National Institute of Child Health and Human Development
Reproductive Medicine Network

PPCOS-PREGNANCY IN POLYCYSTIC OVARY SYNDROME: A 30 WEEK DOUBLE-BLIND RANDOMIZED TRIAL OF CLOMIPHENE CITRATE, METFORMIN XR (EXTENDED RELEASE), AND COMBINED CLOMIPHENE CITRATE/METFORMIN XR (EXTENDED RELEASE) FOR THE TREATMENT OF INFERTILITY IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

Lead Investigator: Richard S. Legro, MD
Pennsylvania State College of Medicine

Co-Investigator: John Nestler, MD
Specialized Cooperative Centers Program in Reproduction Research

TABLE OF CONTENTS

I. PROTOCOL SYNOPSIS

Objectives: To determine the safety and efficacy of clomiphene citrate (Clomid, Serophene), metformin XR (extended release form of metformin with brand name Glucophage XR), or combined therapy of the two in achieving live birth. We hypothesize that: combined clomiphene citrate/metformin XR treatment would result in higher live birth rate than either metformin XR clomiphene citrate alone.

Patient population: 678 infertile PCOS women, (elevated testosterone levels and oligomenorrhea ≤ 8 spontaneous menses/yr., exclusion of secondary causes of hyperandrogenemia) seeking pregnancy, ages 18-39, with at least one patent fallopian tube, normal uterine cavity, and partner with sperm concentration of 20 million/mL in at least one ejaculate.

Study Design: 30 week, double blind, randomized trial of three treatment regimens in infertile PCOS women.

Treatment: After progestin withdrawal, 678 women equally randomized to three different treatment arms: A) metformin XR 1000 mg twice per day, B) clomiphene citrate 50 mg every day for 5 days (day 3-7 of cycle), or C) combined metformin XR 1000 mg twice per day with clomiphene citrate 50 mg/d for 5 days (day 3-7 of cycle) for a total of 30 weeks.

Primary efficacy parameter: Live birth rate

Secondary efficacy parameters: Singleton live birth rate, ovulation rate, cycles to first ovulation, cycles to conception (defined by positive serum HCG), abortion rate, cycles to pregnancy (defined by detection of fetal heart beat), and weeks from pregnancy to live birth.

Statistical Analysis: The primary analysis will use an intent-to-treat approach to examine differences in the live birth rate among three treatment arms. A chi-square test will be used to compare the proportion of live births between the combined clomiphene citrate/Metformin XR group versus the group of either Metformin XR alone or clomiphene citrate alone that has the higher proportion of live births. If this comparison is not significant a chi-square test will be used to compare the proportion of live births between the Metformin XR alone versus clomiphene citrate alone. Logistic regression models will be used in secondary (or supplemental) analyses to evaluate the predictive value of treatment condition, clinical site, prior exposure to either clomiphene citrate or metformin XR, body mass index, and other explanatory variables on binary outcomes (e.g., , abortion, ovulation). Cox proportional hazards models and a Kaplan-Meier method will be applied to compare time to event (eg. Cycles to first ovulation, cycles to pregnancy) between the treatment groups. An interim analysis, incorporating an O'Brien-Fleming adjustment, will be performed when half of the study participants have completed treatment.

Anticipated time to completion: A total of three to four years will be required to complete the study; two year enrollment period, 30 week treatment period, with one year additional observation to determine pregnancy outcomes. This will be accomplished by enrolling 50-90 PCOS women per center over the two-year enrollment period. There are 12 centers currently participating. Other centers may be added.

II. BACKGROUND

Polycystic ovary syndrome (PCOS) is characterized by excess circulating androgen levels and chronic anovulation. The fundamental pathophysiologic defect is unknown, but PCOS is characterized by insulin resistance and compensatory hyperinsulinemia. The evidence for this has been consistent and overwhelming (1-6). Insulin resistance has been found in PCOS women of many racial and ethnic groups implying both that it is a universal characteristic and that a common defect may underlie its prevalence (7-10).

Improving Insulin Sensitivity in PCOS: Improvements in insulin sensitivity in PCOS women, either through lifestyle changes or through pharmaceutical intervention, have consistently resulted in a marked improvement in the reproductive and metabolic abnormalities in PCOS. Resumption of ovulation occurs in up to 60-70% of PCOS women (11-14).

The longest and most varied published experience (in terms of number of studies) with any agent that improves insulin sensitivity in PCOS women has been with metformin, brand name Glucophage. Metformin was approved for the treatment of typeII diabetes by the FDA in 1994, but was used clinically for close to 20 years in other parts of the world. Metformin is a biguanide that works primarily by suppressing hepatic gluconeogenesis, but it also improves insulin sensitivity in the periphery (primarily skeletal muscle). Its use in PCOS women has been accompanied by a reduction in circulating insulin levels, a decrease in androgen levels and resumption of menses (15-17). These beneficial effects have been noted in both lean and obese PCOS women (18). Randomized studies with metformin have consistently shown improvement in insulin action and lowered serum androgens in PCOS women (16;19;20).

When metformin was used specifically to induce ovulation and was compared to clomiphene citrate alone, metformin achieved a significantly higher ovulation rate than clomiphene citrate alone (20). These women were only followed for 35 days on metformin, but achieved a 35% ovulation rate in this period (20). Studies which systematically examined menstrual regularity in PCOS women on metformin over a longer period of time have noted substantial improvement. Regular cycles, suggestive of monthly ovulation, have ranged from 69-96%(17;21;22). Few studies have addressed the issue of pregnancy in PCOS women on metformin and these have been observational and limited by a small sample size. (17;23) A more complete discussion of these trials is found in discussion of the power analysis below under IV B. Sample Size.

The preliminary evidence suggests that metformin in PCOS women is well tolerated, results in spontaneous ovulation, and is without known adverse effects during early pregnancy. Recently a new sustained release form of metformin, metformin XR with brand name Glucophage XR, has become available, and while similarly efficacious in the treatment of type II diabetes as metformin, it has a more favorable side effect profile. There are no published reports of the efficacy and tolerability of metformin XR in women with PCOS.

III. Objectives

A. Primary Research Hypotheses

The primary hypothesis is that improving insulin sensitivity and inappropriate gonadotropin secretion in infertile women with polycystic ovary syndrome (PCOS) with metformin and clomiphene citrate is more likely to result in a live birth than treatment with Metformin alone or clomiphene alone.

Primary Outcome Measure

The primary outcome measure is the presence/absence of a live birth during the study period. The primary analysis of live birth rate within the three treatment conditions will employ an intent-to-treat approach. Hence, 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.

B. Safety Hypothesis

The hypothesis is that adverse event rates between regimens will be comparable.

Safety Measures

Safety measures will be the number and category of reported adverse events

C. Secondary Research Hypotheses

1. Age, body mass index, testosterone, insulin, glucose, and proinsulin levels will be significant predictors of ovulation and conception regardless of treatment.
2. Improvement in body mass index, testosterone, insulin, glucose, and proinsulin levels will be significant predictors of ovulation and conception regardless of treatment.
3. Treatment with metformin XR in combination with clomiphene citrate is more likely to result in singleton pregnancy compared to treatment with Metformin XR alone or clomiphene citrate alone. Singleton pregnancy is defined as presence of an intrauterine gestational SAC with a fetal pole and observable heart motion.
4. Treatment with metformin XR alone or in combination with clomiphene citrate will less likely lead to a first trimester intrauterine fetal demise than treatment with clomiphene citrate alone.

5. Treatment with metformin XR alone is more likely to result in ovulation (increased ovulation rate) compared to treatment with clomiphene citrate. Treatment with both is most likely to result in ovulation.
6. The shortest time to pregnancy will be with metformin XR/clomiphene citrate, followed by metformin XR, followed by clomiphene citrate.

D. Planned / Supplemental Analyses

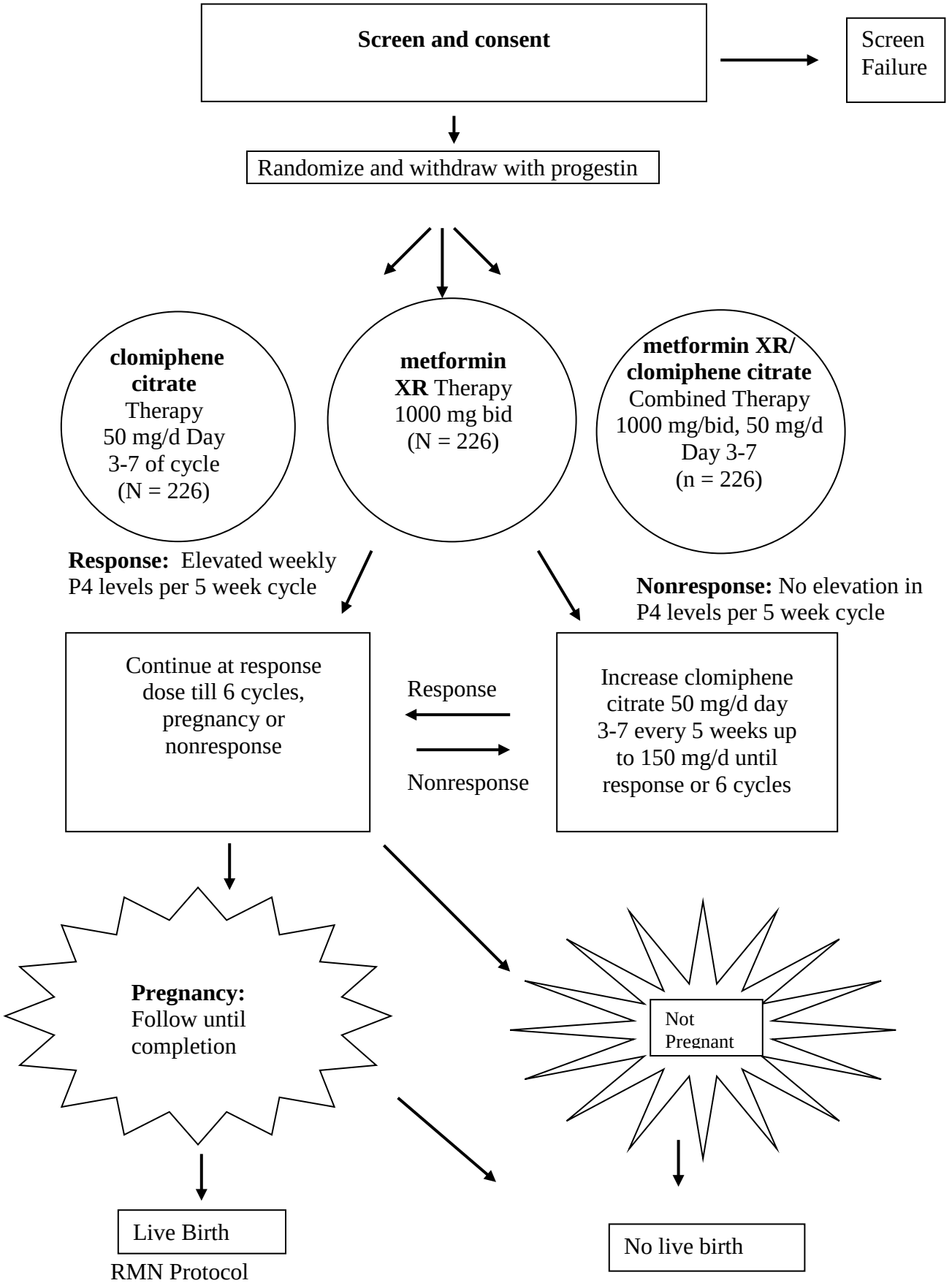
A set of supplemental analyses/sensitivity analyses, will be conducted to evaluate the effect of treatments by excluding randomized patients who were later discovered to be not eligible, or by excluding patients who did not adhere to the assigned drug regimen.

IV. Study Design

A. Overview

The study will be a multicenter, double blind, randomized trial designed to determine the most effective method of achieving a live birth in infertile PCOS women. Patients will be randomized to one of three treatment strategies: 1) metformin XR alone, 2) clomiphene citrate alone, or 3) combined metformin XR and clomiphene citrate.

A1. Schematic of the study design: (see following page)


Screen and consent
Screen Failure
Randomize and withdraw with progestin
clomiphene citrate Therapy 50 mg/d Day 3-7 of cycle (N = 226)
metformin XR Therapy 1000 mg bid (N = 226)
metformin XR/ clomiphene citrate Combined Therapy 1000 mg/bid, 50 mg/d Day 3-7 (n = 226)
Response: Elevated weekly P4 levels per 5 week cycle
Nonresponse: No elevation in P4 levels per 5 week cycle
Continue at response dose till 6 cycles, pregnancy or nonresponse
Response
Nonresponse
Increase clomiphene citrate 50 mg/d day 3-7 every 5 weeks up to 150 mg/d until response or 6 cycles
Pregnancy: Follow until completion
Not Pregnant
Live Birth
No live birth

A.2 Study Population

678 PCOS women actively seeking pregnancy (or 226 per each treatment arm) aged 18-39 years will be enrolled in the 12 sites (eight Reproductive Medicine Network centers and four U54-SCCPRR centers) over a 2-year enrollment period. The overall goal of the inclusion and exclusion criteria is to identify a population of healthy PCOS women with anovulation as the exclusive infertility factor. Local laboratory cutoffs for inclusion/exclusion values may vary according to assay methodology. Where existing medical records are used to verify inclusion or exclusion criteria, the site should keep a copy of these in the source documents. A general list of exclusionary medications requiring a washout period is found in appendix D. This list is not exhaustive and questionable medications can be looked up to see if they belong to one of the families of exclusionary medications or the DCC can be enqueried.

Inclusion Criteria

1. Chronic oligo/anovulation. Oligomenorrhea defined as spontaneous intermenstrual periods of $\geq$ 45 days or a total of $\leq$ 8 menses per year. 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 $\leq$8 menses per year prior to the initiation of this prior therapy will qualify as evidence of oligomenorrhea.
2. Hyperandrogenemia defined by either an elevated total testosterone, or free and weakly bound testosterone, or free testosterone as determined by normative values at each local lab in the multicenter study. Any or all of these measures of circulating testosterone levels can be obtained, although an elevated level of any one of them is sufficient to meet the criteria of hyperandrogenemia. As a general rule, a total

testosterone > 50 ng/dL or a free and weakly bound testosterone > 10 ng/dL should allow entry into the study, but values will vary according to site. (All testosterone values for the purpose of the publication will be repeated by a central lab, but these values will not affect recruitment/enrollment). The cutoff level for these assays will vary from lab to lab, but will be determined prior to the initiation of the study by each P.I. The P.I. will report these values to the DCC at Duke. Because each site may have multiple practice sites, and potentially different labs for each site, there may be more than one testosterone cutoff for a given site. The unifying inclusion criterion is biochemical confirmation of androgen excess. Outside lab values obtained within the last year documenting elevated testosterone levels are sufficient to meet criteria of hyperandrogenemia. All levels drawn at an outside lab must be signed and dated by the P.I. indicating the level is considered clinically elevated and is consistent with hyperandrogenemia.

3. In good general health off of current medications which may confound response to study medications (gonadotropins, thiazolidinediones, etc).
4. Age greater than or equal to 18 years and less than 40 years at the time of obtaining informed consent.
5. Desire to seek pregnancy actively during the study period.

Couple Inclusion Criteria

1. Semen analysis in the man: Sperm concentration of 20 million/mL or greater in at least one ejaculate within the last year. Results obtained within 1 year from date of consent may be used.

2. Ability to have regular intercourse 2-3 times per week during the course of the study.

3. At least one patent tube and normal uterine cavity as determined by sonohysterogram, hysterosalpingogram or hysteroscopy/laparoscopy. Results obtained within 3 years prior to date of consent may be used.

Exclusion Criteria

1. Current pregnancy. For patients who experienced a spontaneous abortion, 3 months must pass prior to randomization.
2. Patients on oral contraceptives, depo progestins, or hormonal implants. A three-month washout period will be required prior to screening for patients on these agents. Longer washouts may be necessary for certain depot contraceptive forms such as Norplant, where the implants are still in place. A one-month washout will be required for patients on oral cyclic progestins.
3. Patients with hyperprolactinemia (defined as two prolactin levels at least one week apart > 30 ng/mL or as determined by local normative values). The goal of eliminating patients with documented hyperprolactinemia is to decrease the heterogeneity of the PCOS population. These patients may be candidates for ovulation induction with alternate regimens (dopamine agonists). A normal level within the year prior to date of consent is adequate for entry.
4. Patients with known 21-hydroxylase deficiency or other enzyme deficiency leading to the phenotype of congenital adrenal hyperplasia. 21-hydroxylase deficiency will be excluded in all patients by a fasting 17-hydroxyprogesterone (17-OHP) level <2 ng/mL(24). If relevant, this level should be determined in the follicular phase (if the patient is in the luteal phase of an infrequent ovulatory cycle). In the case of elevated fasting 17-OHP levels, an ACTH stimulation test will be performed. A 1-hour stimulated value > 10 ng/mL will be an exclusion (24). As 21 hydroxylase

deficiency is a congenital condition, any normal level in the past of 17-hydroxyprogesterone allows entry into this study.

5. Patients with menopausal levels of FSH (> 20 mIU/mL). A normal level obtained within one year prior to date of consent is adequate for entry.
6. Patients with uncorrected thyroid disease (defined as TSH < 0.2 mIU/ML or >5.5 mIU/mL). A normal level obtained within the year prior to date of consent is adequate for entry.
7. Patients diagnosed with Type I or Type II diabetes (defined as a fasting serum glucose > 125mg/dL on two occasions (25)), or patients receiving antidiabetic medications such as insulin, thiazolidinediones, acarbose, or sulfonylureas; patients currently receiving metformin XR for a diagnosis of Type I or Type II diabetes are also specifically excluded. A normal level within the year prior to date of consent is adequate for entry.
8. Patients with liver disease defined as AST or ALT > 2 times normal or total bilirubin >2.5 mg/dL. These labs must be drawn within 30 days prior to randomization.
9. Patients with renal disease defined as BUN > 30 mg/dL or serum creatinine> 1.4 mg/dL. These labs must be drawn within 30 days prior to randomization.
10. Patients with significant anemia (Hemoglobin < 10 mg/dL). This lab must be drawn within 30 days prior to randomization.
11. Patients with a history of deep venous thrombosis, pulmonary embolus, or cerebrovascular accident.
12. Patients with known heart disease (New York Heart Association Class II or higher).
13. Patients who have previously undergone ovarian wedge resection, cautery or other surgical ablative procedure of the ovary, also women who have undergone

hysterectomy. A congenital absence of an ovary or a unilateral oophorectomy is not an exclusion.

14. Patients with a history of, or suspected cervical carcinoma, endometrial carcinoma, or breast carcinoma. If a normal Pap smear result within 12 months prior to date of consent is not available for review, this procedure must be completed during screening.
15. Patients with a current history of alcohol abuse. Alcohol abuse is defined as > 14 drinks/week or binge drinking (≥ 6 drinks at one time).
16. Patients enrolled simultaneously into other investigative studies that require medications, proscribe the study medications, limit intercourse, or otherwise prevent compliance with the protocol. Patients who anticipate taking longer than a one month break during the protocol should not be enrolled.
17. Patients taking other medications known to affect reproductive function or metabolism. These medications include oral contraceptives, GnRH agonists and antagonists, antiandrogens, gonadotropins, anti-obesity drugs, somatostatin, diazoxide, ACE inhibitors, and calcium channel blockers. The washout period on all these medications will be three months.
18. Patients with a suspected adrenal or ovarian tumor secreting androgens.
19. Patients with suspected Cushing's syndrome.
20. Couples with previous sterilization procedures (vasectomy, tubal ligation) which have been reversed. The prior procedure may affect study outcomes, and patients with both a reversed sterilization procedure and PCOS are rare enough that exclusion should not adversely affect recruitment.

B. Sample Size

1. Prior Studies

Direct Comparisons of Combination Therapy vs. either Metformin alone or Clomiphene alone

The following table summarizes the literature comparing combination metformin and clomiphene to either metformin alone, or clomiphene, either from randomized trials or series where the second drug was added if there were no response to the first. A sixth study by Ng et al, (26) enrolled 20 subjects, but reported only median ovulation rates per subject, which were not translatable into cumulative rates.

STUDY	SUBJECTS	# of Cycles	Outcome	Results
Randomized trials				
Nestler et al, 1998 (20)	Metformin alone: n=21 Metformin + CC: n=25 (Unselected)	2	Ovulation	Metformin alone: Cycle 1: 8%, Cycle 2: 8% Metformin + CC: Cycle 1: 48%, Cycle 2: 81%
Kocak et al, 2002, (27)	CC alone: n=28 Metformin + CC: n=28 (All clomiphene resistant)	1	Pregnancy	CC alone: 0% (note: upper bound of 95% CI= 10%) Metformin + CC: 11%
Vandermolen et al, 2001 (23)	CC alone: n=12 Metformin + CC: n=15 (All clomiphene resistant)	6	Pregnancy	CC alone: 7% Metformin+CC: 55%
Nonrandomized				
Batukan Arch Gynecol Obstet 2001 (28)	CC alone: n=24 (3 cycles), followed by Metformin + CC: n=23 (Unselected)	6	Pregnancy	CC alone: 4% Metformin+CC: 65%
Parsanezhad et al Int J Gynecol Obstet 2001; (29)	Metformin alone: n=33 (2 months), followed by Metformin + CC: n=32 (All clomiphene resistant)	3	Pregnancy	CC alone: 3.1% (2 months) Metformin/CC: 25% (3 months)

None of the studies provide data directly applicable to the primary outcome of this protocol, live birth rate after 30 weeks, or 6 cycles, of treatment.

A Markov state-transition model was constructed using the software program DATA 4.0 (TreeAge Software, Williamstown, MA) to convert published ovulation or pregnancy rates from individual studies into estimates of live birth rates after 6 months of therapy. Relevant states for the model are Not Pregnant, Pregnant, Pregnancy Loss, and Live Birth. Since other potential causes of subfertility are ruled out by the protocol's inclusion/exclusion criteria, we assumed a relatively high conception rate of 35% for cycles where ovulation occurs. We assumed that 25% of all conceptions would result in a pregnancy loss, and that rates would not differ between treatment arms. Observed rates were converted to probabilities using the formula:

$$\text{Probability} = 1 - e^{\text{rate}*\text{time}}$$

In order to be conservative with our estimates, a non-zero estimate is required for the pregnancy rate observed in the clomiphene only arm of the Kocak study, in which there were no observed pregnancies. The upper bound of the 95% confidence interval for the observed pregnancy rate in this arm is 10.1%. Differences in ovulation rates in the two groups were over 5- fold (77% for the combined vs. 14 % for the clomiphene only group). Observed differences in ovulation rates in the other studies ranged from 3 fold to 10 fold. For purposes of the simulation, we assumed that the "true" pregnancy rate for the clomiphene only group in the Kocak group was one-third that observed in the combination group, or 3.6%. Again, in order to be conservative, the lower ovulation rate (48%) for the Nestler study was used.

The following table shows the resulting estimated live-birth rates after 6 months of therapy.

STUDY	ESTIMATED LIVE BIRTH RATE AFTER 6 MONTHS OF THERAPY
Nestler et al, 1998 (20)	Metformin alone: 11.6% Metformin + CC: 47.6%
Kocak et al, 2002 (27)	CC alone: 14.7% Metformin + CC: 36.2%
Vandermolen et al, 2001 (23)	CC alone: 5.1% Metformin + CC: 41.3%
Batukan et al, 2001 (28)	Metformin alone: 5.8% Metformin + CC: 48.8%
Parsanezhad et al, 2001 (29)	Metformin alone: 6.7% Metformin + CC: 29.5%

Uncontrolled Series

Clomiphene citrate

In a series of 259 clomiphene-naïve PCOS patients, Imani et al (30) reported an overall 6 month live-birth rate of 30%.

Metformin

Moghetti et al (31) followed 32 oligomenorrheic women for a mean of 8 months on metformin, with 1 dropout for treatment side effects. Seventeen (54.8%) were classified as responders because of return of normal menses, while 14 were nonresponders and remained oligomenorrheic. In a subset of 10 of the responders where progesterone was measured, ovulation was confirmed in 32 of 39 (79%) of cycles. For those women who have normalization, response occurred "within 3 months". Based on these results, estimated overall ovulation rate in response to metformin in unselected oligomenorrheic women would be (0.79)*(0.548), or 43.3%.

Because response did not begin immediately in this trial (consistent with the results of Nestler et al (20), where only 8% of subjects ovulated within the first two cycles), the model was run assuming an 8% response rate for two cycles, followed by a 43% rate for 4 cycles.

Estimated Live Birth Rates based on Available Literature: Summary

Clomiphene Citrate Alone: Estimates range from 7% in clomiphene-resistant women to 30% in women without prior exposure. Because this study will recruit both clomiphene-naïve and previously exposed patients, the overall rate should be somewhat lower than 30%, with the decrease being related to the relative proportions of the two groups.

Assuming that 25% of subjects will have had prior therapy with clomiphene and 75% will be naïve results in an overall estimated rate of

(0.07)*(.25) + (0.30)*(0.75) = 0.2425.

For purposes of sample size estimation, we will assume a rate of **25%** in the clomiphene-only arm.

Metformin Alone: In the Nestler trial, only 8% of women ovulated during the first two months of treatment on metformin alone. In the study of Moghetti et al (31), 54.8% of women had normalization of menses "within 3 months", with 79% of cycles being confirmed ovulatory in a subset of women.

Assuming an 8% ovulation rate for the first 2 months of treatment and a 43% rate for the last 4 months of a 6-month protocol, the estimated live birth rate after 6 months of treatment is 36%. Again, it is unclear what proportion of women will be likely to respond to metformin, or will have had prior treatment with metformin. Because the overall rate is likely to be somewhat lower because of the inclusion of women with prior treatment with metformin, we assume an overall 6 month live birth rate of **30%** in the metformin only arm.

Metformin plus Clomiphene Citrate: Estimated live birth rates derived from the three studies of clomiphene resistant subjects are 29.5%, 36.2% and 41.3%, while the rate derived from estimates in studies of unselected subjects are 47.6% (using a conservative estimate) and 48.8%. Assuming that 25% of subjects will be clomiphene resistant, we conservatively estimate a rate of (0.25)*(0.362) + (0.75)*(0.476) = 0.447, or **45%** for the combination.

2. Minimum Clinically Important Difference

Ideally, the threshold for identifying a minimum difference between treatments is informed by prior data on a variety of factors important for decision making, including efficacy in randomized trials, effectiveness in clinical practice, side effects, patient values for benefits, side effects, and risks of each treatment, health-related quality of life, and costs. Unfortunately, data that would allow estimation of these factors for the clinical situation addressed by this protocol are unavailable.

In the absence of specific data, we assume that any combination of two or more drugs must be superior to either agent alone in order for the combination to be considered the "best" treatment. In other words, given that combination therapy inevitably will cost more than either agent alone, that it will carry the risks of the side effects of both agents, and be less convenient for patients than either alone, the combination of metformin plus clomiphene citrate must be superior to either agent alone by some amount in order for it to become the treatment of choice.

We propose that an absolute difference in live birth rates after 6 months of therapy of **15%** is clinically important.

3. Null Hypotheses

(A) The live birth rate after 30 weeks of therapy with the combination of metformin plus clomiphene citrate is not different from the live birth rate after either metformin alone or clomiphene alone.

(B) The live birth rate after 30 weeks of therapy with metformin alone is not different from the live birth rate after clomiphene alone.

4. Possible Study Outcomes and Interpretation:

a) Null hypothesis (A) is rejected

If the null hypothesis is rejected, we expect that combination therapy is superior to either agent alone, and, barring significant differences in adverse events, combination therapy becomes the treatment of choice.

b) Null hypothesis (A) is accepted, but null hypothesis (B) is rejected

If the difference between combination therapy and one of the single agents is less than 15%, then null hypothesis (A) is accepted. If the observed difference between the single agents is greater than 15%, we expect that metformin would become the treatment of choice, barring substantial differences in adverse events. (This also allows for the possibility that Metformin alone will be at least 15% more effective than clomiphene alone).

c) The null hypothesis is accepted, but no statistically significant differences are observed between any of the treatments.

If the observed differences between all three agents are less than 15%, then the choice of initial treatment will likely be based on side effects, convenience, costs, etc. Although this trial will not be able to definitively answer that issue, data collected from this study will allow planning of additional studies to address issues of cost-effectiveness, health-related quality of life, patient preferences, etc.

Given these considerations, at most two comparisons are needed

- Scenario 1: Point estimate of live birth rate with combination therapy higher than with either agent alone

  Comparisons: (1) Combination therapy vs. highest single agent, (2) single agents to each other.

  Rationale: In order to be preferred, combination therapy needs to be superior to both agents. If combination is not superior to the agent with the highest live birth rate, demonstration of superiority to the other agent is not clinically meaningful. Direct comparison of single agents becomes the critical comparison for decision making.

- Scenario 2: Combination therapy less effective than one of the single agents

  Comparisons: 1

  Rationale: Given cost, complexity, side effects, etc., combination therapy must be demonstrably more effective than single agent therapy in order to be clinically preferable. In this scenario, only the comparison between the two single agents is meaningful.

5. Sample Size Calculations

Primary Endpoint - Live Birth Rate

Assumptions

- 15% absolute difference considered to be clinically meaningful
- 15% dropout rate
- Overall alpha level = 0.05, two-tailed test
- Two planned comparisons

1) Lowest rate vs. second lowest rate

2) Second lowest rate vs. highest rate

- chi^2 test for differences in proportions
- Best estimated live birth rates:
    1) Metformin alone: **30%**
    2) Clomiphene alone: **25%**
    3) Metformin + clomiphene citrate: **45%**

Parameters for sample size calculations:

- Alpha = **0.025**, two-tailed tests since two planned comparisons
- Beta = **0.20**, or 80% power

Sample Size

Treatment 1 (live birth rate)	Treatment 2 (live birth rate)	Required Sample Size	Sample Size with dropout adjustment	Total Sample Size
.50	.35	206/arm	237/arm	711
.45	**.30**	**197/arm**	**226/arm**	**678**
.40	.25	185/arm	213/arm	639
.35	.20	168/arm	193/arm	579

Based on these results, it is concluded that a study with 226 patients/arm is adequately powered to detect an absolute difference of 15% in live birth rates between two treatment arms, given the best estimates of success rates from the available literature.

A continuity correction was not applied to the sample size estimates for the chi-square test statistic. Such a correction is not necessary due to the expected size within each cell (32).

6. Total Sample Size

The study will include 678 patients (226 per treatment arm).

C. Allocation of Intervention

1. Timing

A timeline is provided below:

Figure 1: Study Overview

Time line (~weeks)	*-4 to 0*	*1*	*1-30*	*~~30-70*
Study Phase	*Consent PCOS Screen*	*Progestin withdrawal and Randomization*	*Treatment Phase*	*Pregnancy*
Treatments	None-Complete infertility evaluation as necessary	Treatment Arm 1	Metformin XR	None-Collect pregnancy outcome data
		Treatment Arm 2	Clomiphene citrate	
		Treatment Arm 3	Metformin XR/Clomiphene citrate	
Visits	1 or more visits		2-30 (maximum weekly visits for 6 ovulatory cycles)	As necessary

2. Method

a. Randomization Scheme

Six hundred seventy-eight (678) women will be randomized to one of the three treatment conditions. Using a 1:1:1 treatment ratio, there will be 226 women assigned to each treatment arm. The scheme will be a stratified randomization with permuted blocking within each stratum. Stratification variables will be site and prior exposure to either

clomiphene citrate or metformin XR. The DCC statistician will generate the randomization scheme for the study. Because this is double-blind study, the randomization scheme (including block size) will not be disclosed to the RMN investigators or staff, including the Lead Investigators. Unless otherwise specified, treatment arm data will be presented in a blinded fashion within DSMB reports.

The randomization will be integrated into a central Interactive Voice Response System (IVRS) that will be used by the sites to randomize each patient to a treatment condition. For quality control purposes, a second statistician not affiliated with the study will validate the randomization scheme and its transfer to the IVRS randomization database. The IVRS is maintained by the central randomization service, which will provide daily enrollment updates (without treatment assignment indicated) to the DCC project management team. The DCC statistician will review the randomization data on regular basis to ensure that the scheme is being implemented according to plan. If a patient is randomized but never receives treatment, the patient will be considered enrolled in the study. The randomization slot will not be re-allocated to a new patient due to the intent-to-treat nature of the study.

b. Method for notifying investigator of intervention allocation

Randomization will take place only if the patient has met the eligibility criteria and has completed the baseline evaluation. The site investigators will be provided a 24-hour toll free number that will connect to the centralized IVRS.

D. Intervention

Overview of treatment arms: All patients will receive a kit containing metformin XR/placebo or clomiphene citrate/placebo. The active dose of metformin XR will be

500 mg/tablet consisting of an extended-release tablet given on a two tablets twice per day dosing regimen. The active dose of clomiphene citrate will be 50 mg/tablet, consisting of a generic form. Both investigators and patients will be blinded to the individual contents of the medication kit. Progesterone levels will be obtained at weekly visits. A treatment cycle will consist of either

1) an ovulatory response to medication, as determined by weekly progesterone levels followed by either pregnancy or withdrawal bleed, or
2) an anovulatory response consisting of 5 consecutive weeks of non-ovulation as determined by weekly serum progesterone levels.

The maximum duration of the study will be six treatment cycles, or 30 consecutive weeks on study drug. If the 6th treatment cycle begins prior to 30 weeks on study drug, the 6th cycle may be completed.

Active medication will be provided by the manufacturers. Clomiphene citrate will be overencapsulated, and matching placebo tablets prepared by the research pharmacy. Clomiphene citrate will be dispensed in 50 mg tablets. Metformin XR will be dispensed in 500 mg tablets. Medication will be packaged so that any dosage adjustments made during the study (see below) can be done with the packaging dispensed to each patient.

Table 2: Dosage Regimens

Treatment Arm	Treatment	Bottle "M"	Bottle "C"
1	Metformin XR extended release	Metformin XR 500 mg	Placebo
2	Clomiphene citrate	Placebo	Clomiphene citrate 50 mg
3	Metformin XR/ Clomiphene citrate	Metformin XR 500 mg	Clomiphene citrate 50 mg

1. Intervention 1: Metformin XR Active/Clomiphene Placebo

Description Treatment arm 1 will consist of metformin XR increased in a stepwise fashion to four tablets or 2000 mg/d and clomiphene citrate placebo one tablet or 50 mg daily, day 3-7 of a treatment cycle. Dose of clomiphene citrate placebo will be increased by one tablet per day each cycle if patient is anovulatory, up to a maximum of three tablets placebo per day for five days.

Timing: Metformin XR will be initiated in a step-up fashion on the first day of menstrual bleeding (either spontaneously or after withdrawal with medroxyprogesterone acetate). If metformin can not be started on cycle day 1, it may be started on cycle day 2 or 3 if necessary. Upon initiating treatment patients will begin a dose of metformin XR with one tablet every morning for five days, then one tablet twice per day for five days, then two tablets every morning and one tablet every evening for 5 days, then two tablets twice per day thereafter. This dose will be maintained as tolerated throughout the remainder of the study. The dose will be reduced by one tablet per day upon recommendation of the site investigator should the patient develop side effects (see J. below). When side effects subside, the patient will attempt to increase the dose to the recommended 2000mg/d or to the maximally tolerated dose.

The patient will take the clomiphene citrate placebo tablet(s) for 5 days each cycle beginning on the third day of the menstrual bleed or as instructed by the site personnel (see F. below). During the first cycle this will be administered during the metformin XR step-up therapy. In subsequent cycles this will be initiated on day 3 of menses (or five weeks without menses or ovulation) along with the concurrent stable metformin XR dose. If the patient is unable to start clomiphene citrate on cycle day 3, it may also be started on

day 4 or 5. Patients will be followed on a weekly basis with a serum progesterone level. Dose of the placebo will increased each treatment cycle if there are five consecutive anovulatory weeks as determined by weekly serum progesterone testing. Dose will be maintained at the same level with adequate progesterone response and begun on the third day of menses (see F. below). The maximum duration of the study will be six treatment cycles, or 30 consecutive weeks on study drug.

2. Intervention 2: Clomiphene citrate Active/Metformin XR Placebo

Description Treatment arm 2 will consist of metformin XR placebo, increased in a stepwise fashion to four tablets a day and clomiphene citrate at 50 mg/tablet for 5 treatment days/cycle, beginning with one tablet a day 3-7 of a treatment cycle. Dose of clomiphene citrate will be increased by one tablet each day per cycle if patient is anovulatory, up to a maximum of three tablets each day for five days.

Timing: Metformin XR placebo will be initiated in a step-up fashion. Ideally metformin XR should be started on cycle day 1, but if necessary it may be started on cycle day 2 or 3. Upon initiating treatment patients will begin a dose of metformin XR placebo with one tablet every morning for 5 days, then one tablet twice per day for 5 days, then two tablets every morning and one tablet every evening for 5 days, then two tablets twice daily thereafter. This dose will be maintained as tolerated throughout the remainder of the study. The dose will be reduced by one tablet per day upon recommendation of the site investigator should the patient develop side effects (see J below). When side effects subside, the patient will attempt to increase the dose to 2000mg/d or to the maximally tolerated dose.

The patient will take the clomiphene citrate tablet(s) for 5 days each cycle beginning on the third day of the menstrual bleed or as instructed by the site personnel

(see F. below). Upon initiating treatment with clomiphene citrate, patients will begin a dose of clomiphene citrate 50mg/d for five days beginning on day 3 of treatment. During the first cycle this will be administered during the metformin XR step-up therapy. In subsequent cycles this will be initiated on day 3 of menses along with the concurrent stable metformin XR dose or after five weeks of anovulation and no menses. If the patient is unable to start clomiphene citrate on cycle day 3, it may also be started on day 4 or 5. Patients will be followed on a weekly basis with a serum progesterone level. They will receive monthly allotments of metformin XR/placebo and clomiphene citrate /placebo as appropriate. The maximum duration of the study will be six treatment cycles, or 30 consecutive weeks on study drug.

3. Intervention 3: Metformin XR Active/ Clomiphene Citrate Active

Description Treatment arm 3 will consist of metformin XR 500 mg/tablet, increased in a stepwise fashion to four tablets a day and clomiphene citrate at 50 mg/tablet for 5 treatment days/cycle, beginning with one tablet a day 3-7 of a treatment cycle. The dose of clomiphene citrate will be increased each treatment cycle if patient is anovulatory, up to a maximum of 150 mg/d for five days.

Timing: Metformin XR will be initiated in a step-up fashion. Ideally metformin XR should be started on cycle day 1, but if necessary it may be started on cycle day 2 or 3. Upon initiating treatment patients will begin a dose of metformin XR with one tablet every morning for 5 days, then one tablet twice per day for 5 days, then two tablets every morning and one tablet every evening for 5 days, then two tablets twice per day thereafter. This dose will be maintained as tolerated throughout the remainder of the study. The dose will be reduced by one tablet per day upon recommendation of the site investigator should the patient develop side effects (see J below). When side effects

subside, the patient will attempt to increase the dose to 2000mg/d or to the maximally tolerated dose.

The patient will take the clomiphene citrate tablet(s) for 5 days each cycle beginning on the third day of the menstrual bleed or as instructed by the site personnel after five weeks of anovulation without menstrual bleeding (see F. below). Upon initiating treatment with clomiphene citrate, patients will begin a dose of clomiphene citrate 50mg/d for five days beginning on day 3 of treatment. During the first cycle this will be administered during the metformin XR step-up therapy. In subsequent cycles this will be initiated on day 3 of menses along with the concurrent stable metformin XR dose. If the patient is unable to start clomiphene citrate on cycle day 3, it may also be started on day 4 or 5. Patients will be followed on a weekly basis with a serum progesterone level. The maximum duration of the study will be six treatment cycles, or 30 consecutive weeks on study drug.

Specific Instructions for Adjusting Medication Doses: The goal is to find the optimal dose for achieving regular ovulatory function while minimizing side effects.

Adjusting metformin XR dose downwards

Metformin XR should be reduced by one tablet (500 mg) a day/week if diarrhea or abdominal discomfort develops, which the P.I. attributes to the medication as a probable source.

Adjusting clomiphene citrate dose upwards

Clomiphene citrate dose will be increased upwards by 50 mg/d if a cycle has been anovulatory based on weekly progesterone levels or if there has been an inadequate response. An inadequate response is defined as a serum progesterone level < 5 ng/mL.

E. Enrollment

1. Recruitment

The recruitment strategy for the study is described below and will rely on a combination of local and national strategies.

Local Recruitment Strategies

Hospital/Local Health Care Referrals

Each P.I. will recruit subjects from their individual practice as well as faculty/resident continuity clinic. Ongoing contact with practice and faculty members as well as with residents will be made by the P.I. and nurse coordinators, reminding them of the inclusion criteria, importance of the study, etc. In addition, the P.I. will make contact with other departments in the hospital, i.e. family practice, pediatrics, adolescent medicine, medical endocrinology, who also see and treat these patients and talk about ovulation induction in PCOS. Contacts with local physicians will be made and/or grand rounds will be given to disseminate information about the study and ovulation induction in PCOS.

Local Publicity Office

P.I.s should meet with their local Public Relations official and plan a news release about the study. They should be available for any newspaper, radio, or TV stories that result from this. The full gamut of local media sources should be utilized. Often there is greater yield with more extensive coverage in smaller local outlets, than brief mentions in outlets with larger circulation. News release should mention the uniqueness of a study with pregnancy as an outcome and the benefits improving care of women, as well as the development of new methods for fertility treatment.

Local Advertisements

Advertisements will be placed in local newspapers on a regular basis, and money for this has been budgeted. Suggested copy should advertise for "Are you infertile with 8 or fewer menstrual periods a year…" and not PCOS per se. These should be placed on a regular basis if response is good.

Contact with local PCOS/infertility support groups

The nationwide support group Polycystic Ovarian Syndrome Association, Inc. has a large network of affiliated local support groups, as do the nationwide infertility support groups, such as Resolve or the American Infertility Association (AIA). Contact should be made with local support groups to spread information about the study and participate in local meetings.

National Recruitment Strategies

National Professional Organizations:

Contact should be made with the publicity offices of the Endocrine Society, The American Society for Reproductive Medicine, and the American College of Obstetricians and Gynecologists to issue press releases and mention/support the study in mailings/web sites, etc.

National Support Groups

P.I.s should offer to attend/speak at National Support group annual meetings about the study (i.e. annual meeting of the Polycystic Ovarian Syndrome Association, Inc. held in Minneapolis, MN in 2002). The American Infertility Association (AIA) also holds an annual meeting typically in the New York City area. Ads for the study should also be placed in newsletters of these organizations.

Web sites

The study will be prominently displayed on the RMN web site. Additionally each RMN/SCCPRR center should have a web page devoted to this study with information and contact information. Additionally information should be available at the NICHD web site with links to each RMN center. Links to the NICHD site should be requested at the major PCOS/infertility web sites. An ad should also be placed at "Center Watch" on the web.

National Advertising

A trial ad should be placed in the health section of Women's Day with all our local numbers/contacts as well as the central NICHD number.

Other U54s

This was briefly presented at the annual meeting as the specialized centers in reproduction meeting of the U54. Information about the protocol was sent to the P.I.s at each center. Contact with interested investigators with a special interest in PCOS was made and their participation in the protocol encouraged. We work closely with Lou DePaolo coordinator of the Specialized Cooperative Centers Program in Reproductive Research (SCCPRR) and the National Cooperative Program in Infertility Research (NCPIR) and to coordinate referrals/participation in the study, as many U54s also have a RMN site or are nearby. Four SCCPRR sites are currently participating.

2. Anticipated Period of Recruitment

It is anticipated that recruitment will take 24 months for this study. Three additional sites have been added to the original 9 sites to yield 678 patients randomized in approximately 24 months.

3. Assessment of Eligibility/Screening Period

The goal of screening will be to establish a diagnosis of PCOS and to verify that there are no other major infertility factors in the couple. Costs of this infertility evaluation will not be covered by the study, as these tests are routinely performed as part of a diagnostic evaluation before proceeding with ovulation induction. However in order to provide access to underrepresented groups in medical studies, an evaluation may be provided by sites to subjects without infertility insurance benefits.

It is estimated that the screening evaluation can be completed in one visit, if there has been no prior evaluation. This would consist of the patient bringing in a semen specimen from the partner, obtaining baseline history, physical, and blood tests, and performing an ultrasound with sonohysterogram to determine tubal patency. Sonohysterogram is a procedure where fluid (usually sterile saline) can be infused through an intrauterine catheter that contains a balloon to occlude the cervical canal, such that the contours of the uterine cavity can be examined, and free fluid can be seen accumulating in the posterior cul de sac indicating at least one fallopian tube is patent. This method has been found to have a sensitivity approaching hysterosalpingography for detecting uterine anomalies and tubal patency (around 70-80%)(33;34). Its sensitivity and specificity may be enhanced by adding a stream of air bubbles after the saline infusion (35). Sonohysterography is chosen for this study because of its lower cost compared with hysterosalpingography. The study will be performed by staff at each site proficient in the use of this technique. If for some reason an alternative test such as hysterosalpingography or documentation of tubal patency at the time of laparoscopy is judged to be clinically preferable, these results will be accepted.

If a patient (or her partner in case of the semen analysis) has had any test in the year prior to date of consent that is normal or within normal range for the reference lab,

this may be accepted in lieu of repeating the test for screening. This applies to all inclusion/exclusion criteria except labs that qualify as safety labs (See Safety Screens under table 1 below). In the case of a test for tubal patency, the results will be accepted if performed within the three years prior to date of consent. This protocol acknowledges that there will be site to site variation in terms of the insurance coverage of the diagnostic infertility procedures. A sonohysterosalpingogram is an office-based procedure that utilizes existing ultrasonography technology. Andrology labs exist at every study site and a semenanalysis is a low cost procedure. In order to enhance access to the study for women from a variety of backgrounds, some sites may elect to cover the costs of these tests out of RMN funds. Consent forms should explicitly state what tests will be covered by the study, and which will be the responsibility of the patient.

Physical Exam:

A physical exam with standard pelvic and breast exam will be performed on all patients within 30 days prior to randomization. A hirsutism assessment will be made via the modified Ferriman-Gallwey hirsutism score. All patients should have had a normal Pap smear within the last 12 months from time of consent. If not, one should be performed at the baseline exam. Patients with cytological abnormalities will need to have these resolved prior to study entry.

Ultrasound Exam:

A transvaginal ultrasound will be obtained within 30 days prior to randomization to determine uterine size and/or abnormalities, endometrial thickness, and ovarian size and

morphology. If the patient has had no prior test of tubal patency, this may be the desired time to perform a sonohysterogram to determine tubal patency.

Laboratory Exam:

Women will present fasting for 12 hours at the initial screening visit. Blood work will be sent as described above in the inclusion and exclusion criteria to identify appropriate study subjects. This blood work is found in the table below and will be run in the local lab. It may be prudent to confirm androgen excess before completing the remainder of the laboratory evaluation. Women who deviate from the recommended cutoffs for laboratory screens will proceed with further evaluation as necessary (repeat glucose or perform ACTH stimulation test for elevated fasting 17-hydroxyprogesterone-17-OHP) or be excluded from further study. Costs of these blood tests that determine if the anovulation is consistent with PCOS will be covered by the study. Aliquots of serum from this visit and each subsequent monthly fasting visit will be frozen and maintained for core lab determinations (serum from one 7.5 cc red top tube at baseline and from each subsequent monthly fasting visit). A minimum of 2 ml of serum from these visits will be aliquoted into two 1.5 ml microfuge tubes and will be stored at –20ºC to –80ºC and batched for periodic shipping to the central core lab facility for eventual assay. Aliquots may be temporarily maintained at -20ºC in a non frost-free freezer, but should be moved to a -70ºC or -80ºC freezer as soon as possible. At the Baseline Visit, two 1.5ml tubes of serum should first be aliquoted for the core lab and the remaining serum should be distributed as .5ml aliquots. Only the necessary aliquots (See appendix C) should be sent to the core lab. The remaining aliquots should remain at the study site since they may be used for future research protocols within the RMN. Each individual

site is responsible for obtaining permission from their IRB allowing this serum to be banked for future research.

Table 1: Screening labs of PCOS women

PCOS Diagnostic Labs	PCOS Inclusion/ Exclusion labs	PCOS Metabolic Screen	PCOS Safety Screen
1.Testosterone OR 2. Bioavailable or free testosterone	1. Prolactin 2. TSH 3. 17-OHP 4. FSH	1. Glucose	1. Liver Profile ALT/AST Total Bilirubin 2. CBC 3. Renal Profile BUN/Creatinine 4. Pap smear (if no recent results available). 5. Transvaginal U/S

The determination of eligibility can presumably be made in one visit. Other sites may prefer to perform this in a stepwise fashion over two or more visits (first verify screening labs allow eligibility, then check diagnostic infertility tests). It is anticipated that the eligibility visits can be completed in less than four weeks.

Central laboratory

Screening labs to determine eligibility will be run in the local RMN site labs. Given the variability of assays between labs, secondary analyses of baseline predictive factors and response variables will be performed in a central lab. These assays will be run through the core (Specialized Cooperative Center Program in Reproductive Research- SCCPRR) lab at University of Virginia. Therefore at baseline, an additional red top tube (7.5cc) will be obtained. Sera will be aliquoted and frozen at -20 to –80 degrees C for future assay. Blood work used for monitoring safety and response to therapy such as

progesterone, and hCG assays, will be run in local study site labs. An additional 7.5cc red top tube will be obtained at the monthly fasting visit and serum frozen for assay in the central lab. The purpose of this is to examine the monthly change in insulin/androgen levels in response to therapy. There will be no charge to the patient for these core lab assays.

Preconception Counseling

During the baseline evaluation all patients will be provided with preconception counseling. At a minimum this will consist of offering to verify rubella immune status, and offering HIV screening. In addition, all patients 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. Giving a prescription for folic acid alone is also acceptable. They are to take this pill daily during the study and also during a pregnancy. Sites may provide the prenatal vitamins or folate to patients without pharmacy benefits.

All patients will fill out the American College of Obstetricians and Gynecologists risk factor questionnaire (See Appendix B for copy of the questionnaire) for genetic disease and if indicated, further counseling about genetic risks will be coordinated by the P.I. at each site. Patients 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. Patients who are HIV positive will receive appropriate counseling and will be advised about the need for medical treatment to prevent HIV transmission to their fetus during pregnancy. Thus, all patients who are rubella non-immune or are HIV positive will be eligible to participate in the study. The cost of rubella and HIV screening will be the responsibility of the patient. The site may cover this cost for patients without insurance benefits.

Progestin withdrawal

All patients, except those with evidence of a luteal phase progesterone level on baseline screening, will undergo withdrawal bleeding with progestin. The patient will be given a supply of progestin, for example medroxyprogesterone acetate 5mg x 10 days, and progestin therapy can be instituted immediately after the screening visit at the discretion of the P.I. The P.I. may elect to give another comparable form of oral progestin to institute a withdrawal bleed. Each site will provide this progestin and there will be no charge to the patient.

Screen failures

It is anticipated that patients who may fail screening due to a medically treatable condition, such as hypothyroidism, or mild iron deficiency anemia, may eventually become eligible for re-screening, assuming that other inclusion/ exclusion criteria have not changed (e.g. oligo-amenorrhea as an inclusion criteria, or elevated prolactin levels as an exclusion criteria). These cases will be handled on an individual basis after consultation with the DCC.

Counseling about timing of intercourse

All subjects will receive counseling about the need for regular intercourse during the study by the nurse coordinator at this visit. See guidelines below.

4. Timing of Intercourse Instructions

All subjects (and couples where the male partner is present) will be instructed to have intercourse 2-3 times per week. The optimal frequency will be every 2-3 days. Subjects will be counseled that multiple episodes of intercourse within a short period (i.e.<24 h) can lead to diminishing returns in terms of sperm count and function, and therefore they should not “batch” their intercourse. The monthly visit will include a

query about dates of intercourse. The importance of regular intercourse should be mentioned if the frequency on any week is < 2 episodes/week. An inquiry should result when intercourse has occurred <2 times per week for two consecutive weeks about possible causes. Referrals should be made to appropriate caregivers if there is evidence of sexual dysfunction.

5. Informed consent

Informed consent will be obtained on all subjects at the time of baseline evaluation, after review of the protocol and informed consent form by the IRB at each study site. Consent will be obtained at all sites by the Study or Nurse Coordinator. This will provide a site-specific uniform counseling of the risks and benefits of the protocol. A sample consent form is provided in the appendix. The sample consent form is applicable to the format requested by the IRB at Penn State University, as written by the P.I. Dr. Richard S. Legro. It is anticipated there may be site specific changes in the consent form.

6. Randomization Procedure

All women, unless they have recently ovulated based on luteal phase progesterone levels, will undergo a withdrawal bleed induced by progestin prior to initiating the treatment phase. Women will be given medroxyprogesterone acetate (MPA) orally, 5 mg x 10 days to induce bleeding. They will initiate therapy with their study medication on their first day of bleeding. Women who do not withdraw will be screened with a urinary hCG (home test given to patient) and therapy initiated if hCG is negative two weeks after completing the last medroxyprogesterone acetate pill.

The site investigator will be provided a 24-hour toll free number that will connect to the Interactive Voice Response System (IVRS). After the patient has signed

an informed consent and all required baseline evaluation procedures have been completed, an Investigator or designee will call the IVRS in order to randomize a patient into the trial. The IVRS will query the site for patient eligibility information. If the patient is eligible, the site will be provided with a patient 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.

In the event that emergency unblinding is needed, only the site PI will be able to unblind a patient to treatment by calling the IVRS system. The site PI and DCC staff will receive notification from the central randomization service when emergency unblinding has occurred. With the exception of emergency unblinding, patients will not be unblinded until study findings are released.

F. Study Specific Procedures and or Visits

The screening visit and procedures in the baseline visit are described above under E.3 Assessment of Eligibility: The weekly/monthly monitoring visits are described below.

Description of Weekly/Monthly Visits:

Patients will follow-up weekly after beginning medication. Ideally, the patient will be seen the same day each week, but the weekly visit can take place 4-12 days after the previous weekly visit. A serum progesterone level will be sent weekly and urinary hCG screen as necessary (for example suspected pregnancy, two consecutive positive ovulatory progesterone levels, or one positive level with a missed previous visit, or no menses for 5 weeks). Case report forms (CRFs) will be filled out on a monthly basis. Pelvic exam and/or ultrasound will be performed at the discretion of the principal

investigator. A pill count will be obtained once a month. Medication will be dispensed by the nurse coordinator or appropriate person on a predefined basis.

The weekly visit does not necessarily have to take place on site. It may be accomplished off site by the nurse coordinator at a home or office visit, or at a commercial lab with overnight delivery of the serum specimen to the site laboratory, or at another clinical laboratory with results forwarded to the RMN site. This will facilitate the enrollment of subjects who are at a greater distance from one of the study sites. The progesterone level must be reviewed, however, on a weekly basis by study personnel. Weekly phone or e-mail contact between study personnel and patients is encouraged, however, to increase compliance. Urinary pregnancy tests may also be dispensed to patients who may miss a weekly visit to detect pregnancy.

A patient should be seen in person by study personnel, at a minimum, every four weeks. The monthly visit can be conducted 24-37 days after the previous monthly visit, or in the case of Monthly Visit 1, after the start of study drug. This visit will be performed after an overnight fast. This visit will be important not only to monitor adverse events or side effects, but also to perform pill count, review bleeding and intercourse diaries, dispense medication, obtain a weight and waist to hip ratio, and to draw blood. At this visit it is also important to obtain the serum from a 7.5cc red top in order to examine circulating response variables in the core lab. Blood may also be obtained for the weekly progesterone level at this visit.

<u>Missed Visits:</u>

It is anticipated that subjects may miss a weekly visit from time to time due to schedule conflicts. Missing two consecutive weekly visits becomes more problematic as a potential ovulation may be missed. In this case the P.I. can base further management on

the clinical situation (i.e. no menstrual bleeding and continued anovulatory progesterone levels or subsequent menstrual bleeding and presumptive ovulation.) Missing three consecutive visits may be justification for excluding the patient from further study. This will be determined by the site P.I. after consultation with the DCC.

Medication will be adjusted based on progesterone response and side effects. Medication may therefore be adjusted downwards or eliminated to control side effects. These adjustments can take place independently of the DCC by the P.I at each individual site. For instance, if a patient complains of gastrointestinal symptoms (anorexia, diarrhea, GERD, or abdominal discomfort) and evaluation suggests that medication may be responsible the metformin XR/placebo dose should be backed down by 1 tablet per day every week until symptoms subside. If this necessitates stopping medication completely, the patient will be excluded from further study and discontinued. If symptoms subside, after 1 week an attempt should be made to increase the dose by one tablet every 5 days up to 4 tablets a day. Adjustments will be noted on CRFs.

Responders:

Responders will be identified on the basis of an elevated progesterone level consistent with ovulation (Progesterone $\geq$ 5 ng/mL). One elevated weekly level $\geq$ 5 ng/mL is evidence of response (36). The rationale for this is that it may not be possible to determine a midluteal peak level, but that two-week levels may overlap the peak and be elevated but not reach peak levels. Therefore we have chosen a middle level between two other cited levels of ovulation, 3 ng/mL and 10 ng/mL. After two consecutive elevated progesterone levels or at the discretion of the investigator, a urine hCG level will be checked. If initial result is negative, continue weekly urine hCG levels until menses. Treatment with all medications will cease from the point hCG is detected. If

ovulatory based on serum progesterone levels, women will continue their medication through menses at the same dose and in the same regimen. After menses from an ovulatory cycle, the patient will again take clomiphene citrate/placebo days 3-7 of the cycle as well as the daily metformin XR/placebo. Responders will continue at the same dose until pregnancy, or until a total of six cycles or until 30 weeks is reached.

Nonresponders:

Non-response will be defined as the lack of an elevated progesterone level for any consecutive five week treatment period during the study. If the patient fails to respond to treatment for any five week period, they will be instructed to increase their clomiphene citrate dose after the fifth anovulatory progesterone level to two pills a day for five days while continuing the metformin XR tablet at 2 tablets twice per day. Further non-response for five weeks will lead to an increase in clomiphene citrate to three pills a day for five days. This dose will continue until the completion of a total of six treatment cycles or 30 weeks is reached. This may involve more than one five week period with the maximum clomiphene citrate dose of three tablets a day for five days (See Appendix 1: Study flow sheet below).

Induction of Withdrawal Bleeds for Non-Responders:

There will be no induction of withdrawal bleeds for non-responders during the course of the protocol, unless as dictated by the P.I. at the site. The benefits and/or risks of this for short amounts of time (i.e. less than 6 months) have not been well described in the literature. The use and indication for inducing a withdrawal bleed should be noted on the weekly CRF. During the induction of the withdrawal bleed with medroxy progesterone acetate (5 mg x 10 days), the patient is to continue metformin XR or

placebo, but not take clomiphene citrate or placebo until day 3 of the withdrawal bleed. If there is no spontaneous withdrawal bleed after the use of medroxyprogesterone acetate, a pregnancy test should be checked prior to clomiphene citrate at an arbitrary "day 3", preferably within two weeks of completing the medroxyprogesterone acetate.

Breaks in Study Protocol:

There may be breaks in the protocol for both anticipated and unanticipated reasons. Subjects should be counseled that they should be seeking conception for the full potential 30-week period. If personal or professional commitments will result in more than a four-week break from the protocol, subjects should not be enrolled. A break or breaks, up to four weeks, from clomiphene citrate is allowable to facilitate timing of intercourse if there are other commitments. Metformin XR should be continued during this break. Urinary pregnancy tests can be dispensed to check weekly for pregnancy during these breaks. Longer break(s) should be an indication for dismissing the patient from the protocol as non-compliant.

Study Drug Termination Visit:

Patients who conceive, who complete the study without conception or who withdraw will undergo a study drug termination visit as close to their end of medication as possible. The goal of this visit is to determine that there has been no systemic adverse effects of the medication and to obtain follow up sera to identify predictive factors of success. This visit will consist of completion of a CRF, a brief physical which does not require a breast or pelvic exam, and repeat of the safety labs done at baseline. These safety labs include a CBC with platelets, a renal profile, and liver profile. Additionally,

1 ml of serum from one 7.5cc red top tube will be obtained for assay by the central lab. Serum will be separated, aliquoted, and frozen at –20 to –80 degrees Centigrade until assay by the central lab. A repeat hirsutism assessment will be performed using the Ferriman Gallwey hirsutism scale. Additionally if the patient is pregnant, signed medical release forms should be obtained from the patient to obtain information about the pregnancy outcome-including forms for the prenatal caregiver and the intended hospital for delivery.

Pregnancy Visits:

Conception will be confirmed, if suspected, by measurement of serum hCG. A urinary hCG may be sent as an initial screen. Conceptions 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 conception and number of implantation sites. Patients who conceive will be followed at the site 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. A conception with fetal heart beat is defined as pregnancy. At this point, approximately 6-8 weeks after conception, women will be referred to their prior or referring practitioner, or to an appropriate health care provider. Patients will be reminded to continue their prenatal vitamin or Folic Acid during pregnancy and provided with counseling about prenatal care. Women will be followed throughout pregnancy to determine the abortion rate and through delivery to determine pregnancy outcome. Patients will be informed to notify study personnel of the outcome of the pregnancy. Phone contacts will be initiated if the patient has not contacted study personnel by six weeks beyond the original estimated

date of confinement. Delivery records will be requested to determine the birth weight, length of gestation, and any perinatal complications of mother or infant. If delivery records are not available, patient self -report will be acceptable, however, every effort will be made to obtain records.

Unblinding of Medication Assignments:

Study medication assignments will not be routinely unblinded until the results of the study have been released. Emergency unblinding will be at the discretion of the site P.I.

Timeline for Proposed Protocol

The timeline below is a conservative estimate of the time needed to complete the trial, analyze the data, and achieve publication of the primary publication summarizing the results of the trial. The study should be completed, including pregnancy outcome in all randomized women by year four and data analysis complete by the end of that year. Preparation and submission of the first article should be achieved by the fifth year of the grant.

	Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 5**
Protocol Completion	■				
Subject Recruitment	■	■	■		
Completion of Protocol/Pregnancy		■	■	■	
Data Analysis				■	
Submit Primary Article					■

G. Study Agent Preparation, Administration, and Accountability:

The study agents will be obtained by the DCC through their manufacturer/ distributor. The metformin XR compound to be tested will be the extended release

preparation of metformin XR (known commercially as Glucophage XR and manufactured by Bristol Myers Squibb). 500 mg tablets of metformin XR will be utilized to allow for individual titration of dose. Placebo will be provided by Bristol Myers Squibb. Although data on metformin XR and PCOS is primarily limited to immediate release formulations, there is no reason to expect clinically significant differences in the efficacy of the two formulations in inducing ovulation. Given the greater convenience and potential for reduced side effects, we feel that use of the extended release formulation is justified. The shelf life of Glucophage XR is three years per Bristol Myers Squibb.

Clomiphene citrate 50-mg tablets will be provided by Teva Pharmaceuticals. This dose will also allow for individual titration. A placebo of suitable size and weight to the active clomiphene citrate compound will be constructed by the DCC. The generic formulation of clomiphene citrate manufactured by Teva Pharmaceuticals has a shelf life of two years.

The medication will be packaged into three types of blinded drug kits: 1) Metformin XR and placebo, 2) Clomiphene citrate and placebo, 3) Metformin XR and clomiphene citrate. Over-encapsulated clomiphene citrate and placebo clomiphene citrate tablets will be used. Metformin XR and matching placebo will be used. Additional information will be provided in the trial specific pharmacy inservice manual. The study coordinator at each site will be responsible for distributing the one-month supply drug kits, for verifying pill counts, for determining that there are adequate medication supplies for existing patients and new patients to be randomized. The P.I will ultimately be accountable for administration and accountability of the medication used in this study.

H. Concomitant Medications

An inquiry into concomitant medications will be made at the baseline visit as well as each subsequent visit. Use of an exclusionary agent which affects reproductive function or metabolism, such as an anti-diabetic agent, during the study will be prohibited. These medications include oral contraceptives, GnRH agonists and antagonists, antiandrogens, gonadotropins, anti-obesity drugs, somatostatin, diazoxide, insulin, and calcium channel blockers. However it is possible that the P.I. or other physician will prescribe progestins during the study due to abnormal bleeding/prolonged amenorrhea, which will not qualify as a reason for withdrawal. This list will be examined by the P.I. on a monthly basis to determine a suitable course of action. Potential interactions with study medications should also be checked. For instance multiple medications have been shown to increase peak serum levels of metformin (nifedipine, furosemide, and cationic drugs such as morpine, procianamide, triamterene), although half life of metformin is unaffected.

I. Reporting Adverse Events

All serious adverse events (SAE's) throughout study participation from the start of study drug through one week after the last dose of study medication must be reported. For patients who conceive, all SAEs from the start of study drug through delivery/termination must be reported. SAEs should be reported within 24 hours (or 1 business day) of learning of the event to DCRI Safety Surveillance at:

919-668-8624 (telephone)

919-668-7138 (fax)

DCRI Safety Surveillance will forward reportable events to the NICHD within 1 business day of receipt. The NICHD will report serious, drug-related events to the FDA and all

participating investigators according to regulatory guidelines. The NICHD will forward relevant safety information to the DSMB as needed. The P.I. will be responsible for notifying their IRB of SAEs in accordance with local guidelines.

A serious adverse event is one that is fatal or immediately life-threatening, severely or permanently disabling, requires or prolongs inpatient hospitalization, overdose (intentional or accidental), congenital anomaly, pregnancy loss after 12 weeks gestation or any event so deemed as serious by the P.I. at each RMN site. If any serious event occurs and is thought to be related to the study medication, the study medication will be discontinued. Twenty-four hour unblinding will be available through the IVRS system to break the randomization code for the individual patient if this is required by the P.I. for proper treatment of the patient.

J. Study Medication Related Adverse Events (*Please refer to Appendix E for details*)

There are a number of adverse events that are potentially related to study medication.

Metformin

Gastrointestinal symptoms (diarrhea, nausea, vomiting, abdominal bloating, flatulence, metallic taste in the mouth and anorexia) are the most common reactions to metformin XR and approximately 30% more frequent in women with metformin XR compared to placebo treated patients. These symptoms will be managed with a step down decrease in the daily metformin XR dose (one tablet per day per week) until symptoms resolve. These symptoms alone will not be a reason for withdrawal from the study unless a patient is unable to tolerate any dose of the medication.

There is a small risk of lactic acidosis among women taking this medication. This most commonly occurs in patients with poorly controlled diabetes and impaired renal function. Women with these pre-existing medical problems will be excluded from the

protocol as per the exclusion criteria. There have been reports of lactic acidosis induced by exposure to iodine-containing radiocontrast agents, such as those used for an intravenous pyelogram or even for hysterosalpingogram, if there is intravasation. Metformin XR will be stopped prior to procedures involving exposure to radiocontrast agents to reduce the chance of developing lactic acidosis (one week prior to medication and resume one week after the test). The development of lactic acidosis for any reason will be a reason for discontinuing the study.

Metformin XR is pregnancy category B with no known human teratogenic risk and no known embryonic lethality in humans. Metformin XR has been used throughout pregnancy in a number of studies with no adverse maternal or fetal effects. Because it was approved in other parts of the world over 20 years ago, there is a much longer experience in the larger clinical world. There have been no reported abnormalities associated with its use during early pregnancy in women with diabetes (37-39) or to a women with marked hyperandrogenism during pregnancy (40), or to the small number of PCOS women who have conceived during treatment (17;41) (42) (23). Some authors have given metformin XR during early pregnancy and report a beneficial effect of lowering the spontaneous abortion rate (43), (an approach we are not recommending but noting as further evidence for its safety in early pregnancy). There has been an adverse effect associated with the use of metformin XR during the third trimester of pregnancy in diabetic women with an increased risk of pre-eclampsia reported (32%) compared to other treatment regimens as well as increased perinatal mortality (44). Again this is not the indication or the type of patient we are studying. This protocol does not recommend the use of metformin XR beyond conception.

Other classes of insulin sensitizing agents currently approved for the treatment of Type 2 diabetes, such as the thiazolidinediones, rosiglitazone and pioglitazone, are pregnancy class C and have been associated with embryonic lethality in animal studies. Metformin XR is currently the only pregnancy category B insulin sensitizing agent.

Clomiphene Citrate

Clomiphene citrate should be avoided in patients with pre-existing liver disease given its hepatic metabolism. The development of visual symptoms will be a reason for discontinuing treatment although the etiology of this phenomenon is unknown. The development of persistent ovarian cysts or ovarian enlargement are also reasons for withholding further therapy. Ovarian Hyperstimulation Syndrome (OHSS) is rare in patients receiving prescribed doses of clomiphene citrate but will be another reason to withhold further therapy. Ultrasound of the pelvis will be performed at the discretion of the P.I. at the RMN site should the patient develop abdominal pain or other symptom suggestive of an ovarian cyst. Hot flashes (affecting 10% of patients) are common presumably due to its estrogen antagonism at the hypothalamic-pituitary axis. Mood changes are also frequently reported. Multiple pregnancy rates between 5-10% with the vast majority of these being twins have been reported with the use of clomiphene citrate (45). Although there have been studies suggesting that multiple cycles of clomiphene citrate may be associated with an increased risk of subsequently developing ovarian cancer (46), many other studies have shown no effect on ovarian cancer rates (47;48).

K. Drug Interactions

There are no known clomiphene citrate-metformin interactions. Drug interactions with clomiphene citrate have not been reported. However metformin can interact with a number of other medications that are renally excreted such as furosemide, nifedipine, and

cationic drugs. Cationic drugs (digoxin, morphine, procainaminde, quinidine, trimethoprim, tramterine, ranitidine, cimetidine) are eliminated by renal tubular secretion and theoretically have the potential for interaction with metformin by competing for common renal tubular transport systems. Such interaction between metformin and oral cimetidine have been observed in healthy volunteers, the others remain theoretical according to metformin labeling information.

L. Other Risks of Procedures in the study

Potential risks include those of obtaining the screening tests by venipuncture, and the use of medications. The risk of venipuncture includes discomfort and potential anemia. We are excluding patients at baseline with anemia and the amount of blood we are removing in our weekly tests (<10mL) is unlikely to cause anemia. Some women may additionally require a sonohysterogram or hysterosalpingogram and/or an ACTH stimulation test. There is a potential risk of an idiopathic reaction to Cortrosyn (cosyntropin) used in the ACTH stimulation test, but this is extremely rare (< 0.1%). The risks of an ACTH stimulation test are minimized by utilizing trained nursing personnel with investigator back up during the test. A hysterosalpingogram involves the risk of pain, bleeding, damage to uterus, pelvic infection, interrupting an unrecognized pregnancy, small amount of radiation exposure, and risk of an allergic reaction to the iodine containing radiocontrast. This test should not be performed with the patient on metformin XR therapy. A sonohysterogram involves the risk of pain, bleeding, damage to uterus, pelvic infection, and interrupting an unrecognized pregnancy. These tests are performed routinely as part of an infertility evaluation. It is not anticipated that a laparoscopy will be performed as part of this protocol.

Study women will be carefully screened for medical and reproductive abnormalities prior to randomization. The risks of the medications will be lowered through nursing education and frequent follow-up. Study subjects will be monitored on at least a monthly basis for adverse reactions. Only a limited supply of medication will be dispensed at each visit to encourage compliance with the protocol and prevent a prolonged exposure of an early gestation to medication. All medication will be withheld for a week prior to exposure to iodine containing dyes. All records will be kept with strict confidentiality. No names will be used in the presentation of this data.

M. Study Monitoring

Frequent contact will occur between the P.I. and the DCC representatives to confirm that the study is being performed according to the protocol and that all forms are accurate and complete. An initial study visit will be performed by staff from the DCC after the first patient has been enrolled to ensure compliance with study procedures. Follow-up monitoring visits will be performed every six months. Additionally, there will be meetings between DCC personnel and study coordinators/P.I.'s at the regularly scheduled RMN meetings.

N. Study Centers

There were 9 centers originally participating in the trial, the 8 centers of the Reproductive Medicine Network and an additional a site from a collaborative SCCPRR (Specialized Cooperative Center Program in Reproductive Research) with John Nestler, M.D. as P.I (Medical College of Virginia in Richmond, VA). Three additional SCCPRR sites have been added for a total of 12 centers.

O. Obligations of the Investigator

IRB Review

The P.I is responsible for submitting the approved protocol and consent form to the local IRB for review. Prior to the submission, the informed consent must be forwarded to the DCC for review and approval of any additions and/or changes. The IRB must approve all aspects of the study as detailed in the protocol, including the patient informed consent form. It is anticipated that there will be minor site-specific changes in the consent form. The IRB must periodically review the status of the study at appropriate intervals not exceeding one year. The P.I will also be responsible for submitting revisions to the protocol to the IRB and promptly communicating serious adverse events that result during the study.

P. Maintenance /Retention of the Records

In order to comply with Good Clinical Practice (GCP) requirements, the investigator must maintain for 2 years after the study ends the master patient log that identifies all patients entered into the study such that the patients can be identified by audit. The P.I. must maintain adequate records pertaining to patient files and other source data for a minimum of 5 years after completion of the study.

Q. Data Collection and Management

The completed case report forms (CRF) will follow these guidelines: All data on the CRFs will be entered in ink. No data entry in the original CRF may be deleted or corrected by erasure, use of ink eradication fluid or any other such means. When a data entry is in error, draw a single line through the erroneous entry (the original data must remain discernible) and indicate the correct data in whatever way is appropriate. These changes should be initialed by the P.I. or designated study coordinator. Validity of data

recorded on CRFs during each patient visit will be attested to by one of the investigators by a dated signature on an appropriate CRF page. All questions should be answered. Data management will be as per DCC guidelines.

R. Competency to perform procedure/tests in the protocol

The principal investigator will be responsible for ensuring that laboratory and patient tests performed as part of the study are performed by competent personnel. These tests include semen analysis, ultrasonography of the pelvis, sonhysterograpy and/or hysterosalpingography of the uterus and fallopian tubes. The criteria for determination of competency may vary between sites in the study.

V. DATA ANALYSIS

All statistical tests will test non-directional hypotheses. The level of significance will be set at 0.05 for primary analyses and 0.05 for secondary analysis. An intent-to-treat principle will be employed during the primary analysis of the primary efficacy and safety measures. For the intent-to-treat analyses, randomized women will be included regardless of whether or not the patient received the assigned treatment, the patient was compliant with the treatment plan, and/or the treatment was administered according to randomization assignment.

A. Primary Efficacy Measure

Primary Analysis: A live birth will be defined as delivery of any viable infant. The primary analysis of the primary endpoint will include the following procedures. A chi-square test for equal proportions will be performed to evaluate whether live birth rate in Metformin/clomiphene group is the same as one in the group of either Metformin alone or clomiphene alone that has the higher live birth rate. If the test is not significant, a standard chi-square test will be used to examine differences in proportions in live births

between the groups of Metformin alone and clomiphene alone. The level of significance for all tests included in the primary analysis will be 0.05.

Supplemental analyses: A set of secondary (supplemental/sensitivity) analyses of the primary endpoint will be conducted after the primary analysis has been performed using the intent-to-treat approach. All secondary analyses of the primary endpoint will be considered exploratory since the study has not been adequately powered for this set of supplemental analyses. Due to the exploratory nature of these analyses, the level of significance for the secondary analyses will be set at 0.05 (two-tailed) to reduce the Type II error rate.

Initially, a comparison of the internal consistency of live birth rates across sites will be examined using logistic regression analysis. Sites with similar live birth rates may be collapsed. For the regression model, the data will be examined to determine whether there is a site by treatment interaction effect. The recommended significance level is an alpha of 0.10 since the power to detect interactions is low. In the event that there is a significant interaction term, clinical site will be included as a covariate in all subsequent secondary analyses.

Next, logistic regression models will be used to evaluate the predictive value of treatment condition, clinical sites (if needed), prior exposure to either clomiphene citrate or metformin XR, and other explanatory factors on live birth rate. Examples of potential explanatory variables include: age, prior parity, body mass index, waist hip ratio, baseline proinsulin, insulin, and glucose values, and baseline testosterone values. In addition, we will examine to what extent change in baseline values is related to live birth rate.

Supplemental sensitivity analysis will also include an analysis of 'evaluable only' patients only (e.g., excluding randomized patients who were deemed to be ineligible later

or excluding patients who did not adhere to medication regimen) according to the protocol).

B. Safety Measures

Adverse Event Analysis: Adverse events will be categorized and percentage of patients experiencing adverse events and serious adverse events during the treatment period will be detailed. Chi-square tests will be performed to examine differences in the proportion of total and categories of adverse events within each treatment arm. For each DSMB report, a list and summary of the reported adverse events will be presented in a blinded fashion, unless otherwise formally requested.

C. Secondary Efficacy Measures

Although not powered to address the secondary outcome parameters, such analyses may provide important pilot data for powering future studies**.** Multiple logistic regression models will be used in analyses of secondary efficacy measures to evaluate the predictive value of treatment condition, clinical site (if needed), prior exposure to either clomiphene citrate or metformin XR, body mass index, and other explanatory variables on the secondary binary outcomes (e.g., abortion, ovulation). Generalized estimating equations approach will be used if there are multiple outcomes (eg., multiple ovulations) per patient to account correlation within patient. Examples of potential explanatory variables and covariates are: clinical site, prior exposure to either clomiphene citrate or metformin XR, body mass index, age, among others, total testosterone, insulin, proinsulin, and glucose values. Cox proportional hazards models and a Kaplan-Meier method will be applied to compare time to event (eg., cycles to first ovulation, cycles to first conception, cycles to pregnancy , weeks from viable pregnancy to live birth) between the treatment groups.

Due to the exploratory nature of these analyses, the level of significance for the secondary analyses will be set at 0.05 (two-tailed) to reduce the Type II error rate.

Definition of Secondary Measures

- Two different spontaneous abortion rates will be computed. The first is the number of spontaneous abortions over the number of patients who have conceived (defined by positive serum HCG) and the second is the number of spontaneous abortions over the number of patients who are pregnant (defined by detection of fetal heart beat).
- An ovulation will be defined as an elevated weekly progesterone level separated by at least one week with a nonovulatory progesterone level. A binary variable indication whether the patient ovulated or not will be created for each cycle. Furthermore, cycles to first ovulation will be used as time to event variable.
- Time to pregnancy is defined as the number of cycles required to achieve a viable implantation (detection of fetal heart beat). Weeks from pregnancy to live birth will als be used as a time to event variable for patients who did not have miscarrages.
- Conception. Conception is confirmed by a positive serum hCG value. Cycles to first conception will be used as a time to event variable.

E. Drop outs/Treatment Failures

Dropouts, either by patient choice or physician recommendation due to adverse effects or poor compliance will be analyzed by intention to treat as treatment failures. Subjects who have completed 30 weeks or 6 treatment cycles without a pregnancy will also be considered as treatment failures. Subjects who conceive and are lost to follow up will be considered as a treatment success. It is considered unlikely that the patients will be lost to follow-up after a conception on a treatment regimen.

F. Interim Analysis

Analysis Plan: An interim analysis will be conducted on the primary endpoint when 50% of the study participants have completed treatment. The primary analysis described above will be performed and the O'Brien-Fleming (1979) method will be applied to adjust for at the number of looks at the primary endpoint. The interim analysis should also provide a detailed summary of adverse events reported. The critical value for the primary analysis will be specified in the detailed analysis plan. The results of the interim analysis will be submitted to the Data Safety and Monitoring Committee (DSMC). The interim analysis results will be presented in a blinded fashion to the DSMC, who will then evaluate the findings to determine whether the trial should be continued, modified, or discontinued.

Stopping Rules: The stopping rule for the O'Brien-Fleming method compares the statistic Z_i with $Z^*(N/i)^{.5}$, where $Z^*$is the critical value for the significance level (.05), N is the total number of analyses (N = 2) and i is the analysis number (for the one planned analysis, i = 1).

VI. DISCLOSURE OF DATA

Reproductive Medicine Network Publications Policy

The publication policy proposes guidelines for publications that originate from our collaborative Reproductive Medicine Network. Decisions concerning publications shall be determined by consensus of the collaborating principal investigators (or designate) noted below as the "Network." This policy is designed to promote prompt, exact, quality publications and presentations of Network studies with appropriate academic recognition of those with significant contributions.

I. Major Publications

A major publication is defined as one reporting results of the major hypotheses tested. (For example, does hMG/IUI increase cyclic fecundity in couples with unexplained or male factor infertility?)

A. Authorship: Publications will include the names of investigators from each RMU and the DCC rather than merely identify the "Reproductive Medicine Network". Each RMU will have, on average, one but no more than two authors per publication. The Steering Committee Chair and NIH Research Coordinator will be authors. The principal investigator at each RMU will be responsible for submitting the names of authors from that center. Occasionally, additional authors may be appropriate. In these cases, the final decision
will be by Network consensus. In this way, the number of authors on a given article would be approximately 10, perhaps more.

Other collaborators can be identified prominently by citing the Reproductive Medicine Network in the title as a co-author with an asterisk referring the reader to other names cited on the bottom page of the title page. (In this way these collaborators would not be submerged in the acknowledgement sections typically invisible at the end of the paper.)

B. First Author: It is expected that first authorships would be distributed among principal investigators. The investigator initiating the protocol and chairing the protocol committee will be the first author. The first author would always be expected to prepare the initial draft of the manuscript, after receiving approval from the Network to proceed.

In the event that the initiating protocol investigator denies first author, the next investigator in the rank order of authors (described below) will be the first author.

C. Authorship Order: After the first author, authorship order will be based upon subject recruitment, data accuracy and promptness of data report according to the chart below:

Center	# Subjects Rank	Accuracy Rank	Total Rank	Authorship Order
A	1	4	5	3
B	2	7	9	6
C	3	1	4	2
D	4	2	6	4
E	5	3	8	5
F	6	5	11	7
G	7	6	13	8

Each site's PI will be responsible to document the contributions to the study of that site's author. In the event the journal editor requires fewer authors than 10, the authors included will be subject to the ranking above. Consensus of the Steering Committee may alter inclusions to the explicitly listed authors. Other authors will be listed in the title page footnote.

II. Presentations

Network data should be presented before national organizations by the principal investigators. Organizations that might be appropriate include the American Fertility Society, the Society for Gynecologic Investigation, the American College of Obstetricians and Gynecologists, the American Urology Society and other urology or andrology societies. All presentations will be approved by Network consensus.

Once data are published in at least abstract form, all members of the Network can cite them publicly in lectures. However, investigators should avoid citing specific numbers in review articles and chapters, for this could jeopardize peer review publication.

Authorship, First Author, and Author Order are as described for Major Publications.

III. Minor Publications

Minor studies are defined as those in which the hypotheses would not be the main elements of Network studies, but in which the study database would be utilized. (One example would be determining if sperm velocity predicts successful therapy in a study whose major outcome variable is pregnancy after ovulation induction.) Ideas for "minor studies" will, in general, be proposed by a single individual, who would direct all efforts leading to publication and representation. The results from minor studies would be handled similarly to those from major studies.

A. Authorship: Publications would require acceptance by Network consensus although the list of authors may only identify the Reproductive Medicine Network with footnoted list. Centers may wish to withdraw inclusion from authorship of publications of minor studies in which only data are contributed.

IV. Ancillary Publications

An ancillary study is defined as one that extends the Network's research. (For example, collecting blood for antisperm antibody tests in subjects undergoing intrauterine insemination in an ongoing trial.) A request to conduct an ancillary study must be submitted to the Network Steering Committee for approval and verification that the study is indeed ancillary. Initiation, conduct, and data analysis will use not Network resources. However, publications based on ancillary studies would also require review by the Network.

A. Authorship: The investigator who initiates conducts and writes the ancillary study and those who (s)he names will be the sole authors.

V. Timelines

After conclusion of the protocol, the initiating investigator, designated as first author, will consult with the DCC for data analysis and conclusions. The investigator will draft an abstract for distribution to the Network and discussion at the next teleconference or Steering Committee meeting. This should be completed within two months of study completion. Presentation of such abstract may be discussed at this time.

The first author will prepare a draft manuscript within 6 months of data analysis for Network distribution. After incorporating network comments, discussion at the next Steering Committee meeting should allow preparation for publication to ensue in the following 3 months, such that submission will be possible 9months after data analysis is complete.

VII. Bibliography

1. Burghen GA, Givens JR, Kitabchi AE. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. J Clin Endocrinol Metab 1980;50:113-6.

2. Chang RJ, Nakamura RM, Judd HL, Kaplan SA. Insulin resistance in nonobese patients with polycystic ovarian disease. J Clin Endocrinol Metab 1983;57:356-9.

3. Dunaif A, Graf M, Mandeli J, Laumas V, Dobrjansky A. Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance, and/or hyperinsulinemia. J Clin Endocrinol Metab 1987;65:499-507.

4. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 1989;38:1165-74.

5. Ciaraldi TP, el-Roeiy A, Madar Z, Reichart D, Olefsky JM, Yen SS. Cellular mechanisms of insulin resistance in polycystic ovarian syndrome. J Clin Endocrinol Metab 1992;75:577-83.

6. Ehrmann DA, Sturis J, Byrne MM, Karrison T, Rosenfield RL, Polonsky KS. Insulin secretory defects in polycystic ovary syndrome. Relationship to insulin sensitivity and family history of non-insulin-dependent diabetes mellitus. J Clin Invest 1995;96:520-7.

7. Carmina E, Koyama T, Chang L, Stanczyk FZ, Lobo RA. Does ethnicity influence the prevalence of adrenal hyperandrogenism and insulin resistance in polycystic ovary syndrome? Am J Obstet Gynecol 1992;167:1807-12.

8. Norman RJ, Mahabeer S, Masters S. Ethnic differences in insulin and glucose response to glucose between white and Indian women with polycystic ovary syndrome. Fertil Steril 1995;63:58-62.

9. Dunaif A, Sorbara L, Delson R, Green G. Ethnicity and polycystic ovary syndrome are associated with independent and additive decreases in insulin action in Caribbean-Hispanic women. Diabetes 1993;42:1462-8.

10. Osei K, Schuster DP. Ethnic differences in secretion, sensitivity, and hepatic extraction of insulin in black and white Americans. Diabet Med 1994;11:755-62.

11. Huber-Buchholz MM, Carey DG, Norman RJ. Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. Journal of Clinical Endocrinology & Metabolism 1999;84(4):1470-4.

12. Guzick DS, Wing R, Smith D, Berga SL, Winters SJ. Endocrine consequences of weight loss in obese, hyperandrogenic, anovulatory women. Fertil Steril 1994;61:598-604.

13. Kiddy DS, Hamilton-Fairley D, Bush A, Short F, Anyaoku V, Reed MJ. Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clin Endocrinol 1992;36:105-11.

14. Pasquali R, Fabbri R, Venturoli S, Paradisi R, Antenucci D. Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries. Am J Obstet Gynecol 1986;154:139-44.

15. Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism 1994;43:647-54.

16. Nestler JE, Jakubowicz DJ. Decreases in ovarian cytochrome P450C17-alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. N Engl J Med 1996;335:617-23.

17. Velazquez E, Acosta A, Mendoza SG. Menstrual cyclicity after metformin therapy in polycystic ovary syndrome. Obstet Gynecol 1997;90:392-5.

18. Nestler JE, Jakubowicz DJ. Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17 alpha activity and serum androgens. J Clin Endocrinol Metab 1997;82:4075-9.

19. Crave JC, Fimbel S, Lejeune H, Cugnardey N, Dechaud H, Pugeat M. Effects of diet and metformin administration on sex hormone-binding globulin, androgens, and insulin in hirsute and obese women. J Clin Endocrinol Metab 1995;80:2057-62.

20. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Engl J Med 1998;338(26):1876-80.

21. Morin-Papunen LC, Koivunen RM, Ruokonen A, Martikainen HK. Metformin therapy improves the menstrual pattern with minimal endocrine and metabolic effects in women with polycystic ovary syndrome. Fertil Steril 1998;69:691-6.

22. Glueck CJ, Wang P, Fontaine R, Tracy T, Sieve-Smith L. Metformin-induced resumption of normal menses in 39 of 43 (91%) previously amenorrheic women with the polycystic ovary syndrome. Metabolism: Clinical & Experimental 1999;48(4):511-9.

23. Vandermolen DT, Ratts VS, Evans WS, Stovall DW, Kauma SW, Nestler JE. Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who are resistant to clomiphene citrate alone . Fertility & Sterility. 75(2):310-315, 2001 Feb.

24. Moran C, Knochenhauer ES, Azziz R. Non-classic adrenal hyperplasia in hyperandrogenism: a reappraisal. [Review] [94 refs] . J Endocrinol Invest 1998 Nov;21(10):707-20.

25. Anonymous. American Diabetes Association: clinical practice recommendations 1997 [see comments]. Diabetes Care 1997;20 Suppl 1:S1-70.

26. Ng EH, Wat NM, Ho PC. Effects of metformin on ovulation rate, hormonal and metabolic profiles in women with clomiphene-resistant polycystic ovaries: a randomized, double-blinded placebo-controlled trial. Hum Reprod 2001 Aug;16(8):1625-31.

27. Kocak M, Caliskan E, Simsir C, Haberal A. Metformin therapy improves ovulatory rates, cervical scores, and pregnancy rates in clomiphene citrate-resistant women with polycystic ovary syndrome. Fertility & Sterility 2002 Jan;77(1):101-6.

28. Batukan C, Baysal B. Metformin improves ovulation and pregnancy rates in patients with polycystic ovary syndrome. Archives of Gynecology & Obstetrics 2001 Aug;265(3):124-7.

29. Parsanezhad ME, Alborzi S, Zarei A, Dehbashi S, Omrani G. Insulin resistance in clomiphene responders and non-responders with polycystic ovarian disease and therapeutic effects of metformin. International Journal of Gynaecology & Obstetrics 2001 Oct;75(1):43-50.

30. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. A nomogram to predict the probability of live birth after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. Fertility & Sterility 2002 Jan;77(1):91-7.

31. Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. Journal of Clinical Endocrinology & Metabolism 2000 Jan;85(1):139-46.

32. Grizzle et al. Continuity correction in the chi-square test for the 2 x 2 tables. Am Statistician 1967;21:28-42.

33. Fleischer AC, Vasquez JM, Cullinan JA, Eisenberg E. Sonohysterography combined with sonosalpingography: correlation with endoscopic findings in infertility patients. J Ultrasound Med 1997 Jun;16(6):381-4; quiz 385-6.

34. Darwish AM, Youssef AA. Screening sonohysterography in infertility. Gynecologic & Obstetric Investigation 1999;48(1):43-7.

35. Jeanty P, Besnard S, Arnold A, Turner C, Crum P. Air-contrast sonohysterography as a first step assessment of tubal patency. J Ultrasound Med 2000 Aug;19(8):519-27.

36. Guermandi E, Vegetti W, Bianchi MM, Uglietti A, Ragni G, Crosignani P. Reliability of ovulation tests in infertile women. Obstetrics & Gynecology 2001 Jan;97(1):92-6.

37. Coetzee EJ, Jackson WP. Metformin in management of pregnant insulin-independent diabetics. Diabetologia 1979 Apr;16(4):241-5.

38. Callahan TL, Hall JE, Ettner SL, Christiansen CL, Greene MF, Crowley WF Jr. The economic impact of multiple-gestation pregnancies and the contribution of assisted-reproduction techniques to their incidence [see comments]. N Engl J Med 1994;331(4):244-9.

39. Coetzee EJ, Jackson WP. Pregnancy in established non-insulin-dependent diabetics. A five-and-a-half year study at groote schuur hospital. S Afr Med J 1980 Nov 15;58(20):795-802.

40. Sarlis NJ, Weil SJ, Nelson LM. Administration of metformin to a diabetic woman with extreme hyperandrogenemia of nontumoral origin: management of infertility and prevention of inadvertent masculinization of a female fetus. Journal of Clinical Endocrinology & Metabolism 1999;84(5):1510-2.

41. Diamanti-Kandarakis E, Kouli C, Tsianateli T, Bergiele A. Therapeutic effects of metformin on insulin resistance and hyperandrogenism in polycystic ovary syndrome. European Journal of Endocrinology 1998 Mar;138(3):269-74.

42. Seale FG, Robinson RD, Neal GS. Association of metformin and pregnancy in the polycystic ovary syndrome - a report of three cases . Journal of Reproductive Medicine. 45(6):507-510, 2000 Jun.

43. Glueck CJ, Phillips H, Cameron D, Sieve-Smith L, Wang P. Continuing metformin throughout pregnancy in women with polycystic ovary syndrome appears to safely reduce first-trimester spontaneous abortion: a pilot study . Fertility & Sterility. 75(1):46-52, 2001 Jan.

44. Hellmuth E, Damm P, Molsted-Pedersen L. Oral hypoglycaemic agents in 118 diabetic pregnancies . Diabetic Medicine. 17(7):507-511, 2000 Jul.

45. Asch RH, Greenblatt RB. Update on the safety and efficacy of clomiphene citrate as a therapeutic agent. J Reprod Med 1976 Sep;17(3):175-80.

46. Rossing MA, Daling JR, Weiss NS, Moore DE, Self SG. Ovarian tumors in a cohort of infertile women. N Engl J Med 1994 Sep 22;331(12):771-6.

47. Mosgaard BJ, Lidegaard O, Kjaer SK, Schou G, Andersen AN. Infertility, fertility drugs, and invasive ovarian cancer: a case-control study [see comments]. Fertility & Sterility 1997 Jun;67(6):1005-12.

48. Parazzini F, Negri E, La Vecchia C, Moroni S, Franceschi S, Crosignani PG. Treatment for infertility and risk of invasive epithelial ovarian cancer. Hum Reprod 1997 Oct;12(10):2159-61.

VIII. APPENDIX A.

SCHEDULE OF EVENTS

Event	Baseline	Weekly Visit	Monthly Visit	Study Drug Termination	Pregnancy Visit	Pregnancy Outcome
Obtain consent	■					
Complete Physical	■			■		
Ultrasound	■				■	
Safety labs blood work	■			■		
Metabolic/ Reproductive Blood work	■		■	■		
Progesterone Level	■	■	■	■		
Appropriate CRFs	■		■	■	■	■
Other Procedures	Semen-analysis, tubal patency test		Dispense medication, obtain WT and WHR			

APPENDIX B: Risk Factors for Genetic Disorder

1) Will you be age 35 or older when your baby is due? _ Yes_ No

2) If you or your partner is of Mediterranean or Asian decent, do either of you or anyone in your families has thalassemia? _ Yes_ No

3) Is there a family history of congenital heart defects? _ Yes_ No

4) Is there a family history of Down syndrome? _ Yes_ No

5) Have you ever had a child with Down syndrome? _ Yes_ No

6) If you or your partner is of eastern European Jewish or French Canadian descent, is there a family history of Tay–Sachs disease? _ Yes_ No

7) If you or your partner are of eastern European Jewish descent, is there a family history of Canavan disease? _Yes_ No

8) If you or your partner is African American, is there a family history of sickle cell disease or trait? _Yes_ No

9) Is there a family history of hemophilia? _Yes_ No

10) Is there a family history of muscular dystrophy? _ Yes_ No

11) Is there a family history of cystic fibrosis? _ Yes_ No

12) Is there a family history of Huntington disease? _Yes_ No

13) Is anyone in your or your partner's family mentally retarded? _ Yes_ No

14) If so, was that person tested for fragile X syndrome? _ Yes_ No

15) Do you, your partner, anyone in your families, or any of your children have any other genetic diseases, chromosomal disorders, or birth defects? _ Yes_ No

16) Do you have a metabolic disorder such as diabetes or phenylketonuria? _ Yes_ No

17) Have you had more than two miscarriages in a row? _Yes_ No

18) Have you ever had a baby who was stillborn? _ Yes_ No

19) Is there a family history of alpha-1 antitrypsin deficiency? _ Yes_ No

Appendix C: Core Lab Instructions from UVA Center for Research in Reproduction

PHONE: (434) 982-3675 FAX: (434) 982-0701 EMAIL: LIGANDCORE@VIRGINIA.EDU

ASSAY	TOTAL TESTOSTERONE	SHBG	INSULIN	PROINSULIN	GLUCOSE
METHOD	IMMULITE	IMMULITE	IMMULITE	ELISA	OLYMPUS
SAMPLE TYPE	SERUM	SERUM	SERUM/ HEP. PLASMA	SERUM	SERUM
SAMPLE VOLUME	20 UL X2	10 UL X2	100 UL X2	50 UL X2	50 UL X2
MIN. DEAD VOLUME	100 UL	100 UL	250 UL	NA	NA

NA= NOT APPLICABLE
MINIMUM TOTAL SAMPLE VOLUME NEEDED TO ASSAY ALL TESTS = 2 ML OF SERUM

SAMPLE COLLECTION PROCEDURES:
Normal venipuncture procedures should be followed. Please collect enough blood in a red top tube to give you a minimum of 2 ml of serum. Allow red top to clot 15- 30 minutes before separating. Separate by centrifuging samples 10 minutes at 4ºC or room temperature and 3000 RPMs. Aliquot serum samples into two plastic screw cap 1.5 ml vials with O-rings. Aliquots may be temporarily maintained at -20ºC in a non frost-free freezer, but should be moved to a -70ºC or -80ºC freezer as soon as possible.

SHIPPING INSTRUCTIONS:

1. Notify lab before shipping samples. (Do not ship for weekend delivery.)

2. Ship samples frozen in watertight receptacles on dry ice by Fed-Ex Priority Overnight. Please adhere to all rules regarding shipment of hazardous material from your institution.

3. Ship to the following address: **University of Virginia**
   Center for Research in Reproduction
   OMS Suhling Bldg., RM 6921 Hospital Drive
   Charlottesville, VA 22908
   Attn: Aleisha Schoenfelder, MT

SAMPLE LABELING:

Each sample is to be identified with one of the provided labels. The patient's assigned randomization number, visit number and date specimen obtained are to be entered on the label. Each core lab shipment must be accompanied by the UVA Core Lab Shipment Form.

DATA OUTPUT:
The data will be transferred to the DCC and emailed to the appropriate investigator at the completion of the study as an Excel document, which will include the following information:
Sample #
History #
Average Result
Mean
SD
CV%

Core Lab Blood Draw Schedule
*All blood draws are fasting

Baseline	Monthly Visit	Study Drug Termination Visit
▪ 7.5cc red top to be drawn fasting within 30 days prior to randomization (recommend drawing red top the same time as safety labs)	▪ 7.5cc red top drawn fasting at <u>each</u> monthly visit	▪ 7.5cc red top drawn fasting
▪ Centrifuge for 10 minutes at 3000 RPM	▪ Centrifuge for 10 minutes at 3000 RPM	▪ Centrifuge for 10 minutes at 3000 RPM
▪ <u>Aliquots</u> Fill two 1.5ml vials with serum for core lab Dispense remaining serum into 0.5ml aliquots. Maintain at site for future research.	▪ <u>Aliquots</u> Fill two 1.5ml vials with serum for core lab Remaining serum to be placed into 1 vial and maintained at site.	▪ <u>Aliquots</u> Fill two 1.5ml vials with serum for core lab Remaining serum to be placed into 1 vial and maintained at site.

Appendix D: List of common medications excluded or requiring wash-out period.

PPCOS Excluded Medications

A patient will be excluded from study if they are taking any medication that should not be discontinued and this medication would affect reproductive function or metabolism, or would interact with either study medication.

Angiotensin Converting Enzyme (ACE) Inhibitors
- Prinivil (lisinopril)
- Captoten (captropril)
- Cozaar (losartan)
- multiple others

Antidysrythmics
- Procainamide (Pronestyl)
- Digoxin (Lanoxin)

Antimalarial
- Quinine

Calcium Channel Blockers
- diltiazem (Cardizem, Dilacor, Tiazac, Diltia-XL)
- verapamil (Isoptin, Calan)
- amlodipine (Norvase)
- felodipine (Plendil)
- isradipine (DynaCirc)
- nicardipine (Cardene)
- nifedipine (Procardia, Adalat)
- nifsoldipine (Sular)

Somatostatin
- octreotide (Sandostatin)
- lanreaotide

Diuretics
- Amiloride (Midamor)
- Furosemide (Lasix)
- Triamterene (Dyrenium)

Steroids
- Prednisone (Deltasone)
- Dexamethasone (Decadron)

A short course of steroid therapy for treatment of asthma will not qualify as a reason to withdraw a patient from the study.

Vasodilator
- Diazoxide

Hormonal Medications Requiring 1 month wash-out:

Progestins (Oral or Cyclic)
- medroxyprogesterone acetate (Provera, Cycrin, Amen, Curretab)
- megestrol (Megase)
- norethindrone (Aygestin)
- progesterone gel (Crinone)
- Micronized progesterone (Prometrium)

GnRH Agonists/Antagonists
- Leuprolide (Lupron)
- nafarelin (Synarel)
- buserelin
- gosarelin (Zoladex)
- ganarelix (Antagon)
- cetrorelix (Cetrotide)

Gonadatropins
- Pergonal
- Repronex
- Follistim
- Gonal-F
- Fertinex
- Metrodin

Other medications Requiring 1 month wash-out:

Anti-androgens
- cyproterone (Cyprostat)
- spironolactone (Aldactone)
- flutamide (Eulexin)
- finasteride (Proscar, Propecia)

Anti-obesity
- diazoxide (Proglycem)
- orlistat (Xenical)
- sibutramine (Meridia)
- diethylpropion (Tenuate)
- phendimetrazine (Bontril)
- phentermine (Adipex-P, Fastin, Ionamin)

Hormonal Medications Requiring 3 month Wash-Out Period:

Clomiphene citrate (Clomid, Serophene)

Depot GnRH agonists

Injectable Contraceptives
medroxyprogesterone acetate (Depo Provera)

Oral Contraceptives
Any Brand
Progestins (Not including cyclic)
Any Brand

Other Medications Requiring 3 month Wash-out Period:

Anti-Acne
isotretinoin(Accutane)

Anti-diabetic
acarbose (Precose)
Insulin
sulfonylureas
acetahexamide (Dymelor)
chlorpropamide (Diabinese)
tolazamide (Tolinase)
tolbutamide (Orinase)
glimepiride (Amaryl)
glipizide (Glucotrol)
rosiglitazone (Avandia)
pioglitazone (Actos)
Biguanides
metformin (Glucophage)

Medications with potential longer washouts (Contact DCC)
Contraceptive Implants
Norplant (levonorgestrel implants)

Appendix E
Adverse Events

Adverse events are collected and reported in clinical trials to determine a drug, devise or procedure's safety profile, to evaluate the benefits versus the risks of the intervention, and to provide information to be included on the package insert when a drug is marketed.

Adverse Events
Adverse events are defined as unfavorable medical changes that occur during or after study initiation, that may or may not be related to or caused by study participation. Adverse events can be:

- Physical signs or symptoms
- Abnormal laboratory values
- Changes in vital signs, physical examination findings, or test results such as electrocardiograms
- An increase in the frequency or intensity (worsening) of a condition or illness that was present before study enrollment
- Complications from a procedure or surgery.

Adverse events for PPCOS are not:

- Procedures or surgeries (the medical condition that caused the need for the procedure or surgery is the adverse event)
- Pre-existing conditions or illnesses that do not worsen during the study period.
- Normal complications from pregnancy.

Tips on Reporting Adverse Events in PPCOS

- Monitor for adverse events throughout study participation. For patients who do not conceive, monitor until one week after end of study drug treatment. For patients who conceive, monitor serious adverse events through delivery/termination.
- Report a diagnosis rather than a symptom if possible. For example, report "flu" rather than "headache," "fever," and "cough" as separate symptoms.
- Record adverse events on the AE form.
- Continue to track each event until it is resolved or until one week after study drug termination.

Intensity

The intensity of the event should be evaluated and recorded as one of the following:

- **Mild** intensity – events may or may not be volunteered by the patient. The patient is aware of the event but it is easily tolerated.

- **Moderate** intensity – signifies discomfort sufficient to interfere with normal activities. A change in therapy may or may not be indicated.
- **Severe** intensity – side effects are almost always brought up by the patient, definitely interfere with functioning, and require a medical intervention.

A "severe" adverse event is not the same as a "serious adverse event" or SAE. *Severity is based on the intensity of the event, while seriousness is based upon the event outcome as it poses a threat to the patient's life or functioning.*

Examples of severe adverse events for PPCOS:

1) Diarrhea requiring out-patient IV hydration, alteration in dose of medication or absence from work
2) Abdominal cramping (mittelschmerz) requiring change in activities or absence from work
3) Visual changes/blurry vision requiring stopping of medication

Serious Adverse Events

In clinical trials, a serious adverse event is defined as an experience occurring during the study period that:

- Results in death
- Is life-threatening (at immediate risk of death)
- Results in persistent or significant disability or incapacity
- Requires or prolongs inpatient hospitalization
- Is a congenital anomaly/birth defect
- Other significant medical event that requires intervention in order to prevent one of the events listed above

Any serious adverse event or death must be reported immediately regardless of the circumstances or suspected cause if it occurs or comes to the attention of the investigator at any time during the study. Any SAE occurring at any other time after completion of the study must be promptly reported if a causal relationship to study intervention is suspected.

The investigator is obligated to pursue and provide information as requested by NICHD or the Duke Clinical Research Institute. In general, this will require a description of the event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, including concomitant medications and illnesses, must be provided.

The investigator's assessment of causality must also be provided. If causality is unknown, it should be attributed to study participation.

Examples of Serious Adverse Events for PPCOS:

1) Hospitalization for severe diarrhea/dehydration/OHSS
2) Surgery for ovarian cyst
3) Systemic reaction requiring hospital admission for observation or management
4) Pregnancy loss after 12 weeks gestation

Relationship to Study Participation

The certainty of the relationship of the event to study participation will be recorded as "Possible Related" or Not Possibly Related." The situation surrounding the event should be assessed to determine whether it is related to the study.

REPORTING OF ALL SERIOUS ADVERSE EVENTS FLOWCHART

Site Becomes Aware of Serious Adverse Event

Site notifies DCRI Safety Surveillance of reportable event within 24 hours (or 1 business day) of learning of the event

DCRI Safety Surveillance notifies NICHD of reportable events within 1 business day of receipt.

Site notifies IRB of event

Site completes all follow-up information required by DCRI or NICHD

Other RMN Investigators notify their IRB of the event if required.

IX. Investigator Signature of Agreement

National Institute of Child Health and Human Development
Reproductive Medicine Network

Title: A 30 week double-blind randomized trial of clomiphene citrate, metformin XR, and combined clomiphene citrate/metformin XR for the treatment of infertility in women with polycystic ovary syndrome.

Principal Investigator:

_____

Signature Date

Co-Investigators:

_____

Signature Date

_____

Signature Date

CONSENT FOR CLINICAL RESEARCH STUDY

Penn State College of Medicine
Penn State Hershey Medical Center

Title of Project: A thirty-week double blind randomized trial of Clomiphene Citrate, Metformin XR, and combined Clomiphene Citrate/Metformin XR for the treatment of infertility in women with polycystic ovary syndrome.

Principal Investigator: Richard Legro, M.D.

Other Investigators: William Dodson, M.D., Carol Gnatuk, M.D., Jamie Ober, R.N., Mindy Rivera, Barb Scheetz

Participant's Printed Name:_____

This is a research study. Research studies include only people who voluntarily choose to take part. This consent form gives you information about this research, which will be discussed with you. This consent form may contain words or procedures that you do not understand. You are urged to ask questions about anything that is unclear to you. Discuss it with your family and friends and take your time to make your decision. You will receive a copy of the signed and dated consent form to keep.

1. Purpose of the Study:

The purpose of the study is to determine which of three drug therapies, Clomiphene Citrate alone, Metformin XR alone or the combination of both (Metformin XR and Clomiphene Citrate); will most likely result in pregnancy. Metformin XR is approved by the FDA (Food and Drug Administration) for the treatment of type 2 diabetes, but not for ovulation induction (causing you to release an egg to increase your chance of pregnancy). The FDA has not approved the combination of the two. Both however are commonly used medications to achieve pregnancy in women with polycystic ovary syndrome.

The Reproductive Medicine Network of the National Institutes of Health sponsors this study. There are a total of 12 sites participating in the study and a total of 678 women will participate. Approximately 75 women are expected to participate at the Hershey Medical Center.

2. Procedures to be Followed:

Screening Visit-Visit #1

During the first visit you will be asked questions about your medical history and examined. You understand that you may still participate in the study if you have taken either Metformin XR (brand name is Glucophage) or Clomiphene Citrate (brand names include Serophene and Clomid) in the past. However, you should notify the study personnel that you have, as it may affect the type of drug

you receive in the study. Blood work will be done at this visit to determine if you are eligible for further study. This blood work will consist of androgen (male hormone) levels as well as other hormones to correctly diagnose you with polycystic ovary syndrome. You will also have blood work to ensure that your kidneys and liver are functioning normally and that you are not anemic. A little more than four tablespoons of blood will be drawn at this visit. It is possible that the results of the blood work will show that you are not a candidate to proceed further with the study. An ultrasound of your ovaries and uterus will be performed to look at their appearance and size

If you are a candidate for further study, you will be asked to have your partner provide a sample of his semen and to provide results of any tests you may have had to see if your tubes are open (hysterosalpingogram, laparoscopy, sonohysterogram). These tests are needed to verify that there are no other infertility factors so that you will be receiving the proper therapy and have a reasonable chance for success. If necessary, these tests will be arranged by the doctors performing the study, but will not be reimbursed as part of the study.

Preconceptional Counseling

You will be offered preconceptional counseling during this screening visit to identify any problems that may lead to complications during your pregnancy. You will fill out a questionnaire to identify if there are any genetic diseases in your family and will receive counseling if this is the case. Additionally, you will be offered blood tests to determine if you are immune to rubella (German Measles) or to see if you are infected with the HIV virus. If these tests are abnormal, you will be referred for appropriate treatment and counseling, however they will not prevent you from being eligible for this study. Additionally, you will be given a prescription for prenatal vitamins, which you will take daily. A daily prenatal vitamin has been found to reduce certain types of birth defects.

<u>Randomization and Progestin Withdrawal (Visit #2)</u>

When you have met all of the criteria above for participation, you will have a urine pregnancy test performed to make sure you are not pregnant. If you have not had a recent menstrual period, you will take a 10 day course of Medroxyprogesterone Acetate (Provera 5 mg) to bring on a menstrual period, and then by chance be placed into one of the three treatment groups: Clomiphene Citrate alone, Metformin XR alone, or both Clomiphene Citrate and Metformin XR together. You will have an equal chance of receiving any of these treatments.

The study medication will be given to you at this visit. You will be taking pills from two bottles although it is possible one of the bottles contains a placebo (inactive) pill. One of the treatment groups will take an active pill containing Clomiphene Citrate 50 mg daily for five days every treatment cycle plus a daily placebo. If you don't respond by ovulating, your dose will be increased by one pill (50 mg) a day for five days each cycle to help you ovulate. This will be 100 mg/day in the next cycle if you don't respond, and then further to 150 mg/day if you don't respond. The dose of Clomiphene Citrate will not be increased beyond 150 mg/day so it is possible you will receive Clomiphene Citrate 150 mg/d for five days for up to 4 cycles without responding. This is not the standard practice for giving Clomiphene Citrate, but it is possible you may respond to a later cycle at this dose, even if you haven't responded to an earlier cycle.

The second treatment groups will receive Metformin XR 1000 mg twice a day every day (or two tablets of 500 mg twice a day) at a maximum dose, plus a placebo. The Metformin XR will start at one pill a day for five days and will increase every five days until you are taking a total of 4 pills a day.

The third treatment group will receive both treatments at once, the Clomiphene Citrate for 5 days every cycle and the Metformin XR every day. Neither you nor the doctors and nurses in the study will know which pill you have received until the study has been completed. It is important that you take the pills from both bottles as instructed.

You will be instructed to have intercourse on a regular basis (at least 2 times per week separated by at least 1 day) to increase your chance for pregnancy. Intercourse that occurs too infrequently (less than once a week) or too frequently (more than once a day) may lower your chances for pregnancy.

Weekly Visits (for ~30 weeks or a total of ~32 visits)

You will follow-up on a weekly basis for a brief visit. You will be given a diary to keep track of your menstrual bleeding and any symptoms you have on a daily basis. If you are uncertain about the meaning of any symptoms you develop, you are to call any time, day or night, to report these. At your weekly visits, blood will be drawn to determine if you have ovulated, and your urine may also be checked for pregnancy if you have ovulated recently. A total of 2 tablespoons of blood will be taken each week. The information obtained from the weekly visits may be used to change the dosing of your medication, which will be given to you at these visits. Once a month, in addition to the above procedures, you will have your weight and your waist and hips measured and you will also bring in your medications to have the pills counted. You will continue to be monitored weekly for a total of up to 30 weeks or until you become pregnant. If you become pregnant you will be instructed to stop your study medication and given instructions about what to do next. Once a month, in addition to the above tests and procedures, you may receive another ultrasound of the ovaries if the doctor feels it is important.

Pregnancy

If you do become pregnant the doctors will stop your medication and follow you until they determine your pregnancy is proceeding normally, which will be around 6-8 weeks after conception. At that time you will follow up with your regular doctor for prenatal pregnancy care. If you don't have a doctor who delivers babies, you will be referred to one. Pregnancy care is not provided as part of the study. You understand that you will be contacted to find out the results of your pregnancy and any problems you or your baby may have during pregnancy and delivery. It is important that you provide this information in order to determine the ultimate safety and effectiveness of these treatments.

Final Visit

You will undergo a final visit after you complete the study or become pregnant, whichever comes first. You will bring in any remaining medication, and undergo a history and physical. You will have blood tests to check your liver

and renal function and to measure hormones such as androgens and insulin. A little more than four tablespoons of blood will be drawn at this visit.

3. Discomfort and Risks

Metformin XR

Metformin XR may cause nausea, vomiting, stomach upset, diarrhea, bloating, flatulence, and a metallic taste in your mouth as well as loss of appetite. In studies comparing Metformin XR to sugar pills (placebos), patients receiving placebos were 30% less likely to experience these side effects as patients receiving Metformin XR. You understand that most of the nausea, vomiting and diarrhea are usually associated when you start the medication and in most cases go away the longer you take the medication, and also can be minimized if taken with meals. If you are unable to tolerate the symptoms that occur with this medicine, your symptoms will be managed with a decrease in the daily Metformin XR dose (one pill per day per week) until your symptoms resolve.
If symptoms subside, after one week you will be instructed to increase your dose by one tablet every five days up to four tablets a day.

Very rarely (the chance is about 3 in 100,000), Metformin XR is associated with lactic acidosis, a metabolic condition that is often fatal. This complication usually occurs in patients with diabetes or poor kidney function. The symptoms of lactic acidosis may be fatigue, more difficulty breathing with deep breaths, and confusion. You understand that if you undergo any radiological procedures that involve exposure to iodine containing agents (which includes a hysterosalpingogram- a dye test used to determine if your tubes are open), you are to stop taking the medication for one week prior to the test and not resume it until one week after the test. You are to notify the principal investigator of this study, and the radiologist prior to receiving the radiology study about your use of Metformin XR. Metformin XR is not known to cause any birth defects, but if you suspect you are pregnant you should notify the doctor. Metformin XR will be stopped when you are pregnant.

Clomiphene Citrate

Clomiphene Citrate may cause nausea, vomiting, and constipation. Hot flashes affecting 10% of patients have also been reported. Clomiphene Citrate has been described but rarely causes effects to your liver including an abnormality of your liver enzymes and also inflammation of your liver described as hepatitis. Mood changes are also frequently reported. Clomiphene Citrate may cause visual changes and you should notify the doctor of these immediately. It may also lead to the development of persistent ovarian cysts or ovarian enlargement.

Clomiphene Citrate increases your chance for multiple pregnancy-twins, triplets, etc. Multiple pregnancy rates between 5-10% have been reported with the use of clomiphene citrate and most multiple pregnancies are twins. Multiple pregnancy places you at increased risk for the complications of pregnancy such as diabetes, hypertension, and bleeding. The babies from multiple pregnancy are at increased risk for delivering before their due date, which places them at increased risk for problems with their lungs and other organ systems. You will possibly be receiving Clomiphene Citrate for up to 3 cycles even if you have not initially

responded to it. While this is not usually done clinically because the likelihood of responding is low, it is possible you may respond to one of these additional treatment cycles by ovulating. Clomiphene Citrate is not known to cause any birth defects. Some studies have suggested that prolonged and/or multiple cycles of Clomiphene Citrate may slightly increase the risk of ovarian cancer. More recent studies suggest that there is no increased risk of any cancer in women who use Clomiphene Citrate.

Clomiphene Citrate and Metformin XR together

The combination of these two medications has not been extensively studied in women with polycystic ovary syndrome, but the combination has no known additional risks other than those listed above individually for each agent. Because neither the investigator and research team nor you will know what medication(s) you are on, you should notify them of any of the above symptoms or signs once you start medication.

Medroxyprogesterone acetate (Provera)

If you have not had a recent menstrual period you will be given medroxyprogesterone acetate 5mg to be taken daily for 10 days. Some of the side effects associated with use of this medicine may include nausea, breast tenderness, skin sensitivity to the sun and also a rash.

Procedures

A vaginal probe ultrasound exam of your ovaries involves inserting an ultrasound probe into your vagina to visualize your ovaries and uterus. This may cause some temporary discomfort.

The discomfort associated with removing blood by venipuncture (by needle from a vein) is a slight pinch or pinprick when the sterile needle enters the skin. The risks include mild discomfort and/or a black and blue mark at the site of puncture. Less common risks include a small blood clot, infection or bleeding at the puncture site, and on rare occasions fainting during the procedure.

Pregnancy

Complications may result from pregnancy but there is no evidence to date suggesting the study drugs contribute to these complications.

4. a. PossibleBenefits to You: The medication(s) may help you to become pregnant. The information from these blood tests will let you know more about your health such as the level of hormones involved in reproduction in your blood and your predisposition to diabetes. Abnormalities such as ovarian cysts or abnormalities of the uterine lining may be noted on the initial ultrasound exam. Additionally you may experience regular menstrual bleeding and normal ovulation on these medications. No benefit is guaranteed.

b. Potential Benefits to Society: Information gained from this study may help discover the best and safest way to achieve pregnancy in women with polycystic ovary syndrome.

5. Other Options That Could be Used Instead of this Research: You can elect not to participate and talk with your doctor about other therapies for inducing (causing) ovulation in polycystic ovary syndrome. Clomiphene Citrate can be obtained without being in the study.

6. Time Duration of the Procedures and Study: There will be up to 32 visits over approximately 30-40 weeks. The first two visits and the final visit will last around an hour. The weekly visits will last around 15 minutes, and the monthly visit for an ultrasound of your ovaries will last about 30 minutes if this is performed. If pregnancy occurs you understand you will be contacted 6 weeks past your documented expected delivery date for information related to your pregnancy and delivery for a total study time of up to 71 weeks if you get pregnant on the last month.

7. Statement of Confidentiality:

Health information about you will be collected because you are a part of this research study. By signing this form, you are allowing the people and groups that are listed in the next paragraph to use your health information for purposes related to this research. You are also allowing these groups to share your health information with other specific groups for their use as part of this research study. Your information will only be used or shared as explained in this consent form or when required by law.

The research team may use the following sources of health information:

Personal health history and/or medical records from January 2002 till present

Genetic questionnaire
Daily diary
Hysterosalpingogram result
Physical exam and pap smear results
Blood sample results including HIV tests
Ultrasound results

There may be other health information that is not listed here. Your health information may be used or shared with other specific people or groups in connection with this research study. Research records that identify you will be kept confidential as required by law. You will not be identified by name, social security number, address, phone number or any other direct personal identifier in research records given to someone outside of The Milton S. Hershey Medical Center (HMC) or Penn State College of Medicine (PSU), except when required by law, will not identify you. For records shared outside of HMC/PSU, you will be assigned a code number. The list that matches your name with the code number will be kept in a locked file in Dr. Legro's office.

Representatives of the following people/groups within HMC/PSU are allowed to use and share your health information with other specific groups in connection with this research study:

The principal investigator, Dr. Richard S. Legro, M.D.,
The HMC/PSU Institutional Review Board,

The HMC/PSU Human Subjects Protection Office,
The research team

The people or groups listed in the above paragraph may share your health information with the following persons and organizations outside HMC/PSU for their use in connection with this research study:

The Office of Human Research Protections in the U.S. Department of Health and Human Services
Reproductive Medicine Network of the National Institute of Child Health and Human Development
Duke Clinical Research Institute
Food and Drug Administration

Your information may be shared with groups not listed here if the people who receive your health information are not required by law to protect the privacy of the information.

Your permission for the use and sharing of your health information will continue indefinitely. If you consent to the collection of samples of your blood for future research, the period for the use of the sample is unknown.

People usually have a right to access their medical records. However, while the research is in progress, you may not be allowed to see or copy certain information collected in connection with your participation in this research study. This condition will be effective for the period of the research, and you will be allowed to see your records when the whole research project is complete.

If you wish to participate in this research, you must sign this form. If you do not want to participate you will receive the standard medical care as decided by your physician.

You are free to withdraw your permission for the use and sharing of your health information, but you must do this in writing as indicated in the HMC Privacy
Notice. If you do decide to withdraw, we ask that you contact Dr. Legro in writing and let him know that you are withdrawing from the research study. His mailing address is as follows:

Milton S. Hershey Medical Center
Dr. Richard Legro, M.D.
P.O. Box 850
500 University Drive H103
Hershey, PA 17033

If you withdraw your permission, we will no longer use or share medical information about you for the reasons covered by your written authorization, except when the law allows us to continue using your information. We are unable to take back anything we have already done or shared with your permission and we may continue using and sharing the information obtained prior to your withdrawal as necessary to maintain the soundness of the overall research. Also, we are required to keep our records of the care that we provided to you as long as the laws required.

The samples of your blood and your research records will be labeled with a code number and your initials. Your blood samples may be stored for use in future studies to be conducted by Dr. Legro. Your samples will be stored in a freezer and will be locked in Dr. Legro's Lab at the Hershey Medical Center. Your research information will be kept in a password-protected computer file and locked filing cabinet. Blood samples and your research records are also sent to Duke Clinical Research Institute. There your information will be analyzed and stored in a password-protected computer file. There your blood samples will be stored in a locked freezer.

In the event of any publication resulting from the research, no personally identifiable information will be shared.

8. Right to Ask Questions:

You have been given an opportunity to ask any questions you may have and all such questions or inquiries have been answered to your satisfaction. If you have further questions or concerns related to this study, or if you believe you may have developed an injury that is related to this research, you should contact Dr. Legro (717-531-8478) or the OB/GYN resident on 24-hour call (717-531-8521).

If you have questions regarding your rights as a research subject, you may contact the research protection advocate in the HMC Human Subject Protection Office at 717-531-5687.

If you have any questions or concerns regarding your privacy and the use of your personal health information, please contact Jim Bifano, the HMC Privacy Officer, at 717-531-8059.

At the end of the study you will be informed of your response to the treatments including the frequency of ovulation and the effects on your blood androgen, glucose and insulin levels.

9. Reimbursement and/or Costs of Participation:

The physical exam, ultrasound(s), blood tests, and medications that are required parts of the study will be provided at no cost to you. All study procedures once you have
started the medication, if performed for the purpose of the study, will be paid for by the study. You will not incur any additional costs as a participant in this study. The costs of the semen analysis and test to determine if your tubes are open are not part of this study.

You understand that you, or your insurance carrier, will be responsible for covering the costs of a semen analysis of your partner, and a test to determine if your tubes are open. You or your insurance will be responsible for paying for the blood tests, any further testing or treatment related to the preconceptional counseling, as well as the cost of the prenatal vitamins.

You will receive no monetary compensation for participating in the study. If you conceive, pregnancy care will not be provided as part of the study and you will need to find a doctor to provide this or one will be recommended to you. You

understand that the costs of your prenatal care after this point and costs of your delivery and any complications of your pregnancy will not be covered by this study. Therefore you, or your insurance carrier, will be responsible for these costs.

It is possible that you could develop complications or injuries as a result of participating in this research study. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. It is the policy of this institution to provide neither financial compensation nor free medical treatment for research-related injury. You are not waiving any legal rights you may have by signing this form. Costs for the treatment of research-related injuries will be charged to your insurance carrier or to you. Some insurance companies may not cover costs associated with research studies. If for any reason these costs are not covered by your insurance, they will be your responsibility.

10. Research Funding

The institution and investigators are receiving a grant from the National Institute of Child Health and Human Development Reproductive Medicine Network to support this research.

11. Voluntary Participation

Your participation in this research study is voluntary. If you choose to take part in this research, your major responsibilities will include compliance with visits, medication regiment, and protocol instructions. You do not have to participate in this research. If you choose to take part, you have the right to stop at any time. If you decide not to participate or if you decide to stop taking part in the research at a later date, there will be no penalty or loss of benefits to which you are entitled. In other words, your decision not to participate in this research or to stop taking part in the research will not affect your medical care. You understand that the investigator without your consent may also stop your participation in the research if this is deemed to be appropriate.

12. Storage of Leftover Blood Samples for Future Research Studies:

If you agree, the research team would like to store leftover samples of your blood that is collected as part of this research. These stored samples may be used for future research tests. The storage of leftover blood is optional and will not affect your participation in this research study. You can participate in the main part of the research without agreeing to allow your leftover samples to be stored for future research studies. The results of these tests will not have an effect on your care, nor will the results be put in your health record. If you agree to allow your blood to be kept for future research, you will be free to change your mind at any time. If you have any questions, you should contact Dr. Legro at (717) 531-8478.

You should initial below to indicate your preferences regarding the optional storage of your blood or tissue samples for research purposes.

_____ Your sample[s] **may not** be stored and used for future research studies.

____ Your sample[s] **may** be stored, **but** you want to be contacted again for permission before using your samples for other research studies.

_____ Your sample[s] **may** be stored and used for **any** future research studies involving this or any other project without your future permission.

_____ My sample(s) may be used for this project only. Do not contact me again for permission.

_____ Specific restrictions I place on the use of my sample(s) (if any) are:

_____

*(Initial one)*_____I do or _____ I do not wish to be contacted by investigators with information derived from my sample(s).

If you will be participating in another clinical trial at Hershey Medical Center or elsewhere while in this research you understand you should discuss the procedures and/or treatments with your physician or the investigators. This precaution is intended to protect you from possible adverse effects from interactions of research drugs, treatments or testing.

Signature and Consent to be in the Research

Your signature below means that you have read the above information about this research study and have asked the questions you currently have about this research. By signing this consent form, you indicate that you are voluntarily choosing to take part in this research and have had the opportunity to ask questions and those questions have been answered.

_____ _____ _____ _____
Signature of Participant Date Time Printed Name

Your signature below means that you have read the above information about this research study and have asked the questions you currently have about this research. By signing below, you indicate that you give permission for the participant to take part in this research and have had the opportunity to ask questions and those questions have been answered.

_____ _____ _____ _____
Signature of Participant's Representative Date Time Printed Name

_____
Description of Representative's Authority to Act for Participant

_____ _____ _____ _____
Signature of person who explained this research Date Time Printed Name

ESTIMATED BUDGET FOR REPRODUCTIVE MEDICINE NETWORK UNITS

The budget makes the following assumptions acknowledging that there will be differences between sites. The personnel costs for the additional U54 sites are not included, although material/recruitment costs are contained within the budget below. Assays from the core lab for the purpose of publication are also not included in this budget, but have been submitted separately as a supplement to Dr. Nestler's SCCPRR grant.

Materials Costs

Assays and safety labs are estimated at research and not market rates. We have also projected a cumulative 10% pregnancy rate per cycle. Costs of medications and necessary lab tests for the clinical screening and safety of the patient are included. These include Safety labs (at both initiation and completion of treatment): CBC with platelets ($6.47), renal screen ($9.41), and hepatic profile ($7.61) and serum androgens at baseline (total is $45.50) and end of study.

A monthly cycle will consist of on average 4 weekly progesterone tests ($19.00/test) and 4 urinary hCG screens ($5.00/test) as well as the costs of metformin XR ($60.00/cycle) or clomiphene citrate ($50.00/cycle). Additional medication costs will include the initial withdrawal bleed induced with Provera ($15.00). Recruitment costs of $150.00 per patient has been included. There will be no patient reimbursement for participation in the study. Drug costs, including placebo and packaging, are prepared by the DCC and are not included in this budget estimate. The minimal infertility evaluation of S/A and SHG will be performed as part of a basic infertility evaluation and are not included as study costs. Pregnancy ultrasounds will not be charged to the study.

Recruitment/Patient Costs

It is anticipated that advertisements will attract a large proportion of study subjects and $150.00 per patient is budgeted. There is no patient reimbursement for participation, but substantial benefit from the goal of pregnancy.

Personnel

The P.I. salary was calculated at the NIH salary cap and a 5% overall effort/year and nurse coordinator's at 25% effort/year and an average salary of $40,000/year. Percent effort was estimated for the average 3 year period, although initially this will be larger and later probably much less. Fringe rates were calculated at 28%.

RMN Unit Costs

RMN Lab/Med Costs	Cost per test or subject/ cycle	Cycle1	Cycle 2(10% conception rate)	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Total
# nonpregnant subjects		678	549	494	445	400	360	
Baseline/follow-up Safety labs	46	30,849					30,849	61,698
Serum p4	76	51,528	41,738	37,564	33,808	30,427	27,384	222,448
hCG Screening	20	13,560	10,984	9,885	8,897	8,007	7,206	58,539
Provera cost	15	10,170						10,170
Shipping to core Lab	5	3,390						3,390
Total Lab/Meds Costs		106,107	52,721	47,449	42,704	38,434	34,590	**356,245**
Recruiting Costs	150							**101,700**
Personnel Costs	% Effort	Est Salary		Fringe Benefit (28%)	Subtotal/yr	3 yr cost		
Principal Investigator	20	145,200	29,040	8131	37,171	111,514		
Nurse Coordinator	100	40,000	40,000	11200	51,200	153,600		
Total RMN Personnel						**265,114**		**265,114**

Total RMN Costs **723,059**