ABSTRACT

Introduction: Letrozole showed higher ovulation and live birth rates than clomiphene in infertile women with polycystic ovary syndrome (PCOS). Berberine, a major active component of Chinese herbal medicine rhizomacoptidis, has been used to improve insulin resistance to facilitate ovulation induction in women with PCOS, but there is no study reporting the live birth or its potential as a complementary treatment to letrozole. We aim to determine the efficacy of letrozole with or without berberine in achieving live births among 644 infertile women with PCOS in Mainland China.

Methods and analysis: This is a prospective, randomized, multicentre, double-blinded, controlled design. Infertile women with PCOS were randomized into three-arm, letrozole and berberine, letrozole and berberine placebo, letrozole placebo and berberine. Data and blood were collected at baseline, the third month and sixth month after treatment, or immediately were collected if subject was pregnant. Statisticians and clinical investigators were blinded to treatment allocation and treatment related study results until the central database was locked for final data extraction and analysis determined. The statistical analysis plan described basic analysis principles, methods commonly encountered in data analysis issues, and the specific statistical procedures for analyzing the primary, secondary, and safety outcomes.

Ethics and dissemination: The study was approved by the ethics committee of the First Affiliated Hospital, Heilongjiang University of Chinese Medicine. The study findings will be disseminated through peer-reviewed publications and conference presentations.

TRIAL OVERVIEW

The trial is a multicenter double-blind randomized parallel group with 1:1:1 conducted at nineteen centers in China mainland. A design and protocol paper has previously been published[13]. The purpose of the trial is to determine superior to CC for ovulation and live birth rates in infertile PCOS women[6].

Berberine, the major active component of rhizomacoptidis, is a traditional medicine for treating bacteria-associated diarrhea, intestinal parasitic infections, and ocular trachoma infections for several decades through its antimicrobial activities[7]. The beneficial effects of berberine on metabolism are pleiotropic. In folk medicine of China, berberine is used to treat type 2 diabetes mellitus, obesity, insulin resistance and non-alcoholic fatty liver disease[8-10]. In addition, berberine has also been reported to alleviate dyslipidemia and cardiovascular diseases[11-12].
Table 1. Details of participating centers.

<table>
<thead>
<tr>
<th>ID</th>
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<th>Sites principal investigator</th>
<th>Enrollment number</th>
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<tr>
<td>1</td>
<td>First Affiliated Hospital, Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang</td>
<td>Dr X K Wu, Professor L H Hou</td>
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<tr>
<td>2</td>
<td>Daqing Oilfield General Hospital, Daqing, Heilongjiang</td>
<td>Professor Y Q Gao</td>
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<td>3</td>
<td>Daqing Longnan Hospital, Daqing, Heilongjiang</td>
<td>Professor S M Du</td>
<td>56</td>
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<td>First Affiliated Hospital, Tianjin University of Chinese Medicine, Tianjin</td>
<td>Dr Y Yan</td>
<td>31</td>
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<td>Professor P L Li</td>
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<td>Shanxi Province Hospital of Chinese Medicine, Taiyuan, Shanxi</td>
<td>Professor J F Zhang</td>
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<td>Dr J Y Fu</td>
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<td>Professor H Y Xue</td>
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<td>Dr G Z Yu</td>
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Whether berberine, letrozole or their combination is more likely to result in live birth in infertile PCOS women. A total of 644 Chinese PCOS women attempting pregnancy were enrolled at one of 19 participating sites and randomly assigned to one of three treatment arms. Written informed consent was obtained from each patient prior to her participation in the study.

Inclusion criteria:

- Age of women between 20 and 40 years;
- PCOS diagnosed according to the Rotterdam 2003 criteria (at least 2 of the following 3 criteria);
  - Oligo-ovulation or anovulation,
  - Clinical and/or biochemical signs of hyperandrogenism,
  - PCOS and exclusion of other aetiologies (congenital adrenal hyperplasia, androgen-secreting tumours and Cushing’s syndrome),
- At least one patent tube and normal uterine cavity shown by hysterosalpingogram, hysterosalpingo contrast sonography or diagnostic laparoscopy within 3 years;
- Sperm concentration ≥ 15×10⁷/mL, total motility ≥ 40% or progressive motility ≥ 32%.
- At least 1-year history of infertility.

Exclusion criteria:

- Use of hormonal drugs or other medications including Chinese herbal prescriptions in the past 3 months,
- Patients with known severe organ dysfunction or mental illness,
- Pregnancy, post abortion or postpartum within the past 6 weeks,
- Breastfeeding within the past 6 months,
- Patients with congenital adrenal hyperplasia, clinically suspected Cushing syndrome, or an androgen-secreting neoplasm,
- Not willing to give written consent for the study.

Eligible patients will be randomized into one of three arms: 1) letrozole and berberine placebo; 2) letrozole placebo and berberine; 3) letrozole and berberine. Dose of letrozole was 2.5 mg (1 tablet) on days 3–7 of spontaneous period or withdrawal bleed within the first three treatment cycles and increased to 5 mg (2 tablets) on days 3–7 of the last three treatment cycles. Berberine or berberine placebo was administered orally at a daily dose of 1.5 g for 6 months. Progesterone was tested on day 22 of each treatment cycle to confirm ovulation. All the subjects took the drugs six months until they were pregnant (positive of serum human chorionic gonadotropin refer to local sites range, and they were followed up until delivery. Live birth information was got by telephone calls and abstractions of obstetrical records.

The trial was approved by the ethics committee of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine (2009-LL-001) on April 10, 2009, and then separated approved by local ethic committees in different sites. It was first registered at http://www.chictr.org.cn/ in China on October 8, 2009 (ChiCTR-TRC-09000376). The date of first randomization was 11 November 2009 and the date of last randomization was 28 February 2013. The target for randomization was 660 and the number of subjects randomized was 644. Table 1 gave the details of the nineteen participating centers, including their principal investigators.

**Variable definitions**

**Primary outcome**

The primary outcome is cumulative live birth rate, which is defined as the delivery of a live-born infant after 20 gestational weeks. Singleton live birth, twin live birth and birth weight will be calculated separately. Singleton live birth
rate will be summarized according to the number of single live birth women in total live birth women, twin live birth rate will be calculated by twin live birth women in total live birth women.

Secondary outcomes
The secondary outcomes will be:

1) Ovulation rate: Ovulation was defined as a serum progesterone level according to the standard of the local lab (minimum value of luteal phase) or more than 5 ng/ml. Ovulation rate will be calculated the percentage by the number of ovulated cycles in all tested cycles.

2) Conception rate: Conception is defined as raised serum human chorionic gonadotropin level. Conception rate will be calculated the percentage by the number of conceived subjects in all randomized subjects.

3) Clinical pregnancy rate: Clinical pregnancy means an intrauterine fetal pole with fetal heart motion detected by ultrasonography. It will be calculated the percentage by the number of pregnant subjects in all randomized subjects.

4) Singleton pregnancy rate: It will be calculated the percentage by the number of singleton pregnancy in all pregnant subjects.

5) Twin pregnancy rate: It will be calculated the percentage by the number of twin pregnancy in all pregnant subjects.

6) Pregnancy loss rate: Pregnancy loss is defined as loss of an intrauterine pregnancy before 20 completed weeks of gestation. Pregnancy loss rate is number of pregnancy loss women divided by all women with conception. It is separated to the first trimester pregnancy loss, the second and third trimester pregnancy loss.

7) Changes of demographic characteristics: including body mass index, waist circumference, hirsutism, acne, prematurely bald, follicle numbers in left and right ovaries.

8) Changes in hormonal profile: Follicle-stimulating hormone (FSH), Luteinising hormone (LH), total testosterone (T), prolactin (PRL), estradiol (E2), progesterone (P), LH/FSH, Free androgen index (FAI), sex hormone-binding globulin (SHBG) and Anti Müllerian Hormone (AMH). The fasting blood sample for the tests was drawn at the baseline visit and at the end of the treatment visit at menstrual cycle days 3–7 if not pregnant, after the positive test if pregnant.

9) Changes in the metabolic profile: fasting insulin concentrations, cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). The blood sample for the tests will be drawn at the baseline visit and at the end of the treatment visit.

Safety
There are a number of serious adverse events (SAEs) and adverse events (AEs) that might occur during treatment or gestation. Such as using of letrozole may cause hot flashes, nausea, using of berberine may cause constipation or diarrhea. The pregnant women may have the risk of ectopic pregnancy and other obstetric complications. Therefore, we collected detailed information regarding all SAEs. All the AEs are recorded in case record forms by the treating physician. SAEs are defined as: a) death; b) life-threatening; c) persistent or significant disability/incapacity; d) inpatient hospitalization or causes prolongation of existing hospitalization (except abortion prevention before 12-week gestation); especially in this trial are e) a congenital anomaly/birth defect; f) miscarriage after 12-week gestation; g) ectopic pregnancy; i) neonatal death in 6 weeks.

Glutamic oxalacetic transaminase (AST), glutamic-pyruvic transaminase (ALT), creatinine and urea nitrogen were tested before and after the drugs to assess the safety of letrozole and berberine on subjects.

Randomization and allocation concealment
The randomization was performed through a web-based randomization system operated by an independent data centre, the Institute of Basic Clinical Medicine, China Academy of Chinese Medical Sciences. The randomization was stratified by the participating sites. Participants and all other research and therapy staffs were blinded to intervention allocation.

Sample size
The sample size calculation was based on the live birth rate. A previous study showed that the live birth rate of letrozole was 22%[14] and we hypothesized that a combination of letrozole and berberine can increase the live birth rate to 30%.

According to the sample size of the estimation formula[15],

$$n = \left(\frac{u_a + u_f}{2}\right)^2 \times \frac{2P \times (1 - P)}{(P_0 - P_1)^2}$$

($\alpha = 0.05; \beta = 0.1$)

It is estimated that a sample size of 220 participants per group will be required to give 90% power at 0.05 significance level with a dropout rate of 20%.

Data entry and quality control of data
Case report forms (CRFs) were used. Study assistants were asked to fill in the CRF at each visit, and then entered the data into the web-based data management system in 15 days.

Quality control of data was handled at five different levels. The first level was the real time logical and range checking built into the web-based data entry system. The investigators at the participating sites were required to ensure data accuracy as the first defense. The second was the remote data monitoring and validation that is the primary responsibility of the study data manager and programmer. The data manager conducted monthly comprehensive data checks, as well as regular manual checks (within the database system). Manual checks could identify more complicated and less common errors. The data manager queried sites until each irregularity was resolved. The third level of quality control was the site visits, where data in our database were compared against source documents. Identified errors were resolved between the data coordination center and clinical sites. The
visits assured data quality and patient protection. After the site investigator finished target enrollment and therapy, the CRFs would return to research host unit (Heilongjiang university of Chinese medicine), data in CRFs would be typed in web-based system the second time by data entry staffs to ensure double entry. The system gave difference check to make sure there was no difference between the two entries, and this was the fourth level in data quality control. The fifth level happened before the database was locked and the data manager made a total difference check to guarantee data of double entries were completely consistent.

**Trial profile**

The flow of trial subjects was given in a Consolidated Standards of Reporting Trials (CONSORT) diagram (Figure 1). The number of screened patients who fulfilled trial inclusion criteria, and informed consents were included in the primary and secondary analyses.

**Plan of statistical analysis**

This paper describes the detailed statistical analysis plan (SAP) to avoid outcome reporting bias. It is developed before the database is locked and data analysis is initiated.

**Data management**

The data management was done by HL M and JS G and the data statistics was done by FT. All decisions were recorded in a log book, and all more vital decisions were made in consultation with at least two members of the investigator group (one of whom is the principal investigator).

**Statistical analysis set**

**Intention-to-treat analysis**

The intention-to-treat (ITT) analysis included all participants who were randomized into the treatment arms, regardless of the presence or absence of follow up data. ITT analysis will be reported mainly for efficacy evaluation of primary and secondary analyses.
secondary outcomes. In addition, ITT will be used in the balance testing of demographic data, disease history, and laboratory variables at baseline.

**Per-protocol set**

The per-protocol set is defined as all participants who were randomized into the trial, who met trial eligibility criteria, and who followed their randomized intervention policy at the centre in which they were randomized and without severe protocol violation. In this trial, the severe protocol violations include the following conditions:

- Patients who were randomized to an arm but did not receive any intervention.
- Not meeting the inclusion criteria: including do not meet the criteria of PCOS, just meet one of the Rotterdam 2003 criteria; don’t provided the proof of tubal patency or her partner’s sperm parameters, diagnosed of infertility less than half a year.
- Existing of concomitant medication which may add confounding to the estimation of efficacy and safety, such as some patients was given hCG to induced ovulation.
- Beyond the time window, loss to follow up or dropout; including total treatment cycles more than 10 months or less than 4 months.

**Safety set**

Safety set (SS) includes the subjects received at least one time medication treatment. SS is the main set of safety assessment in this trial.

**Analysis of the primary outcome**

Intention-to-treat analysis will be applied to minimize bias due to dropouts. The number of subjects who have live birth will be reported and it will be performed by comparing the treatment groups using the Pearson chi-square test or Fisher exact test. We will use Kaplan–Meier curves to compare the treatment arms with respect to the primary outcome of live birth, from randomization to live birth.

The KM curves are used to estimate the percentage surviving versus time\(^{17-18}\). It is usually a series of declining horizontal steps with a large enough sample size. An important advantage of the KM curve is that the method can take into account some types of censored data, particularly right-censoring, which occurs if a patient withdraws from a study, is lost to follow-up, or is alive without event occurrence at last follow-up. In this study we use KM curve to estimate the cumulative live birth rate with time from randomization to live birth, it is an ascending trend as the change of time different from general surviving KM curve.

Also we will report the live birth according to different interventions, body-mass index (BMI), age, the menstrual cycles, hirsutism scores and previous infertility duration. A log-rank test will be used to test the interaction between different groups and study treatment with regard to the time from randomization to live birth. All analyses will be performed with the use of SAS software, version 9.2 (SAS Institute).

**Analysis of secondary outcomes**

Dichotomous variables will be summarized by numbers, percentages. Chi-square test or fisher exact test will be used for testing differences between the three treatment groups.

Continuous variables will be summarized using the mean, standard deviation (SD), median inter-quartile range and 95% confidence intervals. According to whether it is normal distribution or homogeneity of variance, analysis of variance or Kruskal-Wallis Test will be used to compare among the three treatment groups. If the P value is <0.05, Mann-Whitney U test will be carried out between the groups.

Adverse events will be categorized and percentage of patients experiencing AE and SAE in this trial will be documented. Chi-square tests will be performed to examine differences in the proportion of total, and categories of adverse events within each treatment arm.

**Missing data**

The decision to withdraw from the study participation can be made by the clinician or the participants.

The number of active withdrawals (broken down by participant, clinical staff, both) will be reported by intervention and center. The reasons for withdrawal will be summarized. Attempts are made to follow up all participants. Follow up information will also be obtained from the patients’ friends, family members or general practitioner or the site investigators. All the analyses will be based on available data only including information secured by the strategies described above.

**Characteristics of patients with baseline comparisons**

We will present the description of baseline characteristics by intervention group. Discrete variables will be summarized by frequencies and percentages calculated according to the number of patients for whom data are available. Where values are missing, the actual denominator will be stated. Continuous variables will be summarized using standard measures of central tendency and dispersion, using either mean ± SD for data with normal distribution, or median and interquartile range for non-normally distributed data.

The baseline variables will be:

- Age of women
- Body mass index
- Waist circumference
- Hip circumference
- Waist hip ratio
- Hirsutism assessed by Ferriman-Gallwey score
- Menstrual pattern
- Previous infertility therapy
- Previous pregnancy
- Ultrasonographic findings
- Fasting serum levels including testosterone, estradiol, free androgen index, FSH, LH, LH/FSH ratio, SHBG and prolactin.
**Outline of figures and tables**

The first figure will be a CONSORT flow chart as specified in Figure 1. The second figure will be a K-M curves relating to live birth rate during the trial period. The first table will be the baseline characteristics of the modified intention-to-treat population, the second table will be the clinical outcomes, and the third table will be the absolute changes in key measures between baseline and last visit. The fourth table will show how many patients in each group had serious adverse events or adverse events, and if there is any statistical difference between different groups.

**Subgroup analysis**

Subgroup analysis and other secondary analysis will be made for subsequent papers after the first primary paper is published.

**CONCLUSIONS**

This article describes the principles of statistical analyses used in the trial for the primary publication of the main outcome measures in order to minimize risk of data-driven results and outcome reporting bias.

We anticipate that this framework will enhance the utility of the reported result and allow readers to better judge the impact.

Provenance and peer review Not commissioned; externally peer reviewed.

**FUNDING**

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**CONTRIBUTORS**

HLM and FT proposed the statistical analysis plan. HLM drafted the manuscript. XKW, EHYN and JPL participated in the design of the trial. JSG conducted the coordination of the trial. HLM, JPL and XKW read, amended and approved the statistical analysis plan and the final manuscript.

**ETHICS APPROVAL**

The study was approved by the Ethics Committee of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine (2009LL-001-02).

**COMPETING INTERESTS**

None.

**PATIENT CONSENT**

Obtained.

**PROVENANCE AND PEER REVIEW**

Not commissioned; externally peer reviewed.

**REFERENCES**


www.wjtcm.org


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