness analysis. We acknowledge that the power of our study for identifying differences in spontaneous abortion and multiple pregnancy rates is low due to the overall low numbers of these events. However even if not significant, any treatment that would have lower multiple pregnancy rates could be seen as having great public health benefit, given the increased perinatal risks and societal costs of iatrogenic multiple pregnancy.

The analysis of other secondary outcomes will entail the application of statistical methods that have been developed for correlated data, since repeated observations will be made over time on each individual, and these methods allow for both within-group and between-group comparisons to be assessed. For secondary outcomes such as hormone levels, a linear mixed-effects model will be fit where the main independent variables will be treatment group, time, and their interaction as well as the designed randomization stratification factors as covariates (37).

We propose not to do an interim analysis. We had planned to perform an interim analysis in the initial PPCOS I trial, but the majority of subjects had been randomized by the time we had accumulated enough live birth outcome data to perform the interim analysis, and it was

omitted. We anticipate that due to similar rapid accrual of subjects, and the up to 9 month period after conception to determine live birth, that an interim analysis would similarly be difficult to perform. The final data analysis will be completed after all pregnancy outcomes in the trial have been collected. Except for emergencies, unblinding of individual study subjects will not take place until all subjects have delivered and reported outcomes to the DCC.

3. Results

3.1 Trial Registration and Conduct

The study protocol, the manual of operations and procedures (MOP), case report forms, informational brochures, advertisements, and informed consent forms have been reviewed and approved by each site participating in the study. We applied for and received an IND from the FDA (IND#: 101671; Serial #: 0002) for the use of letrozole for ovulation. The trial has been registered at clinicaltrials.gov (NCT#: NCT00719186).

The study is coordinated by the Collaborative Center for Statistics in Science with Heping Zhang as PI of the Data Coordinating Center at Yale University. All study sites are monitored on a regular basis, and quality of data tracked as well as protocol deviations and violations. We comply with the guidelines of Good Clinical Practice to ensure high quality of the conduct of the trial, the integrity of the data, and the confidentiality of the study subjects.

3.2 Study Funding, Governance, and Data Sharing

The study is funded through a cooperative agreement by the Eunice Kennedy Shriver National Institutes of Child Health and Human Development (NICHD) and is led by a steering committee consisting of the site PIs of the RMN, the PI of the DCC, an independently-selected chair, and the NIH project scientist. Each of these individuals has one vote. Decisions are reached by majority consensus and formal vote. The steering committee meets four times a year face to face, and monthly via phone conference. Additionally there are subcommittees including a protocol committee, a recruitment committee, a publication committee, a Data and Specimen Committee, etc. Additional sites were added (N = 4), led by investigators who had successfully completed training in a NIH sponsored Clinical Reproductive Endocrinology Scholar's Training program (known as CREST).

The RMN has an Advisory Board of individuals accomplished in research who contribute ideas to the development of protocols, the conduct of the trial and the analysis of data. Additionally, there is an independent Data Safety and Monitoring Board (DSMB) that meets regularly to review the conduct and results of the trial. The DSMB reviews the protocol and makes recommendations during its development and prior to its review by local IRBs. It reviews immediately any serious adverse events and adjudicates these. The DSMB also can recommend termination of the trial due to a variety of reasons including poor recruitment, adverse effects of study medications, or a clear trend in one of the blinded treatment arms in live births exceeding our expectations. There are no formal stopping parameters for this trial.

Drug was purchased, encapsulated, and packaged by subcontract through the RMN by Almac Clinical Services (Durham, NC). No pharmaceutical company contributed drug to this trial. Further, we plan to utilize a core lab at the University of Virginia (Ligand Assay and Analysis Core of the Specialized Cooperative Centers Program in Reproduction and Infertility Research headed by Dan Haisenleder, Ph.D.) to run baseline and monthly visit serum samples (including estradiol, progesterone, testosterone, SHBG, etc.) according to a

common assay for the purposes of analysis of our secondary outcomes and for publication of results.

The study has a formal data and resource sharing policy as stipulated by the NIH. A separate committee handles requests for access to data and samples from the trial. However, no samples or data are available to outside investigators until 12 months after the completion of the data collection or the publication of the primary outcome paper, whichever is earlier. Further, access must be formally requested and approved by the RMN's Resource and Data Access (RADA) Committee. Full details can be found at the home web site of the RMN (<http://c2s2.yale.edu/rmn/>).

3.3 Study Progress and Recruitment

The first site began recruiting in November 2008. There are currently 7 RMN sites and 4 affiliated CREST sites recruiting subjects. Through August 2011, 650 subjects have been randomized. Study subjects have been recruited in a variety of fashions, including through the clinical practices of investigators and associated university or local practices, through advertisement in radio, television, and print media, through posting on a wide array of internet sites including clinicaltrials.gov, Craig's List, University web sites, RMN web sites, and through referral from study subjects themselves and other study coordinators. We anticipate that recruitment will be completed by the end of December 2011.

After recruitment is complete, we estimate an additional 6 months will be required for subjects to complete the protocol, and an additional 9 months for completion of gestation and birth of any pregnancies. Thus we would anticipate preliminary results and a primary outcome manuscript sometime towards the end of 2013.

4.0 DISCUSSION

PPCOSII is a clinical trial designed to test the efficacy of CC vs. letrozole on live birth in women with PCOS. It will be the largest trial utilizing these agents, and the largest infertility trial ever conducted in women with PCOS. There are several unique aspects to this trial. These include the consenting and incorporation of male partners into the protocol, the similarities of the dosing regimens allowing for only one study medication per kit, the serial use of ultrasound to monitor results, the incorporation of multiple secondary hypotheses, and the creation of a specimen repository.

At the request of the DSMB, we consented all male partners for participation in the study. Their participation involved agreeing to contribute a semen specimen for screening, to be a parent, to provide self-reported demographic and biometric information, to participate in regular intercourse with their female partner, and to complete a male quality of life reproductive questionnaire at baseline and end of study. Males have often been ignored in infertility trials, with the primary emphasis traditionally on the female. We sought to incorporate the male partner fully into the trial. We had noted in our previous PPCOS I trial a declining frequency of intercourse with progressive study participation, and the reasons for this were uncertain (38). We theorized a greater involvement of the male may increase compliance with intercourse frequency.

In our original PPCOS I trial, we had two types of study medication, CC or placebo and metformin or placebo, each in a separate kit. Thus, during a given study cycle subjects had to take two different pills. The current study does not require a placebo, because it involves medications that are given according to a common dosing regimen, i.e. begin with one pill a day for 5 days in the early follicular phase, and if the participant responds poorly, the dose can be increased by one pill a day till a maximum of 3 pills a day or a total of 15 pills/cycle

are given. The medication kits are identical appearing, though they contain either CC or letrozole. This feature of the protocol simplifies pill taking for the subjects in our study.

In our previous study, we monitored for ovulation with weekly progesterone levels. This proved burdensome for study subjects, as clinical practice tends to have one monthly monitoring visit to determine ovulation. A long string of visits with serum progesterone levels in the anovulatory range was discouraging to our study subjects and may have contributed to slightly higher than expected dropout rates. In this study, we opted for one midluteal visit that includes serum progesterone level and a transvaginal ultrasound. The purpose of the ultrasound at this timepoint is multifold. It can serve as both a safety and efficacy measure. It can serve as a safety measure by identifying large ovarian cysts, enabling the introduction of a rest cycle to allow for resolution prior to rechallenging the subject with study drug. It can serve as an efficacy measure by capturing the size and echogenicity of ovarian cysts for presumptive determination of ovulation (and multiple ovulation) or endometrial effects (i.e. thickness of the endometrium). These variables, including serial antral follicle counts and ovarian volumes, may also predict response to drug and pregnancy.

Finally based on recommendations from the FDA, we established a pregnancy registry with examination of each newborn by a pediatric dysmorphologist, and three year follow up, including review of pediatric records, growth curves, and yearly administration of a developmental questionnaire. This degree of scrutiny was prompted by concerns about the potential teratogenicity and developmental effects of letrozole when administered periconceptually. In November of 2005, Novartis of Canada, based on data presented at a national infertility meeting, issued a black box warning about the potential teratogenicity of letrozole. However these data have never been published in a peer reviewed journal, so it is not possible to assess their strength or quality.

In response, 5 Canadian fertility centers reviewed their birth outcomes and incidence of congenital malformations on letrozole and compared them to CC (9). The Canadian study involved 911 newborns from women who conceived following CC or letrozole treatment, involved examination of medical files of both mother and newborn, and cross-checked with the parents by telephone calls (9). Overall, congenital malformations and chromosomal abnormalities were found in 14 of 514 newborns in the letrozole group (2.4%) and in 19 of 397 newborns in the CC group (4.8%). The major malformation rate in the letrozole group was 1.2% (6/514) and in the CC group was 3.0% (12/397). One newborn in the letrozole group was found to have a ventricular septal defect (0.2%) compared to 4 newborns in the CC group (1.0%). In addition, the rate of all congenital cardiac anomalies was significantly higher ($P: 0.02$) in the CC group (1.8%) compared to the letrozole group (0.2%).

The authors concluded that there was no difference in the overall rates of major and minor congenital malformations among newborns from mothers who conceived after letrozole or CC treatments. However, it appears that congenital cardiac anomalies maybe less frequent in the letrozole group. It is important to note that these same investigators have pioneered and advocated the use of letrozole as an ovulation induction adjuvant, and thus may have a potential conflict of interest. However these data are invaluable for evaluating the fetal safety of exposure to this medication (and to CC which is approved for this indication with no significant known fetal risks).

An additional concern of AIs is that aromatase action in the brain is thought to be vital to normal neural development and function (39). Interruption of aromatase activity in the developing fetus through preconceptional or postconceptional exposure may result in altered

infant development and therefore we are following the infants carefully for the first three years of life.

Obviously, a study of the size proposed in this protocol will have low power to detect rare congenital malformations; however, it is a needed start and may prove useful when such monitoring becomes the standard. The fate of children resulting from infertility therapy has received increased scrutiny in recent years and most scrutiny has focused on in vitro fertilization (IVF), where results and outcomes are more closely followed than with first-line infertility therapy.

Since our trial was initiated, the best data about the effects of AIs on ovulation induction have come from two industry sponsored trials of anastrozole in women with PCOS and infertility, one using a single dose regimen and the other a 5 day regimen (43, 44). The single dose ranging study utilizing a single dose of anastrozole (administered at 5, 10, 20, and 30 mg) had a lower first cycle ovulation rate than 50 mg of CC (43). The second dose ranging study utilizing a 5 day regimen of anastrozole showed ovulation rates in the first cycle for anastrozole at 1, 5, and 10 mg/d were 30.4% (n = 24), 36.8% (n = 28), and 35.9% (n = 14), respectively, compared with 64.9% (n = 50) for CC at 50 mg/d (44). In up to three cycles of treatment, cumulative ovulation rates did not differ between groups. Overall, the pregnancy rates were lower with anastrozole than CC (though not significantly due to small numbers). However, the efficiency of pregnancy/ovulation was better with anastrozole. In participants who ovulated on either drug, clinical pregnancy rates were higher with anastrozole 5 mg than with CC (anastrozole 1 mg/d, 16.7%; anastrozole 5 mg/d, 25.0%; CC, 20.0%; χ^2 overall $P < .001$) (44).

Overall while these results were discouraging with respect to a greater benefit of AIs for ovulation induction, we are encouraged by the greater efficiency of anastrozole in achieving pregnancy as ovulation remains a surrogate variable in infertility treatment. We were aware of these studies when we designed our trial, but elected to use letrozole as there were more data available about its risk-benefit ratio, and because its patent was soon expiring which, we theorized, would allow greater access to this medication if efficacious. Further, there were data to suggest that anastrozole and letrozole have varying effects when used as ovulation induction agents (45), with letrozole appearing more effective (15). Our primary outcome is also livebirth, not ovulation. We will thus not be able to generalize from our results with letrozole to other AIs, or from CC to other SERMs.

We have multiple secondary hypotheses related to predictive factors including clinical, biometric, biochemical, and genetic factors that may be relevant to individualizing therapy for infertility in women with PCOS. Additionally, we are systematically and thoroughly capturing quality of life in both male and female participants to better understand factors that contribute to stress in infertility and its treatment. We hope to use these findings to better design studies and clinical treatments in the future to maximize participant comfort and minimize stress. We have the ability to extrapolate from our study design (which more closely mimics clinical practice) to perform cost effectiveness analyses of our treatments. Additionally, we are collecting in this trial extra serum and DNA for a specimen repository that we have established for trials in the RMN (46). This is to both serve as a resource for the RMN for post hoc hypotheses that arise after reviewing the data that we then can test, but also for other investigators who can apply for access to stored specimens and data through our RADA committee.

In summary, we soon hope to complete enrollment of this unique trial to discover alternate safe and effective front-line ovulation induction therapies in women with PCOS. Further, we

have extended the continuum of infertility trials from preconception to three years post delivery, while incorporating father, mother and child into our studies.

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Table 1

Overview of study visits

	Screening Visit	Baseline Visit (Day 3)	Mont hly Visit Cycle 1	Men ses Visit 1	Mont hly Visit Cycle 2	Men ses Visit 2	Mont hly Visit Cycle 3	Men ses Visit 3	Mont hly Visit Cycle 4	Men ses Visit 4	Mont hly Visit Cycle 5	Menses Visit 5 and End of Treatment Visit
Visit #	1	2	3	4	5	6	7	8	9	10	11	12
History												
Full Exam												
Brief Exam												
Serum Pregnancy Screen												
Safety/Eligibility Labs												
Fasting Phlebotomy for Study Parameters												
TV U/S												
QOL Measures												
Blood for DNA												
Blood for Repository												

Physical Exam (Full & Brief): Height, weight, hip and waist circumference, blood pressure, facial sebum measurement, urine pregnancy test, local serum progesterone level, medication dispensing and accounting, query for adverse events and concomitant medications, collection and review of intercourse diaries and menstrual diaries.

Fasting Phlebotomy = Serum for the Central Core Laboratory

Transvaginal ultrasound will include endometrial thickness, ovarian volume, antral follicle count and size of ovarian cysts or developing follicles

QOL Measures = FSFI, FSDS, SF-36, prime MD-PHQ, FerriQoL, Sleep Habits and PCOS-QOL questionnaires

Table 2

Per Cycle Ovulation and Live Birth Results in Clomiphene arm of the PPCOS I study (8)

Visit	Ovulations/ subjects	Live Birth/ ovulations
Visit 1	90/209 (43.1%)	10/90 (11.1%)
Visit 2	90/181 (49.7%)	10/90 (11.1%)
Visit 3	86/159 (54.1%)	9/86 (10.5%)
Visit 4	70/141 (49.6%)	10/70 ((14.2%)
Visit 5	58/119 (48.7%)	2/58 (3.4%)
Visit 6	55/99 (55.6%)	6/55 (10.9%)

Table 3
Conception rates with Letrozole alone and vs. Clomiphene Citrate (CC) in women with PCOS in published trials through 2008

Author	Type	Total Subjects	Failed CC	Durations	Conception Rate (Letrozole Group)	Conception Rate (CC group)
	Non-Randomized					
Mitwally et al, 2000	Open label letrozole only	N = 10 (PCOS)	Yes	One cycle	20% (2/10)	N/A
Mitwally et al, 2001 (14)	Open label letrozole only	N = 12 (PCOS)	Yes	One cycle	25% (3/12)	N/A
Elnashar et al, 2006 (16)	Open label letrozole only	N = 44 (PCOS)	Yes	One cycle	13.6 % (6/44)	N/A
	Randomized Controlled trials					
Bayar et al, 2006 (13)	Double blind	N = 46 (anovulatory infertility)	No	Multiple, mean = 2.6 cycles	9% (5/52)	12% (9/67)
Atay et al 2006 (12)	Open label	N = 106 (PCOS)	No	Not stated	21.6% (11/51)	9% (5/55)
Badawy et al, 2008 (47)	Open label	N = 220 (PCOS)	No	Multiple mean = 2.3 cycles	37.6% (82/208)	43% (94/220)
Begum et al, 2009 (47)	Open label	N = 64 (PCOS)	Yes	Up to 6 cycles	40.3% (13/32)	19% (6/32)

Table 4

Power based on varying Letrozole Live Birth Proportions and CC Live Birth Rate from PPCOS I trial.

PPCOS Clomiphene Live Birth Proportion	Letrozole Live Birth Proportion	N per Group (0% Dropout)	N per Group (20% Dropout)	Power (%)
0.20	0.05	300	375	100.0
0.20	0.10	300	375	93.1
0.20	0.15	300	375	36.4
0.20	0.25	300	375	31.1
0.20	0.30	300	375	80.9
0.20	0.35	300	375	98.6