Published in final edited form as:

Fertil Steril. 2014 January ; 101(1): 258–269.e8. doi:10.1016/j.fertnstert.2013.08.056.

The Pregnancy in Polycystic Ovary Syndrome Study II: Baseline Characteristics and Effects of Obesity from a Multi-Center Randomized Clinical Trial

Richard S. Legro, M.D., Robert G. Brzyski, M.D., Ph.D., Michael P. Diamond, M.D., Christos Coutifaris, M.D., Ph.D., William D. Schlaff, M.D., Ruben Alvero, M.D., Peter Casson, M.D., Gregory M. Christman, M.D., Hao Huang, M.D., M.P.H., Qingshang Yan, Ph.D., Daniel J. Haisenleder, Ph.D., Kurt T. Barnhart, M.D., G. Wright Bates, M.D., Rebecca Usadi, M.D., Richard Lucidi, M.D., Valerie Baker, M.D., J.C. Trussell, M.D., Stephen A. Krawetz, Ph.D., Peter Snyder, M.D., Dana Ohi, M.D., Nanette Santoro, M.D., Esther Eisenberg, M.D., M.P.H., Heping Zhang, Ph.D., and for the NICHD Reproductive Medicine Network

Department of Obstetrics and Gynecology, Pennsylvania State University, Hershey, PA (Dr. Legro); Department of Obstetrics and Gynecology, University of Texas Health Science Center at San Antonio, TX (Dr. Brzyski); Department of Obstetrics and Gynecology, University of Colorado, Denver, CO (Drs. Schlaff, Alvero and Santoro); Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI (Dr. Christman, Current Address: Shands Hospital, University of Florida, Gainesville, FL and Dr. Ohi); Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, PA (Drs. Coutifaris, Snyder, Barnhart); Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI (Drs. Diamond, Krawetz); Department of Obstetrics and Gynecology, University of Vermont, Burlington, VT (Dr. Casson); Ligand Core Laboratory University of Virginia Center for Research in Reproduction, Charlottesville, VA (Dr. Haisenleder), Department of Obstetrics and Gynecology, Virginia Commonwealth University, Richmond, VA (Dr. Lucidi); Department of Obstetrics and Gynecology, Stanford Hospital, Palo Alto, CA (Dr. Baker); Department of Obstetrics and Gynecology, Carolinas HealthCare Center for Reproductive Medicine, Charlotte, NC (Dr. Usadi); Department of Obstetrics and Gynecology, University of Alabama, Birmingham AL (Dr. Bates); Urology, SUNY Upstate Medical University, Syracuse, NY (Dr. Trussell); Fertility and Infertility Branch, Eunice Kennedy Shriver NICHD, Rockville, MD (Dr. Eisenberg); and Department of Biostatistics, Yale University School of Medicine, New Haven, CT (Drs. Huang, Yan, and Zhang)

Abstract

Objective—To summarize baseline characteristics from a large multi-center infertility clinical trial.

© 2013 American Society for Reproductive Medicine. Published by Elsevier Inc. All rights reserved.

Corresponding Author: Richard S. Legro, MD, Department of Obstetrics and Gynecology, Penn State College of Medicine, M.S. Hershey Medical Center, 500 University Drive, H103, Hershey PA, 17033, Tel (717)-531-8478, Fax (717)-531-6286, RSL1@PSU.EDU.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

FINANCIAL DISCLOSURE: Richard Legro reports honorarium from Ferring, Germany for lecture; Michael Diamond reports ASRM Board Membership, Consultancy with Halt Medical, Genzyme and grants with Abbvie, Novartis, Boehringer Ingelheim, Ferring, EMD Serono, Biosante; Christos Coutifaris reports NOVA Therapeutics Medical Advisory Board, pending grants with NIH, payment for lectures from Ohio State University, Yale University, University of Michigan and reimbursement for attending Executive Board meetings from ASRM; Daniel Haisenleder reports employment with NIH and pending grants with NIH; JC Trussell reports ownership of equities in Pfizer, Merck, Astellas, J and J.

Design—Cross-sectional baseline data from a double-blind randomized trial of 2 treatment regimens (letrozole vs. clomiphene).

Setting—Academic Health Centers throughout the U.S.

Interventions—None

Main Outcome Measure(s)—Historical, biometric, biochemical and questionnaire parameters.

Participants—750 women with PCOS and their male partners took part in the study.

Results—Females averaged ~30 years old and were obese (BMI 35) with ~20% from a racial/ethnic minority. Most (87%) were hirsute and nulligravid (63%). . Most of the females had an elevated antral follicle count and enlarged ovarian volume on ultrasound. Women had elevated mean circulating androgens, LH:FSH ratio (~2), and AMH levels (8.0 ng/mL). Additionally, women had evidence for metabolic dysfunction with elevated mean fasting insulin and dyslipidemia. Increasing obesity was associated with decreased LH:FSH levels, AMH levels and antral follicle counts but increasing cardiovascular risk factors, including prevalence of the metabolic syndrome. Males were obese (BMI 30) and had normal mean semen parameters.

Conclusions—The treatment groups were well-matched at baseline. Obesity exacerbates select female reproductive and most metabolic parameters. We have also established a database and sample repository that will eventually be accessible to investigators.

Keywords

insulin resistance; hirsutism; infertility; ovulation induction; metabolic syndrome

INTRODUCTION

The Pregnancy in Polycystic Ovary Syndrome II (PPCOS II) study is a randomized, double-blinded clinical trial sponsored by the National Institutes of Health/Eunice Kennedy Shriver National Institute of Child Health and Human Development (NIH/NICHD), conducted at 14 centers in the United States. Briefly, the purpose of the trial is to determine which method of first line ovulation induction with oral agents, clomiphene citrate or letrozole, is most likely to result in live birth in infertile women with polycystic ovary syndrome (PCOS). The trial rationale, summary of the protocol, and statistical analysis plan have been described previously.(1) The PPCOS II trial builds on methodology that we developed during our PPCOS I trial,(2) which tested clomiphene, metformin, or the combination of both for ovulation induction, with live birth as the primary outcome.(3) That trial led to the choice of clomiphene as the primary comparator for the PPCOS II trial.

This report summarizes the baseline demographic and biomedical characteristics of the randomized subjects with PCOS, both by treatment arm and as a cohort in the PPCOS II trial. Further, because we systematically examined and characterized the female subjects with PCOS, the baseline data provides insight into the infertility and medical history, biometric, ultrasonographic, biochemical, and psychosocial aspects of the syndrome. We also consented and collected data on male partners relating to their sperm parameters, sexual function, and quality of life in the study, thus expanding the concept of a couple, rather than an individual, participating in an infertility trial. Since obesity coexists in a large number of women with PCOS, we also examined key phenotypic parameters in these women with PCOS by established BMI categories.

MATERIALS AND METHODS

Study Design and Overview

PPCOS II is a multi-center, double-blind clinical trial of clomiphene citrate (CC) vs. letrozole for 5 cycles of ovulation induction (24 weeks). Enrollment began in February 2009 and was completed in January of 2012. After progestin withdrawal, 750 women were equally randomized to two different treatment arms: A) clomiphene citrate 50 mg every day for 5 days (day 3–7 of cycle), or B) letrozole 2.5 mg every day for 5 days (day 3–7 of cycle), for up to 5 cycles. Because both drugs were prescribed in a similar fashion, i.e., giving for 5 days to initiate follicular development and increasing stepwise by one additional pill if anovulatory (i.e. 50 mg for clomiphene and 2.5 mg for letrozole), we utilized study drug that was over encapsulated and identical in appearance. Dose was increased in subsequent cycles in both treatment groups for non-response or poor ovulatory response up to a maximum of 150 mg (3 pills) of clomiphene a day (x 5 days) or 7.5 mg (3 pills) of letrozole a day (x 5 days). All subjects who conceived were followed for the outcome of pregnancy including live birth. The protocol was approved by the local IRB at all sites and participants (males and females) all gave written informed consent. The study was overseen by a NICHD appointed Data and Safety Monitoring Board.

Participants

We report on the 750 women and their male partners who were randomized into the study. Inclusion/exclusion criteria were applied both to women with PCOS and couples to identify other infertility factors.

Inclusion Criteria for Female Subjects—Women diagnosed with PCOS based on a modified form of the Rotterdam criteria(4,5) were enrolled. All women were required to have ovulatory dysfunction combined with either hyperandrogenism and/or polycystic ovaries:

1. Chronic anovulation or oligomenorrhea was defined as spontaneous intermenstrual intervals of ≥ 45 days or a total of ≤ 8 menses per year, or for women with more regular menses but suspected anovulatory bleeding, a midluteal (~21 days from last bleeding episode) serum progesterone level < 3 ng/mL which was considered to be indicative of chronic anovulation.
2. Hyperandrogenism was defined as either hirsutism or hyperandrogenemia. Hirsutism was determined by a modified Ferriman-Gallwey Score > 8 at screening exam.(6) Hyperandrogenemia was determined from serum measurements performed at local labs (using pre-determined local cutoffs within the year prior to participation).
3. Polycystic Ovaries on Ultrasound: We used the revised Rotterdam criteria for diagnosing polycystic ovaries (PCO).(7) PCO were defined as either an ovary that contains 12 or more follicles measuring 2–9 mm in diameter, or an increased ovarian volume (> 10 cm³ without concomitant cysts) on at least one ovary, for entry into the study.

Female Exclusion Criteria—We excluded subjects with medical conditions that may mimic PCOS including active thyroid disease, congenital adrenal hyperplasia, prolactin excess, androgen secreting tumor or Cushing's syndrome. We also excluded medical conditions that represent contraindications to study drug and/or pregnancy. These conditions included poorly controlled Type I or Type II diabetes (this included taking treatment drugs likely to confound study drug such as metformin, thiazolidinediones, and insulin);

undiagnosed liver disease or dysfunction (based on serum liver enzyme testing); renal disease or abnormal serum renal function; significant anemia; a history of deep venous thrombosis, pulmonary embolus, or cerebrovascular accident; uncontrolled hypertension, known symptomatic heart disease; history of or suspected cervical carcinoma, endometrial carcinoma, or breast carcinoma; undiagnosed vaginal bleeding, and use of other medications known to affect reproductive function or metabolism. Women who had bariatric surgery were required to have a stable weight before participating, which in our experience occurs between 6–12 months post-operatively.(8)

Couple Inclusion/Exclusion Criteria—For the male, we required a sperm concentration of 14 million/mL in at least one ejaculate within the last year, with at least some motile sperm, and the ability and desire to have regular intercourse during the ovulation induction phase of the study. For the female with PCOS, we required at least one patent tube and normal uterine cavity as determined by sonohysterogram, hysterosalpingogram, or hysteroscopy/laparoscopy within the last 3 years. An uncomplicated intrauterine non-IVF pregnancy and uncomplicated delivery and postpartum course resulting in live birth within the last three years also served as sufficient evidence of a patent tube and normal uterine cavity. A further exclusion criterion for both males and females was a prior sterilization procedure.

Baseline Evaluation and Procedures

History and Physical Exam—A full infertility and medical history was obtained using standardized forms in all participants. Males self-reported height and weight. Height, weight and waist and hip circumferences were recorded in females to the nearest 0.1 cm, 0.1 kg and 0.1 cm. They were weighed while dressed in light clothing, without shoes. Blood pressure was determined in the arm in the sitting position after a 5-minute rest. An acne assessment was performed by trained personnel using an Investigators Global Assessment and an acne lesion count.(9) Sebum was measured in the middle forehead using a Sebumeter (SM 815, CK Electronic GMBH, Koln Germany).(10) Hirsutism assessment and transvaginal ultrasound exam were performed as noted above.

Bloodwork—Fasting blood was obtained for hormonal assays which were batched and analyzed at the Ligand Assay & Analysis Core Laboratory at the University of Virginia as in the PPCOS I study.(2) Due to the costs and the volume of assays we *a priori* chose the following schema for assay quality control: All assays were run in singlet. A total of 5% of the samples were randomly chosen for double assay, and all outliers were repeated. A summary of the quality control data is found in Supplemental Table 1. All assays had intra- and inter-assay coefficients of variation (CV) below 10%. We have previously reported a blinded laboratory study showing that our total testosterone assay (used in both PPCOS I and II studies) had similar, if not superior, precision and quality control measures as commonly utilized LC/MS/MS assays of total testosterone.(11) The free androgen index (FAI) is calculated from measurable values for total T and SHBG, as previously described, (12) using the following equation: $(FAI = \text{Total testosterone in nmol/L} / \text{SHBG in nmol/L}) \times 100$. Glucose levels were determined on a glucose analyzer using the glucose oxidase method. Liver and renal function tests as well as CBC (Complete Blood Counts) were performed as safety labs at each site (data not shown). After further informed consent, additional serum and DNA (whole blood and blood spots) were collected for storage in the central RMN biologic specimen repository.

Administration of Standardized Questionnaires—Medical Quality of Life (QOL) in males and females was determined by the Short Form 36 (SF-36),(13) and infertility QOL with the FertiQol survey.(14,15) The SF-36 is a multi-purpose, short form self-administered

health survey instrument designed to evaluate functional health status over the past 4 weeks. The eight health concepts were selected from 40 included in the Medical Outcomes Study (MOS) and chosen to represent the most frequently measured concepts in widely used health surveys and those most affected by disease and treatment.(16) The score on each of the eight health concepts ranges from 0 (worst) to 100 (best). Norm-based scoring the SF-36, version 2 is based on a mean of 50 and an SD (sample standard deviation) of 10 for all measures.

Both females and males were screened for mental disorders, functional impairment, and recent psychosocial stressors with the PRIME-MD Patient Health Questionnaire (PHQ).(17) This is a 16-item, self-administered instrument that assesses depression, anxiety, eating disorders and reproductive-related stress and menstrual/pregnancy issues among other mental health conditions.(18) The FertiQOL is a 36-item (2 overall life and physical health, 24 core and 10 treatment-related), self-administered questionnaire. It was designed to measure quality of life in men and women who are experiencing fertility problems.(14) Besides 2 general items (rating health and satisfaction with quality of life), it contains 24 specific items covering four subscales of QoL: Mind-Body (interference with work, etc); Relational (commitment to partner, etc); Social (isolation, etc); Emotional (coping). A higher score on one of the subscales means a better QoL with subscale scores ranging from 0–100. Both partners also completed a Sleep Habits questionnaire, the standard measure used to collect data for the 10-year long multi-center NHLBI Sleep Heart Health Study.(19) The Sleep Habits Questionnaire was adapted from the Sleep Heart Health Study.(20) The questionnaire addresses several categories of sleep disorders: (1) sleep duration weekdays and weekends (2 items); (2) snoring (3 items); (3) breathing pauses/apnea (4 items); (4) insomnia symptoms (3 items); (5) sleep symptoms, including insufficient sleep, daytime sleepiness, nightmares, leg jerks, leg cramps, and need for sleep aids (12 items); (6) sleep quality, including perception of depth and duration of sleep (3 items); and (7) restless legs syndrome (10 items). There is an additional category that assesses potential sleep disruptors, including sinusitis, room noise, temperature, pain, and frequent toileting (9 items).

Female specific questionnaires included the Female Sexual Function Inventory (FSFI) along with the Female Sexual Distress Scale (FSDS).(21) The FSFI is a 19-item self-administered questionnaire that has been validated in 259 female subjects. It was designed to evaluate sexual function in both hetero-and homosexual women as a clinical trials assessment instrument that addresses the multidimensional nature of female sexual function(21) and has been validated in women with female sexual arousal disorder, female orgasmic disorder and hypoactive sexual desire disorder.(22) Scores on 6 domains of sexual function as well as a total score are evaluated. The domains include: desire (2 items), arousal (4 items), lubrication (4 items), orgasm (3 items), satisfaction (3 items), and pain (3 items). Each question was answered using a 5-point Likert scale, and scores were transformed by multiplying domain scores by a weighting factor. Women with a FSFI total score below 26.55 were categorized as experiencing sexual dysfunction, whereas those above this cut-off score were categorized as functionally normal.(23) The Female Sexual Distress Scale (FSDS) is a 13-item index of sexual distress. Items were scored from 0–4 (never, occasionally, frequently or all the time) and describe negative associations with sexuality (guilt, inadequacy, frustration, distress).(24)

Female subjects completed a PCOS Quality of Life questionnaire.(25,26) The PCOS Quality of Life scale was developed to assess the impact of the diagnosis of PCOS on women. It is a validated, 26-item, self-administered questionnaire that assesses 5 domains important to women with PCOS: emotions; body hair; weight; infertility concerns and menstrual problems. Items are scored on a 7-point Likert scale ranging from none to 'severe' or 'all of the time'. Lower scores indicate lower health related quality of life.

Male sexual function was assessed with the International Index of Erectile Function (IIEF) which is a multidimensional scale for assessment of erectile dysfunction. It is composed of 15 questions that were scored from 0–5. Lower scores indicate lower erectile function. The measure addresses the relevant domains of male sexual function (erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction). (27)

Data Management and Analyses

All data entry, data management, and analyses were coordinated or performed at the Collaborative Center for Statistics in Science at Yale University, which serves as the Data Coordinating Center for this study. The presence of the metabolic syndrome was assessed by utilizing the ATPIII criteria (waist circumference, blood pressure, fasting glucose, HDL-C and triglyceride levels).(28) Categorical variables are presented as a frequency and percentage within each group. A chi-square test was used for testing differences between the two treatment groups. Fisher's exact test was used if any frequency count was fewer than 5. For all continuous variables, the mean and SD within each group is shown, a Wilcoxon rank sum test was used for testing differences between the two treatment groups, and boxplots were presented for PCOS phenotypes between the two treatment groups. All analyses were performed by using the Statistical Analysis System, version 9.2 (SAS Institute, Cary, NC).

RESULTS

Clinical and Demographic Characteristics of Subjects and their Partners

Female subjects were on average close to 30 years old and had Class II obesity (mean $\pm$ SD BMI: 35.1 ± 9.3) with approximately 20% from a racial or ethnic minority (Table 1). Most (87%) were hirsute. Over 40% of females had a smoking history, though only 15% were currently smoking at the baseline visit. Close to 90% of the female subjects had used alcohol at some point, though only slightly more than 60% of the female subjects were current drinkers at baseline. Men had higher rates of current and past smoking and drinking. Over a quarter of females had a history of psychiatric disease (Supplemental Table 2).

More than half of the female subjects (64%) had never been pregnant, and only a fifth had a prior live birth (Table 2). On average subjects had been attempting conception for nearly three and a half years (42 months). Twenty percent of the women had sexual dysfunction as determined by FSFI. Most of the men had a semen concentration well within the normal range, with only a fraction (6%) that had a semen concentration less than 20 million/ml. Men also had a slightly higher rate of fathering pregnancies with any partner compared to a history of any conception with their current female partners (42% vs. 36%).

Ultrasound and Biochemical Measures

At least one ovary was visualized in almost all subjects (Table 3). On average, most of the subjects had an elevated antral follicle count and an enlarged ovarian volume. Only about a fifth had a follicle or cyst greater than 10 mm in diameter. Normal ovaries were relatively common (about a tenth), whereas hyperthecotic ovaries (defined as at least one enlarged ovary i.e. $> 10 \text{ cm}^3$ and an antral follicle count < 12 and no dominant follicle $> 10 \text{ cm}$ diameter) were very rare (2–3%). The right ovary was on average about one cubic centiliter larger than the left ovary. Serum testing showed a biochemical phenotype consistent with PCOS (Supplemental Table 3). Women had elevated mean androgen levels, an elevated mean LH to FSH ratio (~ 2), and an increased mean level of AMH. About half of the subjects had an elevated total testosterone level ($> 50 \text{ ng/dL}$). Mean levels of estradiol and progesterone indicated that women were likely in an early follicular phase hormonal environment, and were on average anovulatory. Additionally, women had evidence for

metabolic dysfunction with elevated mean fasting insulin levels, dyslipidemia and elevated mean hsCRP levels (Supplemental Figure 1).

Quality of Life

Overall, medical QoL questionnaires, the SF36 and the PHQ, showed that at baseline women had consistently lower quality of life indicators than their male partners (Supplemental Table 4). Overall QoL from the mental component of SF-36 was 5.6 points lower in females. The discrepancy was mostly reflected in a higher prevalence of depression, depression related symptoms, panic attacks and anxiety, where rates were 3–4 times higher in females than males. However, absolute rates were still at or under 5% for these disorders in women. In the PHQ, sleep quality was comparable between men and women. Scores on the Fertil-QOL were also lower in women than men, with the greatest difference in the emotional domain in women (60 in females vs. 84 in males). The infertility domain in the PCOSQ represented the one domain associated with the lowest quality of life in the female subjects.

Effects of Obesity on the PCOS Phenotype

In examining obesity effects by increasing BMI category (Table 4), we note that obesity is significantly associated with increasing blood pressure, fasting glucose and insulin levels, worsening dyslipidemia (decreased HDL-C and increased triglyceride levels) and increasing prevalence of metabolic syndrome. Overall 34% of subjects had metabolic syndrome (254/747). The largest percentage increase in the metabolic syndrome is noted in the Class I Obesity group (BMI 30–34.9 group) versus the non obese group (BMI < 30), with lesser increases in the higher BMI categories. Increasing obesity is also associated with more severe hirsutism, a lower LH to FSH ratio, a lower AFC and lower circulating SHBG and AMH, as well as a progressive decline in quality of life by both the physical component of SF-36 and PCOS-Q questionnaires.

Effects of Hyperandrogenism on the PCOS Phenotype

We compared characteristics of subjects who met entry criteria without hyperandrogenism (i.e. they had ovulatory dysfunction combined with polycystic ovaries but no biochemical or clinical hyperandrogenism) (N = 42) to those with hyperandrogenism (N = 699) (Supplemental Table 5). As expected by this division, subjects without hyperandrogenism (N = 42) had significantly lower levels of circulating androgens, elevated SHBG levels, and less hirsutism. In addition, women who fit this non- NIH definition of PCOS were significantly thinner, had lower insulin and triglyceride levels, and higher quality of life measures.

DISCUSSION

Participants in the PPCOSII trial were well matched for a wide spectrum of baseline characteristics, an important goal of any randomized clinical trial. Further, we have collected extensive phenotypic data on a large multi-center, racially and ethnically representative population (of the U.S.) of women with PCOS, including uniform baseline biochemical serum measurements of key reproductive and metabolic hormones in a central lab. This report includes baseline data from one of the largest randomized trials conducted in this population. We have also systematically consented and collected historical, biometric, and semen analysis results on male partners, to advance our understanding of the role of males in such infertility trials. In addition, we have collected extensive psychosocial, sexual function, and quality of life measures on both the female and male participants to improve our understanding both of the effect of PCOS and infertility on our female subjects as well as their male partners. The extent and breadth of our baseline data combined with the collection

of similar data longitudinally at later time points in our trial represent an unprecedented dataset with which to better understand PCOS and response to treatment.

Our diagnostic criteria for the diagnosis of PCOS was modified based on the Rotterdam criteria and required oligovulation with either hyperandrogenism or polycystic ovaries. This definition contrasts with the PPCOSII diagnostic criteria, which were based on the 1990 NIH criteria,(29) and required oligo-ovulation and hyperandrogenism. Despite this difference in definition, the cohort from the current trial matches the PPCOS I cohort well, with a mean age within a year and nearly identical mean BMIs, similar volumes in right and left ovaries, and similar circulating total testosterone levels (by the same assay). Thus, women in the PPCOS II trial have a reproductive and metabolic phenotype very similar to the PPCOS I cohort. This suggests that the small difference in diagnostic criteria had little effect on the type of female subject who participates in a PCOS infertility trial. We have again identified a cohort of women with chronic anovulation, most of whom are hyperandrogenic, both by clinical and by biochemical criteria, thus satisfying the diagnostic criteria of the NIH consensus conference,(29) Rotterdam,(4,30) the AE-PCOS Society,(31) and the recent diagnostic recommendations of the NIH Prevention Workshop Expert Panel (http://prevention.nih.gov/workshops/2012/pcos/docs/PCOS_Final_Statement.pdf). Compared to recent large multicenter trials of infertile women with PCOS conducted in Scandinavia (the first with N = 150 subjects(32) and the second N = 320(33)), our subjects were of similar age (within a year), but with a higher BMI of 35 (compared to mean BMI of 24(32) and 27(33)). Our participants were also markedly more hyperandrogenic either by clinical assessment (mean Ferriman Gallwey score was 5) or by biochemical assay (mean Free Androgen Index was 8 vs. 5(33)). These recent Scandinavian studies had less extensive phenotyping than the RMN trials and represented pragmatic clinical trials.

One of the significant strengths of the PPCOS II trial is that we have utilized the same NIH supported core lab (Ligand Core Lab, University of Virginia) and the same assays described in PPCOS I. The assay of testosterone in females remains a problematic issue in endocrinology and many have recommended that testosterone should only be assayed with liquid chromatography extraction and mass spectrometry.(34) We used a very well validated, longstanding RIA to measure testosterone as we have previously shown that this RIA has equal (or superior precision) to commonly utilized LC/MS/MS assays.(11)

We designed our diagnostic criteria to identify a group of women with anovulatory infertility and no other significant infertility factors. Their male partners had semen analysis parameters within WHO norms. Although there was evidence of sexual dysfunction in both males and females, it affected only a fraction of the individuals. In addition, intercourse diaries that were collected were prospectively reviewed for compliance at each cycle visit. This is similar to the methodology we pioneered in our PPCOS I trial.(35,36) Approximately 15% had only a single fallopian tube patent on tests that can distinguish individual tubal patency (i.e. hysterosalpingogram, or diagnostic laparoscopy). The prognosis for fertility is likely comparable in a woman with one vs two tubes patent. The finding of unilateral tubal patency may also represent a false positive in many cases, as tubal spasm or technique related limitations can result in failure to document tubal patency. The majority of women underwent sonohysterogram as the method of screening for tubal patency.

We report an extensive array of baseline demographic, biometric, and reproductive variables, as well as quality of life factors for men participating in the trial. We note that a greater proportion of men smoke and drink alcohol than do their female partners. There are multiple potential reasons for this sex difference, including ignorance about potential reproductive effects, the male partner's relative distance from the study (and conception and pregnancy), and lack of effective counseling of the male partner on our part. These baseline

data are meaningful for assessing the differences between male and female responses in infertility diagnosis, especially treatment related changes. In addition, we have collected (and expanded upon) these parameters in men participating in our larger trial of unexplained infertility (AMIGOS trial with N= 900 couples);(37) this will allow for meaningful comparisons between these two studies. With the consent of males now a standard practice in our infertility trials, we are both obligated and dedicated to better understanding male fertility and infertility through more intense study.

Our cohort represents the phenotypic spectrum of PCOS found in the U.S. and parallels that found in other large multi-center trials in women with PCOS.(3,38) The prevalence of the metabolic syndrome mirrors that of another multi-center industry sponsored study (33%). (39) This study also excluded most cases of type 2 diabetes from the study, such that the prevalence is likely larger among an unselected cohort of women with PCOS.(40) In subdividing our cohort by BMI categories, we can document in a dose response fashion the adverse effects of obesity on reproductive and metabolic aspects of the PCOS phenotype. Our findings echo previous studies examining the effects of obesity on PCOS, with elevation in cardiovascular risk factors including the metabolic syndrome as BMI increases. (39,41) An interesting finding consistent with previous studies is a lack of association between BMI categories and race and ethnicity, which suggests that the phenotype may be influenced more by increasing BMI than race or ethnicity.(42)

Our findings also support previous findings of the association of obesity with suppression of hypothalamic gonadotropin secretion(43) as well as AMH levels.(44) Our study provides novel data about the decrease in ovarian AFC associated with increasing obesity. These findings of parallel suppression of AFC and AMH with increasing obesity blur the power of these diagnostic markers of ovarian reserve in obese women with PCOS.(45) We did not measure free testosterone or non-SHBG bound testosterone in our study, but obesity is associated with a stepwise decrease in SHBG levels and corresponding increase in the FAI, with constant total testosterone levels, implying that the bioavailability of testosterone is increased by obesity. Further, when we consider these obesity related effects in this cohort along with the novel finding of relatively stable total testosterone despite massive weight loss after bariatric surgery in women,(8) the lack of association between BMI and facial sebum levels, yet a clear obesity related increase in hirsutism, we may hypothesize that hirsutism in PCOS is more associated with hyperinsulinemia and metabolic dysfunction (i.e., SHBG levels which can affect testosterone bioavailability, may be affected by obesity and decreased insulin sensitivity(46)).

Obesity has been cited as one of the major factors, if not the major factor, depressing quality of life in women with PCOS.(47,48) We have demonstrated both in a general medical and a specific PCOS validate QoL questionnaire, that QoL declines with increasing BMI. It is therefore difficult to disentangle the effects of BMI from signs and symptoms in PCOS in figuring out what is causing adverse quality of life. However, multiple studies from the obesity literature have documented a marked improvement in quality of life with weight loss,(49,50) whereas the improvement with treatment of hyperandrogenism and menstrual irregularity is less dramatic.(26,51,52)

Given the space limitations of the journal, it is impossible to discuss the implications of all of the baseline data. The outcomes of the trial will certainly influence and inspire other more detailed analyses of the data, and we have an extensive list of secondary hypotheses to explore. We also report our systematic acquisition of data and specimens as a resource for collaborative research with other investigators in the U.S. and throughout the world.(53) We have established a separate specimen repository that contains serum and DNA from participating men, women, and infants who provided additional consent for their de-

identified samples to be used in other studies.(54) These specimens were collected, processed, and stored in a standardized fashion to increase their practical value. Additionally, the full database, including outcome data and remaining specimens, will be available to qualified investigators after publication of the main outcome results through a transparent application and review process established by the Reproductive Medicine Network (see <http://c2s2.yale.edu/rmn/>). We have also created a pregnancy registry that will examine infants whose parents consent to additional study for congenital defects, yearly developmental screening, and review of medical records and growth curves for the first three years of life. Thus, these data and specimens may serve as a rich and long-lasting resource to a diverse group of investigators in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

In addition to the authors, other members of the NICHD Reproductive Medicine Network were as follows: *Pennsylvania State University College of Medicine, Hershey*: C. Bartlebaugh, W. Dodson, S. Estes, C. Gnatak, J. Ober; *University of Texas Health Science Center at San Antonio*: C. Easton, A. Hernandez, M. Leija, D. Pierce, R. Robinson; *Wayne State University*: A. Awonuga, L. Cedo, A. Cline, K. Collins, E. Puscheck, M. Singh, M. Yoscovits; *University of Pennsylvania*: K. Barnhart, K. Lecks, L. Martino, R. Marunich; *University of Colorado*: A. Comfort, M. Crow; *University of Vermont*: A. Hohmann, S. Mallette; *University of Michigan*: M. Ringbloom, J. Tang; *University of Alabama Birmingham*: S. Mason; *Carolinas Medical Center*: N. DiMaria; *Virginia Commonwealth University*: M. Rhea; *Stanford University Medical Center*: K. Turner; *Yale University*: D. DelBasso, Y. Li, R. Makuch, P. Patrizio, L. Sakai, L. Scashill, H. Taylor, T. Thomas, S. Tsang, M. Zhang; *Eunice Kennedy Shriver National Institute of Child Health and Human Development*: C. Lamar, L. DePaolo; **Advisory Board**: D. Guzick (Chair), A. Herring, J. Bruce Redmond, M. Thomas, P. Turek, J. Wactawski-Wende; **Data and Safety Monitoring Board**: R. Rebar (Chair), P. Cato, V. Dukic, V. Lewis, P. Schlegel, F. Witter.

FUNDING SOURCE:

This work was supported by National Institutes of Health (NIH)/*Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Grants U10 HD27049 (to C.C.), U10 HD38992 (to R.S.L.), U10HD055925 (to H.Z.), U10 HD39005 (to M.P.D.), U10 HD33172 (to M.P.S.), U10 HD38998 (to W.D.S.), U10 HD055936 (to G.M.C.), U10 HD055942 (to R.G.B.), and U10 HD055944 (to P.R.C.); U54-HD29834 (to the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core of the Specialized Cooperative Centers Program in Reproduction and Infertility Research); General Clinical Research Center Grants MO1RR10732 and construction grant C06 RR016499 (to Pennsylvania State University). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NICHD or NIH.

REFERENCES

1. Legro RS, Kunselman AR, Brzyski RG, Casson PR, Diamond MP, Schlaff WD, et al. The Pregnancy in Polycystic Ovary Syndrome II (PPCOS II) trial: rationale and design of a double-blind randomized trial of clomiphene citrate and letrozole for the treatment of infertility in women with polycystic ovary syndrome. *Contemp Clin Trials*. 2012; 33:470–481. [PubMed: 22265923]
2. Legro RS, Myers ER, Barnhart HX, Carson SA, Diamond MP, Carr BR, et al. The Pregnancy in Polycystic Ovary Syndrome study: baseline characteristics of the randomized cohort including racial effects. *Fertil Steril*. 2006; 86:914–933. [PubMed: 16963034]
3. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. *N Engl J Med*. 2007; 356:551–566. [PubMed: 17287476]
4. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004; 81:19–25.
5. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod*. 2004; 19:41–47. [PubMed: 14688154]

6. Hatch R, Rosenfield RL, Kim MH, Tredway D. Hirsutism: implications, etiology, and management. *Am J Obstet Gynecol.* 1981 Aug 1;140:815–830. [PubMed: 7258262]
7. Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definitions. *Hum Reprod Update.* 2003; 9:505–514. [PubMed: 14714587]
8. Legro RS, Dodson WC, Gnatuk CL, Estes SJ, Kunselman AR, Meadows JW, et al. Effects of gastric bypass surgery on female reproductive function. *J Clin Endocrinol Metab.* 2012; 97:4540–4548. [PubMed: 23066115]
9. Leyden JJ, Shalita A, Thiboutot D, Washenik K, Webster G. Topical retinoids in inflammatory acne: a retrospective, investigator-blinded, vehicle-controlled, photographic assessment. *Clin Ther.* 2005; 27:216–224. [PubMed: 15811485]
10. Kim MK, Choi SY, Byun HJ, Huh CH, Park KC, Patel RA, et al. Comparison of sebum secretion, skin type, pH in humans with and without acne. *Arch Dermatol Res.* 2006; 298:113–119. [PubMed: 16786345]
11. Legro RS, Schlaff WD, Diamond MP, Coutifaris C, Casson PR, Brzyski RG, et al. Total Testosterone Assays in Women with Polycystic Ovary Syndrome: Precision and Correlation with Hirsutism. *J Clin Endocrinol Metab.* 2010; 95:5305–5313. [PubMed: 20826578]
12. Miller KK, Rosner W, Lee H, Hier J, Sesmilo G, Schoenfeld D, et al. Measurement of free testosterone in normal women and women with androgen deficiency: comparison of methods. *J Clin Endocrinol Metab.* 2004; 89:525–533. [PubMed: 14764757]
13. Ware, JESK.; Kosinski, M.; Gandek, B. SF-36 health survey manual and interpretation guide. Boston: New England Medical Center: The Health Institute; 1993.
14. Boivin J, Takefman J, Braverman A. The fertility quality of life (FertiQoL) tool: development and general psychometric properties. *Hum Reprod.* 2011; 26:2084–2091. [PubMed: 21665875]
15. Boivin J, Takefman J, Braverman A. The Fertility Quality of Life (FertiQoL) tool: development and general psychometric properties. *Fertil Steril.* 2011; 96:409. 15 e3. [PubMed: 21458806]
16. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care.* 1992; 30:473–483. [PubMed: 1593914]
17. Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire. *JAMA : The Journal of the American Medical Association.* 1999; 282:1737–1744. [PubMed: 10568646]
18. Spitzer RL, Williams JB, Kroenke K, Hornyak R, McMurray J. Validity and utility of the PRIME-MD patient health questionnaire in assessment of 3000 obstetric-gynecologic patients: the PRIME-MD Patient Health Questionnaire Obstetrics-Gynecology Study. *Am J Obstet Gynecol.* 2000; 183:759–769. [PubMed: 10992206]
19. Kump K, Whalen C, Tishler PV, Browner I, Ferrette V, Strohl KP, et al. Assessment of the validity and utility of a sleep-symptom questionnaire. *Am J Respir Crit Care Med.* 1994; 150:735–741. [PubMed: 8087345]
20. Baldwin CM, Choi M, McClain DB, Celaya A, Quan SF. Spanish translation and cross-language validation of a sleep habits questionnaire for use in clinical and research settings. *J Clin Sleep Med: JCSM : official publication of the American Academy of Sleep Medicine.* 2012; 8:137–146.
21. Rosen R, Brown C, Heiman J, Leiblum S, Meston C, Shabsigh R, et al. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. *J Sex Marital Ther.* 2000; 26:191–208. [PubMed: 10782451]
22. Meston CM. Validation of the Female Sexual Function Index (FSFI) in women with female orgasmic disorder and in women with hypoactive sexual desire disorder. *J Sex Marital Ther.* 2003; 29:39–46. [PubMed: 12519665]
23. Wiegel M, Meston C, Rosen R. The female sexual function index (FSFI): cross-validation and development of clinical cutoff scores. *J Sex Marital Ther.* 2005; 31:1–20. [PubMed: 15841702]
24. Derogatis LR, Rosen R, Leiblum S, Burnett A, Heiman J. The Female Sexual Distress Scale (FSDS): initial validation of a standardized scale for assessment of sexually related personal distress in women. *J Sex Marital Ther.* 2002; 28:317–330. [PubMed: 12082670]

25. Cronin L, Guyatt G, Griffith L, Wong E, Azziz R, Futterweit W, et al. Development of a health-related quality-of-life questionnaire (PCOSQ) for women with polycystic ovary syndrome (PCOS). *J Clin Endocrinol Metab.* 1998; 83:1976–1987. [PubMed: 9626128]
26. Guyatt G, Weaver B, Cronin L, Dooley JA, Azziz R. Health-related quality of life in women with polycystic ovary syndrome, a self-administered questionnaire, was validated. *J Clin Epidemiol.* 2004; 57:1279–1287. [PubMed: 15617954]
27. Rosen RC, Riley A, Wagner G, Osterloh IH, Kirkpatrick J, Mishra A. The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. *Urology.* 1997; 49:822–830. [PubMed: 9187685]
28. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation.* 2004; 109:433–438. [PubMed: 14744958]
29. 1 ed.. Boston: Blackwell Scientific Publications, Inc.; 1992.
30. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to Polycystic Ovary Syndrome (PCOS). *Hum Reprod.* 2004; 19:41–47. [PubMed: 14688154]
31. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline. *J Clin Endocrinol Metab.* 2006; 91:4237–4245. [PubMed: 16940456]
32. Kjotrod SB, Carlsen SM, Rasmussen PE, Holst-Larsen T, Mellembakken J, Thurin-Kjellberg A, et al. Use of metformin before and during assisted reproductive technology in non-obese young infertile women with polycystic ovary syndrome: a prospective, randomized, double-blind, multi-centre study. *Hum Reprod.* 2011; 26:2045–2053. [PubMed: 21606131]
33. Morin-Papunen L, Rantala AS, Unkila-Kallio L, Tiitinen A, Hippelainen M, Perheentupa A, et al. Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. *J Clin Endocrinol Metab.* 2012; 97:1492–1500. [PubMed: 22419702]
34. Rosner W, Vesper H. Toward excellence in testosterone testing: a consensus statement. *J Clin Endocrinol Metab.* 2010; 95:4542–4548. [PubMed: 20926540]
35. Pagidas K, Carson SA, McGovern PG, Barnhart HX, Myers ER, Legro RS, et al. Body mass index and intercourse compliance. *Fertil Steril.* 2010; 94:1447–1450. [PubMed: 19540480]
36. Pagidas K, Carson SA, McGovern PG, Barnhart HX, Myers ER, Legro RS, et al. Intercourse compliance, ovulation, and treatment success in the National Institute of Child Health and Human Development-Reproductive Medicine Network's Pregnancy in Polycystic Ovary Syndrome (PPCOS) Trial. *Fertil Steril.* 2010; 94:1444–1446. [PubMed: 19540477]
37. Diamond MP, Mitwally M, Casper R, Ager J, Legro RS, Brzyski R, et al. Estimating rates of multiple gestation pregnancies: sample size calculation from the assessment of multiple intrauterine gestations from ovarian stimulation (AMIGOS) trial. *Contemp Clin Trials.* 2011; 32:902–908. [PubMed: 21787883]
38. Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, et al. Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. *J Clin Endocrinol Metab.* 2001; 86:1626–1632. [PubMed: 11297595]
39. Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghazzi MN. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2006; 91:48–53. [PubMed: 16249284]
40. Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2005; 90:1929–1935. [PubMed: 15623819]
41. Taponen S, Martikainen H, Jarvelin MR, Sovio U, Laitinen J, Pouta A, et al. Metabolic cardiovascular disease risk factors in women with self-reported symptoms of oligomenorrhea and/or hirsutism: Northern Finland Birth Cohort 1966 Study. *J Clin Endocrinol Metab.* 2004; 89:2114–2118. [PubMed: 15126528]

42. Ladson G, Dodson WC, Sweet SD, Archibong AE, Kunselman AR, Demers LM, et al. Racial influence on the polycystic ovary syndrome phenotype: a black and white case-control study. *Fertil Steril*. 2011; 96:224–229 e2. [PubMed: 21723443]
43. Arroyo A, Laughlin GA, Morales AJ, Yen SS. Inappropriate gonadotropin secretion in polycystic ovary syndrome: influence of adiposity. *J Clin Endocrinol Metab*. 1997; 82:3728–3733. [PubMed: 9360532]
44. Piouka A, Farmakiotis D, Katsikis I, Macut D, Gerou S, Panidis D. Anti-Mullerian hormone levels reflect severity of PCOS but are negatively influenced by obesity: relationship with increased luteinizing hormone levels. *Am J Physiol Endocrinol Metab*. 2009; 296:E238–E243. [PubMed: 18957615]
45. Dewailly D, Gronier H, Poncelet E, Robin G, Leroy M, Pigny P, et al. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. *Hum Reprod*. 2011; 26:3123–3129. [PubMed: 21926054]
46. Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Ritmaster RS, et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. *J Clin Endocrinol Metab*. 1991; 72:83–89. [PubMed: 1898744]
47. Alvarez-Blasco F, Luque-Ramirez M, Escobar-Morreale HF. Obesity impairs general health-related quality of life (HR-QoL) in premenopausal women to a greater extent than polycystic ovary syndrome (PCOS). *Clin Endocrinol*. 2010; 73:595–601.
48. Elsenbruch S, Benson S, Hahn S, Tan S, Mann K, Pleger K, et al. Determinants of emotional distress in women with polycystic ovary syndrome. *Hum Reprod*. 2006; 21:1092–1099. [PubMed: 16459352]
49. Mathus-Vliegen EM, de Wit LT. Health-related quality of life after gastric banding. *Br J Surg*. 2007; 94:457–465. [PubMed: 17310505]
50. Karlsen TI, Lund RS, Roislien J, Tonstad S, Natvig GK, Sandbu R, et al. Health related quality of life after gastric bypass or intensive lifestyle intervention: a controlled clinical study. *Health Qual Life Outcomes*. 2013; 11:17. [PubMed: 23406190]
51. Harris-Glocker M, Davidson K, Kochman L, Guzick D, Hoeger K. Improvement in quality-of-life questionnaire measures in obese adolescent females with polycystic ovary syndrome treated with lifestyle changes and oral contraceptives, with or without metformin. *Fertil Steril*. 2010; 93:1016–1019. [PubMed: 19781696]
52. Ladson G, Dodson WC, Sweet SD, Archibong AE, Kunselman AR, Demers LM, et al. The effects of metformin with lifestyle therapy in polycystic ovary syndrome: a randomized double-blind study. *Fertil Steril*. 2011; 95:1059–1066. e1-7. [PubMed: 21193187]
53. Casson PR, Krawetz SA, Diamond MP, Zhang H, Legro RS, Schlaff WD, et al. Proactively establishing a biologic specimens repository for large clinical trials: an idea whose time has come. *Syst Biol Reprod Med*. 2011; 57:217–221. [PubMed: 21951166]
54. Krawetz SA, Casson PR, Diamond MP, Zhang H, Legro RS, Schlaff WD, et al. Establishing a biologic specimens repository for reproductive clinical trials: technical aspects. *Syst Biol Reprod Med*. 2011; 57:222–227. [PubMed: 21899384]

Table 1

Subject demographic and biometric characteristics by treatment arm

	Female			Male		
	Clomiphene	Letrozole	All	Clomiphene	Letrozole	All
Age (yr)						
N	376	374	750	362	362	724
Mean(SD)	28.8(4.0)	28.9(4.5)	28.9(4.3)	31.5(5.3)	31.5(6.6)	31.5(6.0)
Body Mass Index (kg/m ²)						
N	376	374	750	354	356	710
Mean(SD)	35.1(9.0)	35.2(9.5)	35.1(9.3)	30.2(6.8)	30.5(7.1)	30.4(7.0)
Waist circumference(cm)						
N	374	374	748			
Mean(SD)	106.4(20.0)	105.5(20.8)	105.9(20.4)			
Blood pressure (mmHg)						
Systolic						
N	375	374	749			
Mean(SD)	119.7(12.7)	119.4(13.1)	119.6(12.9)			
Diastolic						
N	375	374	749			
Mean(SD)	77.1(9.6)	77.0(9.1)	77.1(9.4)			
Hirsutism						
N	376	374	750			
Mean(SD)	16.9(8.5)	17.0(8.6)	17.0(8.5)			
FG < 8	52/376(13.8%)	45/374(12.0%)	97/750(12.9%)			
FG 8–16	138/376(36.7%)	138/374(36.9%)	276/750(36.8%)			
FG > 16	186/376(49.5%)	191/374(51.1%)	377/750(50.3%)			
Acne						
N	376	373	749			
Mean(SD)	9.0(16.3)	8.3(15.9)	8.7(16.1)			
<5	219/376(58.2%)	218/373(58.4%)	437/749(58.3%)			
5–10	67/376(17.8%)	68/373(18.2%)	135/749(18.0%)			
>10	90/376(23.9%)	87/373(23.3%)	177/749(23.6%)			

	Clomiphene	Letrozole	All	Clomiphene	Letrozole	All
Sebum(mcg/cm ²)						
N	365	365	730			
Mean(SD)	106.5(55.2)	108.8(55.8)	107.7(55.5)			
<100	187/365(51.2%)	193/365(52.9%)	380/730(52.1%)			
100–200	153/365(41.9%)	145/365(39.7%)	298/730(40.8%)			
>200	25/365(6.8%)	27/365(7.4%)	52/730(7.1%)			
Level of education						
High school graduate or less	86/376(22.9%)	87/374(23.3%)	173/750(23.1%)	146/362(40.3%)	135/362(37.3%)	281/724(38.8%)
College graduate or some college	242/376(64.4%)	249/374(66.6%)	491/750(65.5%)	188/362(51.9%)	200/362(55.2%)	388/724(53.6%)
Graduate degree	48/376(12.8%)	38/374(10.2%)	86/750(11.5%)	28/362(7.7%)	27/362(7.5%)	55/724(7.6%)
Annual household income						
<\$50,000	144/376(38.3%)	156/374(41.7%)	300/750(40.0%)			
>=\$50,000	174/376(46.3%)	169/374(45.2%)	343/750(45.7%)			
Wish to not answer	58/376(15.4%)	49/374(13.1%)	107/750(14.3%)			
Ethnicity						
Not Hispanic or Latino	308/376(81.9%)	314/374(84.0%)	622/750(82.9%)	296/361(82.0%)	287/362(79.3%)	583/723(80.6%)
Hispanic or Latino	68/376(18.1%)	60/374(16.0%)	128/750(17.1%)	65/361(18.0%)	75/362(20.7%)	140/723(19.4%)
Race						
White	302/376(80.3%)	288/374(77.0%)	590/750(78.7%)	283/362(78.2%)	278/360(77.2%)	561/722(77.7%)
Black	44/376(11.7%)	56/374(15.0%)	100/750(13.3%)	54/362(14.9%)	64/360(17.8%)	118/722(16.3%)
Asian	12/376(3.2%)	12/374(3.2%)	24/750(3.2%)	10/362(2.8%)	10/360(2.8%)	20/722(2.8%)
American Indian or Alaska Native	4/376(1.1%)	3/374(0.8%)	7/750(0.9%)	3/362(0.8%)	2/360(0.6%)	5/722(0.7%)
Native Hawaiian or other Pacific Islander	2/376(0.5%)	0/374(0.0%)	2/750(0.3%)	3/362(0.8%)	1/360(0.3%)	4/722(0.6%)
Mixed race	12/376(3.2%)	15/374(4.0%)	27/750(3.6%)	9/362(2.5%)	5/360(1.4%)	14/722(1.9%)

Table 2

Reproductive history and screening for other infertility factors in female and male participants

	Clomiphene	Letrozole	All
Female pregnancy history			
Prior conception	134/376(35.6%)	139/374(37.2%)	273/750(36.4%)
Prior live birth	73/376(19.4%)	75/374(20.1%)	148/750(19.7%)
Prior loss	89/376(23.7%)	85/374(22.7%)	174/750(23.2%)
Female infertility history			
Patient had a diagnosis of infertility	323/376(85.9%)	325/374(86.9%)	648/750(86.4%)
Patient had prior therapy for infertility	202/376(53.7%)	214/374(57.2%)	416/750(55.5%)
How long has the patient been attempting conception (months)?			
N	362	353	715
Mean(SD)	42.5(37.6)	40.9(38.0)	41.7(37.8)
Previous study drug exposure **			
None	205/376(54.5%)	177/374(47.3%)	382/750(50.9%)
Letrozole only	4/376(1.1%)	0/374(0.0%)	4/750(0.5%)
Clomiphene Citrate only	158/376(42.0%)	186/374(49.7%)	344/750(45.9%)
Letrozole and Clomiphene Citrate	9/376(2.4%)	11/374(2.9%)	20/750(2.7%)
Total score of FSFI			
N	368	369	737
Mean(SD)	30.0(4.4)	29.7(4.9)	29.9(4.6)
Sexual dysfunction	71/368(19.3%)	78/369(21.1%)	149/737(20.2%)
Total score of FSDS			
N	368	368	736
Mean(SD)	6.7(8.4)	7.0(8.7)	6.8(8.6)
Female tubal patency			
HSG			
Both Tubes Patent	90/106(84.9%)	95/111(85.6%)	185/217(85.3%)
Only one Tube Patent	16/106(15.1%)	16/111(14.4%)	32/217(14.7%)
Laparoscopy/Surgery			
Both Tubes Patent	3/7(42.9%)	3/10(30.0%)	6/17(35.3%)
Only one Tube Patent	4/7(57.1%)	7/10(70.0%)	11/17(64.7%)
SHG			
At least one Tube Patent	261/261(100.0%)	250/250(100.0%)	511/511(100.0%)
Having an IUP within the last three years	2/2(100.0%)	3/3(100.0%)	5/5(100.0%)
Male pregnancy history			
Have ever created a pregnancy with partner	152/362(42.0%)	154/362(42.5%)	306/724(42.3%)
Sperm Concentration (million/mL)			
N	376	374	750
Mean(SD)	75.4(59.2)	84.4(76.4)	79.9(68.4)
14–20,000,000/ML	22/374(5.9%)	21/370(5.7%)	43/744(5.8%)
20–50,000,000/ML	133/374(35.6%)	123/370(33.2%)	256/744(34.4%)

	Clomiphene	Letrozole	All
> 50,000,000/ML	219/374(58.6%)	226/370(61.1%)	445/744(59.8%)
Total score of IIEF			
N	359	349	708
Mean(SD)	69.2(5.7)	69.3(6.4)	69.3(6.1)
Erection (time)			
N	359	349	708
Mean(SD)	4.8(0.5)	4.8(0.7)	4.8(0.6)

**
P-value between 0.01 and 0.05.

Table 3

Transvaginal ultrasound results in female participants by treatment arm

	Clomiphene	Letrozole	All
Left ovary not visualized	1/376(0.3%)	3/373(0.8%)	4/749(0.5%)
Right ovary not visualized	6/376(1.6%)	2/373(0.5%)	8/749(1.1%)
Neither ovary visualized	0/376(0.0%)	1/373(0.3%)	1/749(0.1%)
Morphology			
Left: Normal	50/373(13.4%)	41/365(11.2%)	91/738(12.3%)
PCOS	310/373(83.1%)	317/365(86.8%)	627/738(85.0%)
Hyperthecotic	13/373(3.5%)	7/365(1.9%)	20/738(2.7%)
Right: Normal	38/369(10.3%)	39/367(10.6%)	77/736(10.5%)
PCOS	325/369(88.1%)	321/367(87.5%)	646/736(87.8%)
Hyperthecotic	6/369(1.6%)	7/367(1.9%)	13/736(1.8%)
Both ovaries: Neither ovary polycystic	25/367(6.8%)	16/362(4.4%)	41/729(5.6%)
Polycystic/normal combination	37/367(10.1%)	48/362(13.3%)	85/729(11.7%)
Both polycystic	305/367(83.1%)	298/362(82.3%)	603/729(82.7%)
Left ovarian volume(cm ³)			
N	373	370	743
Mean(SD)	11.3(5.8)	11.8(6.7)	11.6(6.3)
Right ovarian volume(cm ³)			
N	368	371	739
Mean(SD)	12.5(7.2)	12.6(7.6)	12.6(7.4)
Patients with at least one follicle/cyst>10	58/375(15.5%)	77/367(21.0%)*	135/742(18.2%)
Size of largest follicle/cyst (cm ³)			
N	50	70	120
Mean(SD)	5.8(5.8)	3.7(5.1)	4.6(6.0)
Antral Follicle Count: Right Ovary			
<5	9/368(2.4%)	9/362(2.5%)	18/730(2.5%)
5–10	38/368(10.3%)	35/362(9.7%)	73/730(10.0%)
10–15	78/368(21.2%)	77/362(21.3%)	155/730(21.2%)
>15	243/368(66.0%)	241/362(66.6%)	484/730(66.3%)
Antral Follicle Count: Left Ovary			
<5	6/372(1.6%)	5/357(1.4%)	11/729(1.5%)
5–10	52/372(14.0%)	46/357(12.9%)	98/729(13.4%)
10–15	74/372(19.9%)	73/357(20.4%)	147/729(20.2%)
>15	240/372(64.5%)	233/357(65.3%)	473/729(64.9%)
Antral Follicle Count: Both Ovaries			
N	365	354	719
Mean(SD)	46.5(28.5)	47.4(27.4)	46.9(27.9)
<5	1/365(0.3%)	1/354(0.3%)	2/719(0.3%)
5–10	5/365(1.4%)	5/354(1.4%)	10/719(1.4%)
10–15	14/365(3.8%)	12/354(3.4%)	26/719(3.6%)

	Clomiphene	Letrozole	All
>15	345/365(94.5%)	336/354(94.9%)	681/719(94.7%)

*
P-value between 0.05 and 0.1.

Table 4

PCOS phenotypic characteristics by BMI category

Category	Parameter	BMI<30	BMI 30–34.9	BMI 35–39.9	BMI 40	P value [*]
Demographic	Age (yr)					
	N	233	145	127	245	
	Mean(SD)	28.3(3.8)	28.8(4.4)	28.8(3.9)	29.5(4.7)	0.003
	Caucasian (%)	186/245(75.9)	97/127(76.4)	119/145(82.1)	188/233(80.7)	0.382
	Latino (%)	31/245(12.7)	24/127(18.9)	28/145(19.3)	45/233(19.3)	0.170
Biometric	Body Mass Index (kg/m ²)					
	N	245	127	145	233	
	Mean(SD)	24.8(3.2)	32.7(1.4)	37.4(1.5)	46.0(5.1)	<0.001
	Waist circumference(cm)					
	N	244	127	144	233	
	Mean(SD)	84.0(11.0)	103.0(9.9)	112.4(10.5)	126.5(11.5)	<0.001
	Systolic					
	N	245	127	145	232	
	Mean(SD)	112.8(10.9)	121.3(12.8)	121.4(12.3)	124.6(12.4)	<0.001
	Diastolic					
	N	245	127	145	232	
	Mean(SD)	72.8(8.5)	77.3(9.4)	78.7(8.9)	80.4(8.9)	<0.001
	Hirsutism					
	N	245	127	145	233	
	Mean(SD)	14.4(8.2)	17.1(8.7)	18.3(8.6)	18.9(8.2)	<0.001
Imaging	Sebum(mcg/cm ²)					
	N	237	123	141	229	
	Mean(SD)	109.6(54.4)	113.5(55.0)	105.7(56.9)	103.7(56.0)	0.173
	Total ovarian volume(cm ³)					
	N	240	125	143	227	
	Mean(SD)	23.9(11.8)	24.1(10.4)	25.4(11.7)	23.2(11.1)	0.600
	Antral Follicle Count: Both Ovaries					
	N	235	124	139	221	

Category	Parameter	BMI<30	BMI 30-34.9	BMI 35-39.9	BMI 40	P value [*]
Laboratory	Mean(SD)	51.0(26.2)	47.0(27.1)	46.9(28.4)	42.6(29.4)	<0.001
	Endometrial Thickness(mm)					
	N	243	126	144	232	
	Mean(SD)	6.3(2.7)	6.3(2.7)	7.3(3.4)	7.0(3.0)	<0.001
	AMH(ng/mL)					
	N	245	127	145	231	
	Mean(SD)	10.0(8.1)	9.1(8.4)	7.5(5.1)	5.7(5.0)	<0.001
	Total Testosterone(ng/dL)					
	N	245	127	145	231	
	Mean(SD)	51.9(28.6)	58.5(29.7)	55.9(28.9)	55.9(28.3)	0.050
	SHBG(nmol/L)					
	N	245	127	145	231	
	Mean(SD)	47.4(29.0)	29.9(17.4)	28.1(13.4)	25.4(16.3)	<0.001
	DHEAS(ug/dL)					
	N	245	127	145	231	
	Mean(SD)	168.5(79.9)	180.0(101.0)	156.2(86.3)	147.6(87.1)	<0.001
LH to FSH ratio						
	N	245	127	145	231	
	Mean(SD)	2.9(9.8)	2.7(8.5)	1.6(1.0)	1.4(0.7)	<0.001
	Fasting glucose(mg/dL)					
	N	245	127	145	231	
	Mean(SD)	82.5(13.1)	85.7(11.2)	87.6(12.2)	88.8(12.8)	<0.001
	Fasting insulin(uIU/mL)					
	N	245	127	145	231	
	Mean(SD)	8.4(14.1)	16.4(11.8)	26.3(49.5)	28.0(17.8)	<0.001
	HDL cholesterol(mg/dL)					
	N	245	127	145	231	
	Mean(SD)	41.9(11.4)	37.7(10.2)	36.2(10.3)	34.7(8.8)	<0.001
	Triglycerides(mg/dL)					
	N	245	127	145	231	
	Mean(SD)	92.9(47.6)	128.3(61.4)	127.5(60.3)	128.1(56.7)	<0.001

Category	Parameter	BMI<30	BMI 30-34.9	BMI 35-39.9	BMI 40	P value [*]
QoL	Metabolic syndrome (%)	24/244(9.8)	54/127(42.5)	61/145(42.1)	115/231(49.8)	<0.001
	Score of SF36					
	Physical component					
	N	237	124	141	227	
	Mean(SD)	55.2(5.9)	53.4(6.4)	54.0(5.4)	51.3(6.7)	<0.001
	Mental component					
Total score of PCOSQ	N	237	124	141	227	
	Mean(SD)	50.1(8.7)	47.8(10.8)	49.6(9.4)	49.6(9.6)	0.693
	N	237	126	142	228	
	Mean(SD)	4.4(1.2)	3.7(1.1)	3.7(1.1)	3.5(1.0)	<0.001

* Spearman correlation test was used for continuous variables and chi-square test was used for categorical variables.