

# Accuracy and efficacy of diagnosis using clinical characteristics integrated with metabolomics in patients with polycystic ovary syndrome: study protocol for a randomized controlled trial

**Cheng-Ming Ni**

The Affiliated Wuxi People's Hospital of Nanjing Medical University

**Wen-Long Huang**

Department of endocrinology, Jiangyin People's Hospital

**Yan-min Jiang**

The Affiliated Wuxi People's Hospital of Nanjing Medical University

**Juan Xu**

Department of endocrinology, Jiangyin People's Hospital

**Ru Duan**

The Affiliated Wuxi People's Hospital of Nanjing Medical University

**Yun-Long Zhu**

xi Maternal and Child Health Centers Clinical Hospital

**Xu-Ping Zhu**

The Affiliated Wuxi People's Hospital of Nanjing Medical University

**Xue-Mei Fan**

Tsinghua University

**Guo-An Luo**

Tsinghua University

**Yi-ming Wang**

Tsinghua University

**Yan-Yu Li**

The Affiliated Wuxi People's Hospital of Nanjing Medical University

**Lan Xu** (✉ [1378771946@qq.com](mailto:1378771946@qq.com))

**Qing He**

Wuxi People's Hospital

---

**Study protocol**

**Keywords:** polycystic ovary syndrome (PCOS), metabolomics, clinical signs and symptoms, accuracy and efficacy

**Posted Date:** December 13th, 2019

**DOI:** <https://doi.org/10.21203/rs.2.12197/v2>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published on February 11th, 2020. See the published version at <https://doi.org/10.1186/s13063-020-4060-6>.

# Abstract

## Background

Polycystic ovary syndrome (PCOS) is defined as a complex endocrine syndrome, and the mechanisms underlying its various clinical signs and symptoms are still poorly understood. It is critical to precisely diagnose the phenotypes of PCOS in order to provide patients with individualized therapy[1, 2]. However, the criteria by which to diagnose different phenotypes, which are mostly based on symptoms, physical examination, and laboratory evaluation, remain unclear. The aim of this study is to compare the accuracy and precision of metabolomic markers with common clinical characteristics to determine a more effective way to diagnose and treat two subgroups, one based on clinical indexes and another based on metabolomic indexes[3], of PCOS patients. The effects of different the interventions based on the two subgroups will also be observed.

## Methods

This is a prospective, multicenter, analyst-blinded, randomized controlled trial. There will be one healthy control group and two parallel experimental arms in this study: (1) people without PCOS (health control group); (2) PCOS patients diagnosed based on clinical indexes (group 1); (3) PCOS patients diagnosed based on metabolomic indexes (group 2). A total of 276 eligible people will be recruited, including 60 healthy people and 216 PCOS patients, who will be randomly assigned to different diagnosis groups in a 1:1 ratio. Patients in the two different diagnosis groups will be divided into two different subgroups based on their clinical characteristics (group 1 based on clinical indexes, group 2 based on metabolomic indexes); thereafter, they will receive a 6-month different treatment. The primary outcome for experimental groups will be the treatment effect of PCOS.

## Discussion

The purpose of this trial is to determine whether integrated metabolomic indexes are more accurate and effective than clinical characteristics in the diagnosis of the phenotypes of reproductive females with PCOS. This trial will therefore contribute to the provision of a solid foundation for the precise clinical diagnosis of two PCOS subgroups, as well as for future research on individualized PCOS therapy.

## Background

Polycystic ovary syndrome (PCOS) is defined as a complex endocrine syndrome that exhibits chronic ovulatory disorders, hyperandrogenism, insulin resistance, and metabolic disorders. The global prevalence of PCOS is approximately 5%–10% [4, 5]; it is one of the most common endocrine and metabolic disorders in Chinese reproductive woman. There is a strong association between PCOS, insulin resistance, and dyslipidemia, which includes obesity, type 2 diabetes, and metabolic syndrome (MetS)<sup>[6–10]</sup>. Evidence also suggests that PCOS patients might have a higher prevalence of asthma<sup>[11]</sup>, non-alcoholic fatty liver disease (NAFLD)<sup>[12]</sup>, and mental disorders<sup>[13]</sup>, such as depression and anxiety<sup>[14]</sup>.

However, the underlying mechanism is still poorly understood, thus making clinical diagnosis and treatment very difficult.

To determine whether a patient has PCOS, careful assessment of hyperandrogenism, hyperandrogenaemia, ovulatory function, and ovarian morphology is required. The recent criteria are mostly based on the combination of criteria/statements which are disputed, and due to the heterogeneity of clinical manifestations and complex pathogenesis of PCOS, the criteria for diagnosis mainly aimed at ruling out other diseases. Most importantly, the accuracy of the diagnosis and evaluation methods for PCOS used to assess the individual criteria is even more critical.

It is well recognized that there are diverse phenotypes of PCOS<sup>[15-17]</sup>, and thus treatment should be precise and individualized. The European Society of Endocrinology<sup>[2]</sup> proposed that there were four phenotypes of PCOS, including a type without hyperandrogenemia which is controversial. It was also suggested that abnormalities of enzymatic steroidogenic, prolactin excess and thyroid problems need to be excluded before the diagnosis. The classification of subtypes and treatment of PCOS in China is mainly based on the clinical indexes, such as sex hormone index (e.g. FSH, LH, LH/FSH, free testosterone, DHEAS, androstenedione and SHBG), 6 item serum lipids indexes (e.g. total cholesterol, triglycerides, LDL-C, HDL-C, Apo(A), Apo(B)), the results of OGTT tests and IRT tests<sup>[1]</sup>. No one could deny that based on the existing diagnostic criteria, it is likely to cause a considerable number of misdiagnoses. Therefore, it is urgent to explore a new detection method to standardize the classification of phenotypes and to establish an efficient diagnosis and treatment strategy for PCOS. The development and application of new technologies, such as genomics, proteomics, and metabolomics, provide a new direction for the clinical diagnosis of PCOS phenotypes. However, in genomics, although the findings of Genome-wide association study (GWAS) research have implications, the direct PCOS pathogenic gene has not been found and cannot be used for phenotyping. In terms of proteomics, although some scholars have identified potential biomarkers for PCOS phenotyping, the clinical application has been limited because proteins, or amino acids, are highly susceptible to interference, and thus the results are not stable. Due to the high sensitivity and specificity of metabolomic technology, it can be assumed that some metabolic markers can be used to distinguish whether PCOS patients are accompanied by high androgens.

Recent studies have adopted plenty of different measurements trying to classify the phenotypes, such as nuclear magnetic resonance (NMR)<sup>[18]</sup>, liquid chromatography-mass spectrometry<sup>[19]</sup>, and others<sup>[20, 21]</sup>. Based on our previous study<sup>[3]</sup>, with the employment of ultra-performance liquid chromatography/quadrupole-time of flight-mass spectrometry (UPLC/Q-TOF-MS), we found there were two subgroups of PCOS patients. One group mainly included those with hormone metabolism disorders while the other included those with lipid metabolism disorders.

Thus, our group designed this trial to determine whether the integration of metabolomic indexes and clinical characteristics would provide a more accurate and effective method for diagnosing the PCOS phenotypes. Additionally, we hoped that it would provide motivate for further research on individualized PCOS therapy.

## Methods/design

# Study design

This is a prospective, multicenter, analyst-blinded, randomized controlled trial. There will be one healthy control group and two parallel experiment arms in this study: (1) people without PCOS (health control group); (2) PCOS patients diagnosed based on clinical indexes (group 1); (3) PCOS patients diagnosed based on metabolomic indexes (group 2). A total of 276 eligible people will be recruited, including 60 people without PCOS in the healthy control group while the other 216 patients with PCOS will be randomly assigned to different diagnosis groups in a 1:1 ratio. After the group assignment, necessary medical detection, relative information, and biological samples of the healthy control group, as well as the two diagnosis groups, will be collected. Additionally, the blood samples for the participants in all of the groups will be tested using metabolomics for further studies, while the analysis results will be totally blinded to the participants in the groups and their research doctors. Based on our previous study<sup>[3]</sup>, we found there were two subgroups of PCOS patients; one included participants mainly with hormone metabolism disorders (subgroup 1), while the other included participants mainly with lipid metabolism disorders (subgroup 2).

As a result of this, patients in the two diagnosis groups will be automatically divided into the two different subgroups according to their characteristics. The main difference is the division of group 1 diagnosed based on indexes recommended by the statement<sup>[1]</sup>, such as clinical symptoms, signs, and common clinical indexes (including ultrasonography), while group 2, will be selected mainly based on metabolomic indicators. All patients will receive corresponding treatment according to their subtypes, one being darin-35 and the other being metformin. The interventions for all experimental groups will last for 6 months and the results will be evaluated if three constant sessions( 3 months) of treatment is finished.

Outcome assessment will be conducted and data will be analyzed by someone who is blinded to the assignment of the participants. The design of study is briefly illustrated in the flow chart in Fig. 1, and the study timetable is presented in Figs. 2 and 3.

The main treatment center will be the Department of Endocrinology and Metabolism in Wuxi People's Hospital affiliated with Nanjing Medical University, which will enroll 60 participants in the healthy control group and 108 participants in experiment group. Two other treatments centers—Wuxi Maternal and Child Health Centers Clinical Hospital and Jiangyin People's Hospital—will enroll the other 108 participants.

Fig 1–3

## Participants and recruitment

People without PCOS will mainly be enrolled in the healthy control group excluded other disorders may influence the sex related hormone and metabolism conditions. For the experiment group, all eligible

participants will be diagnosed with PCOS according to the 2018 Chinese Endocrine Society clinical practice consensus on PCOS [1], which is based on the globally accepted criteria published by Rotterdam [22, 23]. Patients who are of reproductive age and can obey the protocol including strict contraception over the course of 6 months will be informed of this trial. If the potential participant expresses interest, a face-to-face interview about the whole trial process will be conducted in a reception room for clinical research subjects in the three hospitals. Patients who meet the inclusion criteria are eligible for enrollment in the trial if they are willing to provide written informed consent. We will publicize the trial to potential participants in two ways: 1) experimental groups will be recruited by approaching patients with PCOS admitted to an outpatient department or inpatient ward in each center, the official microblog and WeChat platforms of each center will also be used; 2) healthy control groups will be directly recruited by approaching volunteers in the health examination center of Wuxi People's hospital.

## Inclusion criteria

Participants who meet all the following criteria will be enrolled in the experimental group:

- Diagnosed with PCOS according to the 2018 Chinese Endocrine Society clinical practice consensus. (Diagnosed with the presence of at least two of the following three conditions, and the exclusion of other etiologies must be followed: 1. Clinical or biochemical hyperandrogenism; 2. Oligo-anovulation [menstrual cycle length > 35 days and < 8 menstrual cycles per year]; 3. Polycystic ovaries morphology having at least 25 small follicles [2–9 mm] in the whole ovary, and/or increased ovarian volume  $\geq 10$  ml.
- 2) Aged 18–45 years, reproductive female.
- 3) Able and willing to comply with the intervention and follow-up evaluations.
- Provides written informed consent prior to being enrolled.

## Exclusion criteria

Participants will be excluded if they meet any of the following criteria:

- Currently receiving treatment in another experimentally study, or just finished another trial less than 30 days ago.
- Treated with daine–35, metformin, or other forms of estrogen, progesterone, lipid-regulating, and hypoglycemic drugs (such as glucocorticoids, spironolactone, antibiotics, bacteria regulators, and anti-inflammatory drugs) within 12 weeks.
- An allergy or intolerance to daine–35, metformin, or any of its components.

- Suffers from congenital adrenal cortical hyperplasia, hypercorticosis, androgen-secreting tumors, Cushing's syndrome, thyroid dysfunction, hypogonadotropin deficiency, hyperprolactinemia disease, premature ovarian insufficiency, functional hypothalamic amenorrhea, or diabetes.
- A medical history of malignant tumors, in particular a gynecological malignant tumor history of surgery, radiation, and chemotherapy.
- Suffers from liver function damage (ALT and AST above 1.5 times of the normal limits of the laboratory index) and chronic liver disease.
- Suffers from hematopenia or thrombotic disease.
- Is pregnant or expects to be pregnant during the study.
- In an unstable medical, physical, or mental state.
- Has a medical history of symptomatic ventricular arrhythmias with torsion ventricular tachycardia.
- Unable to complete the procedures outlined in the study protocol.
- Any other situation that would interfere with the study evaluation, procedures, or completion.

## Dropout criteria

The dropout criteria are defined as follows:

- The subject quits.
- Safety issues (e.g. adverse events, failure of contraceptive measures, and accidents).
- Lost to follow-up.

4) Researchers remove the participant from the study (e.g. poor compliance, such as severe liver dysfunction, complication considerations, or serious adverse events.).

## Suspension criteria

The comprehensive suspension criteria will be as follows:

- 1) A significant safety problem is found, such as the liver function of the patient is seriously impaired or sudden onset of other life-threatening illness that be unable to continue the study.
- 2) The diagnostic and therapeutic effect is poor, such as menstrual/metabolic disorders did not improve (we will evaluate this in the 3<sup>rd</sup> month of therapy).
- 3) A major mistake ( such as patients taking the wrong doses, taking or using medicine without following doctor's advice) is made that will affect the results of the study.
- 4) There is a huge problem in funding or management, such as withdrawal of funding.

# Randomization

The Department of Good clinical practice of Wuxi People's Hospital will lead the randomization process by using a random number generator in the Statistical Package for Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago, IL, USA).

Random sequences will be placed in opaque envelopes, numbered in order, and then will be sent to a clinical researcher and doctors.

The envelopes will be opened sequentially to decide upon the allocation for participants. In this trial, the doctors and the trial participants will be blinded to the group assignment.

## Blinding

In the experimental groups, participants will not be informed of the group assignment, the type of diagnosis, or the treatment that they will receive. Data managers, and the statistician will be blinded in this trial. But the trial administrator ( The steering committee, the Department of Good Clinical Practice in Wuxi People's Hospital) will be not blinded in order to be responsible for the assignment of patients in different subgroups and monitor the whole study process. Besides, the clinical research doctors will be blinded to the assignment of group 1 whose treatment will be decided based on their clinical practice, while they have access to the diagnosis and treatment allocation for group 2. The clinical doctors will be responsible for learning how to use the blinding method to communicate with participants to ensure the diagnosis and treatment blinding. The blinding procedure will be conducted until the data are locked and the trial is completed. Additionally, unblinding will only be permitted in the case of a medical emergency or the participants quits the trial. All cases of unblinding will be documented.

## Interventions

### Healthy control group

Participants will be recruited if they do not have PCOS, metabolism conditions, or any other disorders which may influence their sex related hormones and if they are not using any drugs which could also influence sex hormones. Necessary medical physical diagnosis will be conducted to confirm the qualification. Then, the trial team will collect information regarding the medical history, will conduct a physical examination, and collect blood samples from all qualified participants for further investigation.

### Clinical indexes group

For the clinical indexes group, blood samples and medical information of all qualified participants will be collected. According to the clinical indexes and the Chinese Endocrine Society clinical practice consensus on PCOS in 2018, the participants will be divided into two subgroups; one in which participants have hormone metabolism disorders (subgroup 1) and the other in which participants mainly have lipid metabolism disorders (subgroup 2). Based on several guidelines and clinical experience, subgroup 1 will receive oral Daine-35 once a day for 21 days; thereafter this will be paused for 7 days. Subgroup 2 will receive oral metformin (2000–2500mg) once a day based on their clinical conditions. The whole treatment session will last for at least 6 months. During the trial, blood samples will be collected three times (the week 0, Week 15±1, and Week 26±1) in order to conduct metabolomic detection to assess the changes. However, the detection information will be blinded to all the participants and the clinical research doctors in case it influences the results. All clinical research doctors will be required to have majored in endocrinology and metabolism or gynecological endocrinology for at least 10 years. They also must be employed as an attending doctor for more than 5 years. Finally, they must receive professional training on clinical trials and pass a test to ensure their consistency performance in study methods.

## **Metabolomic indexes group**

For the metabolomic indexes group, the majority of the interventions will be similar to those from the clinical indexes group. The main difference is that the division of the subgroup will be based on metabolomic indexes. The doctors in this group will be informed of the allocation of the subgroup and give the corresponding treatment scheme to all of the participants. It should be mentioned that there will be no chance for doctors to change the scheme during the trial and they will still be blinded to the metabolomic results to minimize the possibility of any influence on treatment decisions.

## **Concurrent treatment of patients**

All other treatments for PCOS are banned during the trial, including oral contraceptives, the broad-spectrum lipid drug, and any other drugs that might influence the results. Participants may receive any treatment that is not related to PCOS and should be instructed to use condoms when having sexual intercourse. Any change in concurrent treatment will be recorded at every visit.

## **Outcome measurements**

For the healthy control group, they will remain enrolled in the trial until all their samples are collected. For the experimental groups, the primary outcome will be the changes in their relative PCOS condition evaluated using clinical indexes. The main clinical index that will be evaluated will be the LH/FSH ratio.

The secondary outcomes will be assessed using the other indexes, signs, and symptoms as described in the consensus of the Chinese Endocrine Society in 2018<sup>[1]</sup>, such as eight sex hormone-related indexes (FSH, LH, PRL, E2, P, Ts, AMH, and VitD); nine blood lipid related indicators (TC, TG, HDL, LDL, ApoA1, Apo B, Lp[a], FFA and hsCRP); endocrine-related indicators (GLU [0h,30min,120min], INS [0h,30min,120min], and BUA). There are also three metabolic markers will help us to evaluate the outcomes, which contains palmitoyl sphingomyelin, cGMP, and DHEAS. Ultrasonography examination is also included; the number and diameters of the follicles of the bilateral ovary will be measured.

The safety outcome will be any severe impairment in liver function or the inability to tolerate (such as nausea and vomiting which caused by metformine) related therapeutic drugs. It will be monitored after each treatment session (one month) via the hepatic function test. The exploratory outcome will be the entire recovery of PCOS after treatment within 26 weeks.

## Safety evaluation

The occurrence of adverse events (AEs) will be evaluated at each visit; this will include every unexpected or unfavorable response that occurred during or after treatment. These events may not have a causal relationship with this study. However, the investigators need to ensure that all AEs are reported and recorded in the subject's medical records. In this trial, AEs are defined as (1) disorders that will hinder one's ability to work or be life threatening (especially liver dysfunction) (2) lead to hospitalization or prolong a department or hospital stay. Remedial treatment should be given immediately to resolve the observed AE, and all AEs will be reported to the responsible units, ethical committees, and to the trial administrator to determine whether the participant ought to remain in or drop out of the trial. No matter what is decided, all participants with an AE will be followed up until the event has been resolved or the condition has become chronic or stable.

The study will not add the times of collection of blood samples and the drugs of treatment in this trial is suggested by Consensus of Chinese endocrinologists for the diagnosis and treatment of polycystic ovary syndrome. In this situation, our study do not add any extra harms to participants. However, once serious damage related to the study is happened, we will take the relevant expenses (including the diagnosis and treatment) and follow up the situation of the participants until recovery.

## Follow-up

To evaluate the accuracy and efficacy of diagnosis, the safety, and the superior effects of individual interventions, a 6 month telephonic follow-up (or via ace-to-face, emails, text messages, or WeChat) will be conducted after the trial. During the follow-up period, no participants will undergo special therapy with the exception of routine cervical care. At weeks 30, 34, 38, and 41, the outcome assessor will call participants to investigate the PCOS condition, asking mainly about menstrual cycle length and

frequency, the appearance of hyperandrogenism (such as weight loss, acne, excessive facial and body hair), and pregnancy. Participants are welcomed to inform the assessors of their clinical symptoms and AEs via face-to-face, emails, phone, text messages, or WeChat at the relevant time points.

## **Blinding and credibility tests**

The findings from metabolomic detection on blinding will be completed at weeks 0, 15, and 26. The implementation of the blinding strategy will be crucial for the trial. The credibility rating for both the diagnosis methods and different types of treatments will be estimated using a credibility test at week 15, 26, and at week 41 during the follow-up period.

## **Data collection and monitoring**

Data on and basic personal medical characteristics will be collected and recorded by screeners when participants are recruited. Clinical signs and symptoms, physical examination findings, metabolomic detection results, short- and long-term outcomes, assessment of diagnosis accuracy and treatment efficiency, and details of the AEs will be recorded by trial assessors and clinical researchers in case report forms (CRFs).

Completed CRFs will be checked and reviewed by a five-person steering committee, which is composed of two supervisors of the major trial center, a data statistician and two data administrators. Committees are completely independent from the research team and constantly blinded to the group allocation.

All data entry and management will be conducted in an OpenClinica System (version 3.12) database. The committee members are qualified in data analysis and will have been trained uniformly.

To ensure the accuracy of the data, two data administrators will independently enter and validate data. If there are issues with the information in the CRF, the data administrators will bring this to the attention of the steering committee. Any revisions will be modified by the administrators according to the feedback provided by the committee. Once the accuracy of data is confirmed, the electronic database will be locked while the real-time tracking and monitoring will still be opened.

The steering committee and the department of Good Clinical Practice in Wuxi People's Hospital will be in charge of monitoring the whole trial processes (e.g., checking the progress of recruitment, participant data, including CRFs, the protocols for researchers, informed consent forms, and any other study specific files.) and are going to audit trial conduct at least one time per month.

All these information will be made available to investigators whose proposed use of the data has been approved by the committee, for up to 15 years following publication. Findings will be presented at conferences and published in peer-reviewed journals. A summary of the findings will be provided to participants if they request via phone.

# Sample Collection and Handling

The actual dates and times of sample collection must be recorded in the laboratory requisition form. Instructions for the collection, handling, storage of samples are found in the laboratory manual that will be provided.

Standard operation of plasma separation for clinical patients:

- (1) fresh anticoagulant peripheral blood was collected with the EDTA anticoagulant tube.
- (2) the fresh peripheral blood was centrifuged at 4°C at 1700G for 10min, and the upper plasma was carefully collected to avoid touching the lower layers of red blood cells and white blood cells.
- (3) the collected plasma was centrifuged at 4°C at 2000G for 10min and the supernatant was taken.
- (4) divide the plasma and freeze it in -80°C refrigerator for later use.

## Precautions for plasma separation:

- (1) the plasma separation process is performed on the ice to maintain the integrity of the blood cells.
- (2) the whole separation operation shall be completed within 4 hours.
- (3) the process of blood collection and plasma separation should be handled carefully to avoid the risk of hemolysis.

After the study, all the samples will be destroyed.

## Statistical analyses

The dataset will include a safety set, a full analysis set (FAS), and a per protocol set. The safety set will be designed for the participants who were randomly assigned and received at least one session of treatment. The FAS will include all medical data related to the trial which will indicate the intervention conducted; however, individuals who miss the primary outcome evaluation will be excluded. In the per protocol dataset, participants will be included if they received at least three constant sessions of treatment.

In this trial, the FAS will be used for the basic analysis. For missing data, we will take imputation adjustment approach and the last observation analysis will be selectively used to handle the missing data.

A sensitivity analysis will then be used to compare the results from the per protocol analysis and the different intervention analyses to evaluate the impact of the missing data on the trial results.

Descriptive statistics about the quantitative indicators, such as means, standard deviations (SD), medians, minimum and maximum, will be presented. Variance analysis will be used to compare the quantitative indexes among different groups (health control group; group 1 and group 2). Student-Newman-Keuls will be used in analysis of two subgroups.

Classification indicators are described by the number and percentages of each category. The chi-square test or exact probability method was used to compare the qualitative indexes among the three groups, and the chi-square segmentation method will be used for the pair-wise two subgroups comparison.

SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) will be used to analyze all trial data. All statistical tests were double-sided. Statistical significance will be considered when the p value is  $\leq 0.05$ .

## Sample size calculation

Based on the literature and the findings from a previous small pilot study which used metabolomic techniques, the sensitivity and specificity needed to classify the control and PCOS groups are 100% and 86%. The sensitivity and specificity between the control group and subgroup 1 is 96.7% and 100%, while the rate between the control group and subgroup 2 is 100% and 86.2%. The sensitivity and specificity to divide PCOS into two different subtypes are 90.9% and 87.5%. In order to meet the lower Statistical limit, each subgroup should include at least 30 people.

According to a presumptive maximum dropout tolerance of 20%, with a significance level of 0.05 and power of 0.80, we calculated that the required sample size in the experimental groups were 216. In this trial, there are 60 people in healthy control group and 108 people in each experimental group across the three centers.

## Quality control

During the trial, quality control will be carried out by the steering committee. All researchers are required to attend training on trial methods, techniques, and protocol regulations in order to maintain the consistency and validity of the data. Any modifications or corrections required should be discussed, decided upon and submitted to the steering and ethics committees. In addition, details should be kept through the trial.

## Discussion

PCOS is defined as a syndrome which combines various signs and symptoms, such as androgen excess, ovarian dysfunction, and polycystic ovarian morphology. Considering its high prevalence among reproductive women in China, and given that its complications may lead to endometriosis, endometrial

cancer, and some malignant diseases, it might be one of the most common and severe endocrine and metabolic disorders.

Currently, the diagnosis of PCOS is still based on the exclusion of other diseases<sup>[24-29]</sup>, which includes thyroid dysfunction, non-classical congenital hyperplasia, and androgen secreting tumors. Additionally, it is recognized that there are several phenotypes of PCOS which present with distinct characteristics and such patient should receive individualized treatment<sup>[1]</sup>. However, with the lack of classification criteria, it is very difficult for doctors to make a precise diagnosis and develop a suitable treatment strategy.

Of note, researchers have made attempts to determine whether there are some biomarkers which could be used to screen as predictive measures for the different PCOS phenotypes.

Zhang L et al.<sup>[30]</sup> found that the phenotype with hyperandrogenism had a higher homeostasis model assessment of insulin resistance (HOMA-IR) than other phenotypes (without androgen excess). Further detection showed that serum irisin levels are associated with hyperandrogenism, but not with oligo-anovulation or PCO morphology. Additionally, Minoee S et al.<sup>[31]</sup> pointed out that the testosterone-to-androstenedione ratio was associated with the insulin resistance (sensitivity 0.83, specificity 0.42) and metabolic syndrome (MetS) (sensitivity 0.85, specificity 0.70) among PCOS patients, indicating the strong connection between insulin resistance and hyperandrogenism. This might assist clinicians and researchers to classify the specific phenotypes of PCOS. However, Božić-Antić I et al.<sup>[32]</sup> designed a cross-sectional clinical study of 365 women with PCOS and 125 healthy controls in a Caucasian population. In this study, PCOS patients were divided into groups based on four phenotypes according to the European Society of Human Reproduction and Embryology (ESHRE) /American Society for Reproductive Medicine (ASRM) criteria<sup>[33]</sup>. Their findings demonstrated that all phenotypes had the same prevalence of MetS, with different cardiovascular risk, particularly phenotype B. The difference might partly be related to the epigenetic and environmental influences. Moreover, the free androgen index (FAI)<sup>[34]</sup>, serum anti-Müllerian hormone (AMH) level<sup>[35-38]</sup>, PPARGC1A (a leukocyte methylation promoter)<sup>[39]</sup>, and some other indexes were also found to be potential biomarkers for the prediction in women with PCOS and the classification of its phenotypes. Although many efforts are investing in changing the predicament surrounding clinical diagnosis, the precise classification criteria have yet to be developed. However, there has been a turning point in detection methodology in accordance with the advent of metabolomics.

As a result, this trial was designed to explore a more specific and sensitive method to determine the subtypes of PCOS. Based on the preliminary study, our team found that clinical indicators such as LH/FSH, ApoA1, AMH, and INS levels have a good correlation with the metabolomic results. Such findings indicate the potential to develop a diagnosis and therapeutic strategy based on an integrated biomarker system.

To investigate this further, we will conduct a prospective cohort study, expanding the sample size of PCOS patients and adding the detection frequencies. This study is a multi-center, randomized, open label and blinded clinical study, aiming to standardize the classification diagnosis and precise treatment of

PCOS. The sensitivity and specificity of integration with metabolomic indexes will be compared using clinical indexes. It will help to improve the empirical classification, find the predictive biomarkers, and hopefully, with integration of some of the metabolomic indexes, it may assist in establishing an integrated biomarker system which will develop precise diagnosis and treatment strategies for different PCOS subgroups in the future.

## **Study limitations**

The main difficulty inherent to this trial is to make consistent diagnosis of group 1 because the diagnosis is made by several different doctors. To avoid the influence as much as possible, our group will organize a diagnosis team which will consist of three professional doctors who have majored in endocrinology and metabolism or gynecological endocrinology for at least 10 years and achieved the position of assistant director physician. Once the clinical research doctors cannot make their diagnosis of the participants' subtype, the case will be submitted to the team to provide the final diagnosis. Another methodological difficulty is to ensure that the usage of treatment is in accordance with the scheme. In this trial, we will ensure that medication reminders are sent and participants who miss the treatment for a continuous 7 days will be excluded from the trial.

## **Trial Status**

The protocol for this trial is version number 2.0. Recruitment was initiated in February, 2019 and is anticipated to be completed in August, 2019. This trial is now in the period of screening and some participants are undergoing observation. Ethical approval was granted in 2018, and the study is expected to be completed at the end of December 2019.

## **List Of Abbreviations**

AE: Adverse event; PCOS: The polycystic ovary syndrome; CRF: Case report form; FAS: Full analysis set; RCT: Randomized controlled trial; SPSS: Statistical Package for Social Sciences.

## **Declarations**

## **Ethics approval and consent to participate**

Central ethical approval has been confirmed from the ethics committee of Wuxi People's Hospital, which is affiliated with Nanjing Medical University (ref approval no. 2017-IIT-08-01) and we will not begin recruiting at other centres in the trial until local ethical approval has been obtained. Informed consent will be requested in writing before participating the trial and this issue will be in charge of the clinical trial communicator.

# Consent for publication

Not Applicable.

## Availability of data and materials

The datasets analysed during the current study will be available from the corresponding author upon reasonable request. And the way of sharing it (include metadata and protocol) will through clinical trial management public platform( <http://www.medresman.org>). The member of the research team, such as the sponsor, Qing He and Lan Xu, the core member of the team, such as Chengming Ni and Xuemei Fan will have the eligibility to write the papers relevant to this trial. And we don not intend to use any professional writers.

## Competing interests

The authors declare that they have no competing interests. There is no financial and other competing interests for principal investigators for the overall trial and each study site.

## Funding

This study is supported by the Science and Technology Development Fund of Wuxi (Z201807). The funders had no role in the design or conduct of the trial.

## Authors' contributions

CN and WH participated in planning the study protocol and drafted the manuscript.

YJ and JX participated in designing the outcome measurements and assessing the outcomes.

YZ participated in assessing treatment efficacy.

XZ participated in recruiting and screening eligible patients in the outpatient department.

YL was responsible for generating and distributing the random numbers.

RD was responsible for monitoring the data during the trial.

XF, GL and YW were responsible for the detection and analysis of metabolomics.

LX and QH participated in designing the trial and the critical revision of the manuscript. They are the sponsor of the trial and they will have ultimate authority over the trial.

All the authors have read and approved the final manuscript.

## Acknowledgements

We thank Master.Lu Yu, Chao Liu, Xuezhi Lv ( The Affiliated Wuxi People's Hospital of Nanjing Medical University) for their helpful advice to this work.

## References

- 1.Chinese Medical Doctor Association of Endocrine and Metabolic diseases. Consensus of Chinese endocrinologists for the diagnosis and treatment of polycystic ovary syndrome. *Chin J Endocrine Metabolism.* (2018),34(1):1–7.
- 2.Conway G., D. Dewailly, E. Diamanti-Kandarakis, et al.The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. *Eur J Endocrinol* (2014), 171(4): P1–29.
- 3.Fan X., J. Jiang, Z. Huang, et al. UPLC/QTOFMS based plasma metabolomics and clinical characteristics of polycystic ovarian syndrome. *Mol Med Rep* (2019), 19(1): 280–292.
- 4.Marcondes R. R., M. Maliqueo, R. Fornes, et al. Exercise differentially affects metabolic functions and white adipose tissue in female letrozole- and dihydrotestosterone-induced mouse models of polycystic ovary syndrome. *Mol Cell Endocrinol* (2017), 448: 66–76.
- 5.Zhai K. L., G. Zhuo, H. B. Chi and Z. Lan. Comparisons of prevalence and clinical and environmental characteristics between Tibetan and Han Women with polycystic ovarian syndrome in Tibetan Plateau. *Zhonghua Yi Xue Za Zhi* (2017), 97(37): 2928–2931.
- 6.Lazaridou S., K. Dinas and K. Tziomalos. Prevalence, pathogenesis and management of prediabetes and type 2 diabetes mellitus in patients with polycystic ovary syndrome. *Hormones (Athens)* (2017), 16(4): 373–380.
- 7.Boyle J. A., J. Cunningham, R. J. Norman, et al. Polycystic ovary syndrome and metabolic syndrome in Indigenous Australian women. *Intern Med J*(2015), 45(12): 1247–1254.
- 8.Hillman J. K., L. N. Johnson, M. Limaye, et al. Black women with polycystic ovary syndrome (PCOS) have increased risk for metabolic syndrome and cardiovascular disease compared with white women with PCOS. *Fertil Steril* (2014), 101(2): 530–535.
- 9.Ranasinha S., A. E. Joham, R. J. Norman, et al. The association between Polycystic Ovary Syndrome (PCOS) and metabolic syndrome: a statistical modelling approach. *Clin Endocrinol (Oxf)* (2015), 83(6):

879–887.

- 10.Lim, S. S., R. J. Norman, M. J. Davies and L. J. Moran. The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. *Obes Rev* (2013), 14(2): 95–109.
- 11.Htet T. D., H. J. Teede, B. de Courten, et al. Asthma in reproductive-aged women with polycystic ovary syndrome and association with obesity. *Eur Respir J* (2017), 49(5).
- 12.Kumarendran B., M. W. O'Reilly, K. N. Manolopoulos, et al. Polycystic ovary syndrome, androgen excess, and the risk of nonalcoholic fatty liver disease in women: A longitudinal study based on a United Kingdom primary care database. *PLoS Med* (2018), 15(3): e1002542.
- 13.Damone A. L., A. E. Joham, D. Loxton, et al. Depression, anxiety and perceived stress in women with and without PCOS: a community-based study. *Psychol Med*(2018), 1–11.
- 14.Kogure G. S., V. B. Ribeiro, I. P. Lopes, et al. Body image and its relationships with sexual functioning, anxiety, and depression in women with polycystic ovary syndrome. *J Affect Disord* (2019), 253: 385–393.
- 15.Lizneva D., L. Suturina, W. Walker, et al. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. *Fertil Steril* (2016), 106(1): 6–15.
- 16.Huang C. C., Y. J. Tien, M. J. Chen, et al. Symptom patterns and phenotypic subgrouping of women with polycystic ovary syndrome: association between endocrine characteristics and metabolic aberrations. *Hum Reprod* (2015), 30(4): 937–946.
- 17.Torchen L. C., R. S. Legro and A. Dunaif. Distinctive Reproductive Phenotypes in Peripubertal Girls at Risk for Polycystic Ovary Syndrome. *J Clin Endocrinol Metab*(2019).
- 18.Castiglione Morelli, M. A., A. Iuliano, S. C. A. Schettini, et al. NMR metabolic profiling of follicular fluid for investigating the different causes of female infertility: a pilot study. *Metabolomics* (2019), 15(2): 19.
- 19.Li J., L. M. Xie, J. L. Song, et al. Alterations of Sphingolipid Metabolism in Different Types of Polycystic Ovary Syndrome. *Sci Rep* (2019), 9(1): 3204.
- 20.Gent R., T. du Toit, L. M. Bloem and A. C. Swart. The 11beta-hydroxysteroid dehydrogenase isoforms: pivotal catalytic activities yield potent C11-oxy C19 steroids with 11betaHSD2 favouring 11-ketotestosterone, 11-ketoandrostenedione and 11-ketoprogesterone biosynthesis. *J Steroid Biochem Mol Biol* (2019), 189: 116–126.
- 21.Zhang B., S. Shen, T. Gu, et al. Increased circulating conjugated primary bile acids are associated with hyperandrogenism in women with polycystic ovary syndrome. *The Journal of Steroid Biochemistry and Molecular Biology* (2019), 189: 171–175.

22. Rotterdam, Eshre Asrm-Sponsored Pcos Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* (2004), 81(1): 19–25.
23. 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(1): 41–47.
24. Kyritsi E. M., G. K. Dimitriadis, I. Kyrou, et al. PCOS remains a diagnosis of exclusion: a concise review of key endocrinopathies to exclude. *Clin Endocrinol (Oxf)* (2017), 86(1): 1–6.
25. Azziz R., E. Carmina, Z. Chen, et al. Polycystic ovary syndrome. *Nat Rev Dis Primers* (2016), 2: 16057.
26. Azziz, R., E. Carmina, D. Dewailly, et al. Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen and P. Society. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril* (2009), 91(2): 456–488.
27. Bozdag G., S. Mumusoglu, D. Zengin, et al. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod* (2016), 31(12): 2841–2855.
28. Lie Fong, S. J. S. E. Laven, A. Duhamel and D. Dewailly. Polycystic ovarian morphology and the diagnosis of polycystic ovary syndrome: redefining threshold levels for follicle count and serum anti-Mullerian hormone using cluster analysis. *Hum Reprod* (2017), 32(8): 1723–1731.
29. Stankiewicz, M. and R. Norman. Diagnosis and management of polycystic ovary syndrome: a practical guide. *Drugs*(2006), 66(7): 903–912.
30. Zhang L., X. Fang, L. Li, et al. The association between circulating irisin levels and different phenotypes of polycystic ovary syndrome. *J Endocrinol Invest* (2018), 41(12): 1401–1407.
31. Minooe S., F. Ramezani Tehrani, M. Tohidi and F. Azizi. Role of androgen ratios in the prediction of the metabolic phenotype in polycystic ovary syndrome. *Int J Gynaecol Obstet* (2017), 137(2): 110–115.
32. Bozic-Antic I., D. Ilic, J. Bjekic-Macut, et al. Lipid accumulation product as a marker of cardiometabolic susceptibility in women with different phenotypes of polycystic ovary syndrome. *Eur J Endocrinol* (2016), 175(6): 551–560.
33. Fauser B.C., B.C. Tarlatzis, R. W. Rebar, et al. Consensus on women’s health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril* (2012), 97(1): 28–38 e25.
34. Ozga K. M., M. Krzyczkowska-Sendrakowska, A. Hubalewska-Dydejczyk, et al. The value of Free Androgen Index depends on the phenotype of polycystic ovary syndrome, one centre experience. *Endokrynol Pol.*(2019).

35. Romualdi D., C. Di Florio, V. Tagliaferri, et al. The Role of Anti-Mullerian Hormone in the Characterization of the Different Polycystic Ovary Syndrome Phenotypes. *Reprod Sci* (2016), 23(5): 655–661.
36. Song D. K., J. Y. Oh, H. Lee and Y. A. Sung. Differentiation between polycystic ovary syndrome and polycystic ovarian morphology by means of an anti-Mullerian hormone cutoff value. *Korean J Intern Med* (2017), 32(4): 690–698.
37. Tal R., D. B. Seifer, M. Khanimov, et al. Characterization of women with elevated antimullerian hormone levels (AMH): correlation of AMH with polycystic ovarian syndrome phenotypes and assisted reproductive technology outcomes. *Am J Obstet Gynecol* (2014), 211(1): 59 e51–58.
38. Gupta M., R. Yadav, R. Mahey, et al. Correlation of body mass index (BMI), anti-mullerian hormone (AMH), and insulin resistance among different polycystic ovary syndrome (PCOS) phenotypes - a cross-sectional study. *Gynecol Endocrinol* (2019), 1–4.
39. Zhao H., Y. Zhao, Y. Ren, et al. Epigenetic regulation of an adverse metabolic phenotype in polycystic ovary syndrome: the impact of the leukocyte methylation of PPARGC1A promoter. *Fertil Steril* (2017), 107(2): 467–474 e465.

## Figures

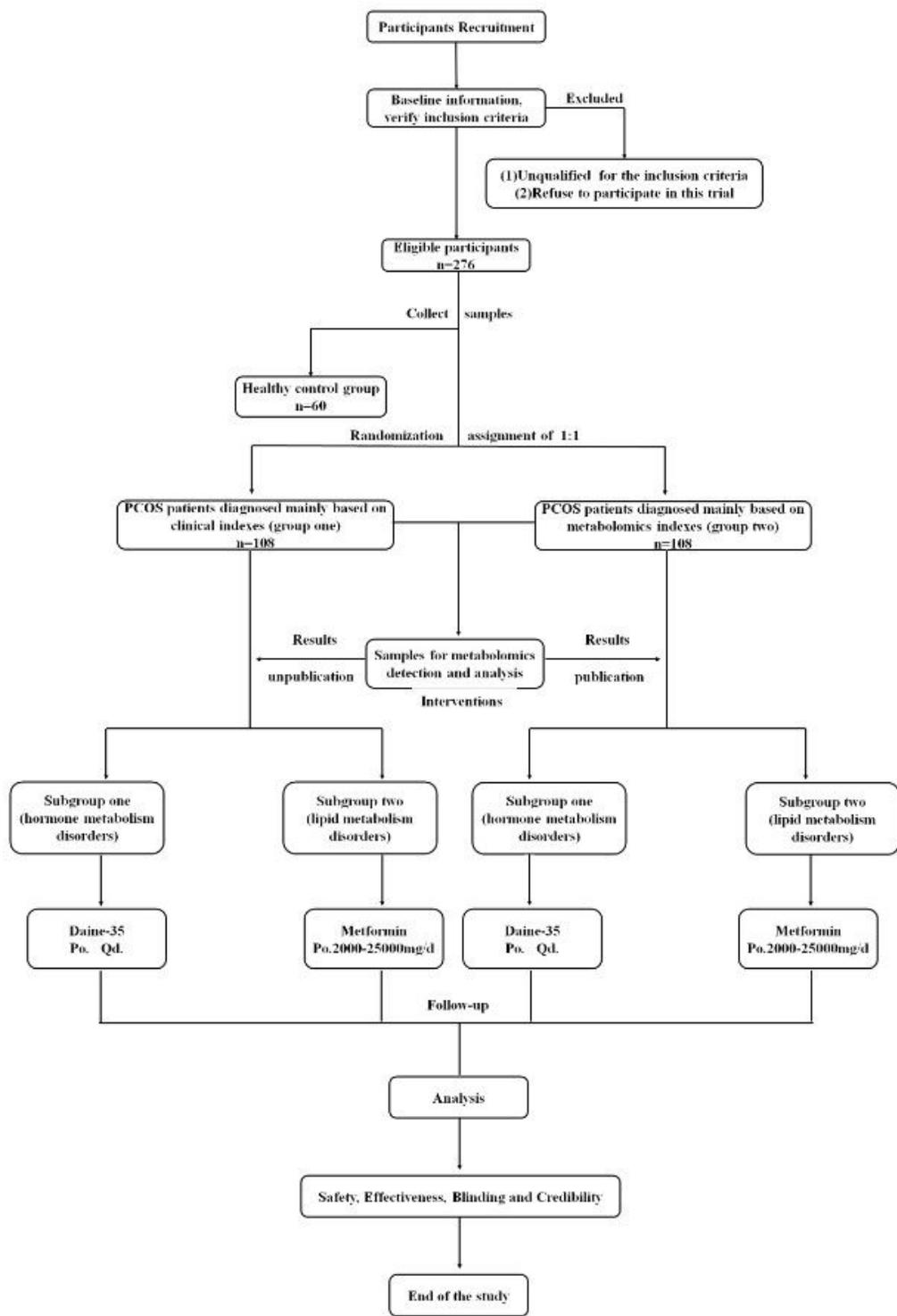


Figure 1

Timeline (Designed for group one and group two)

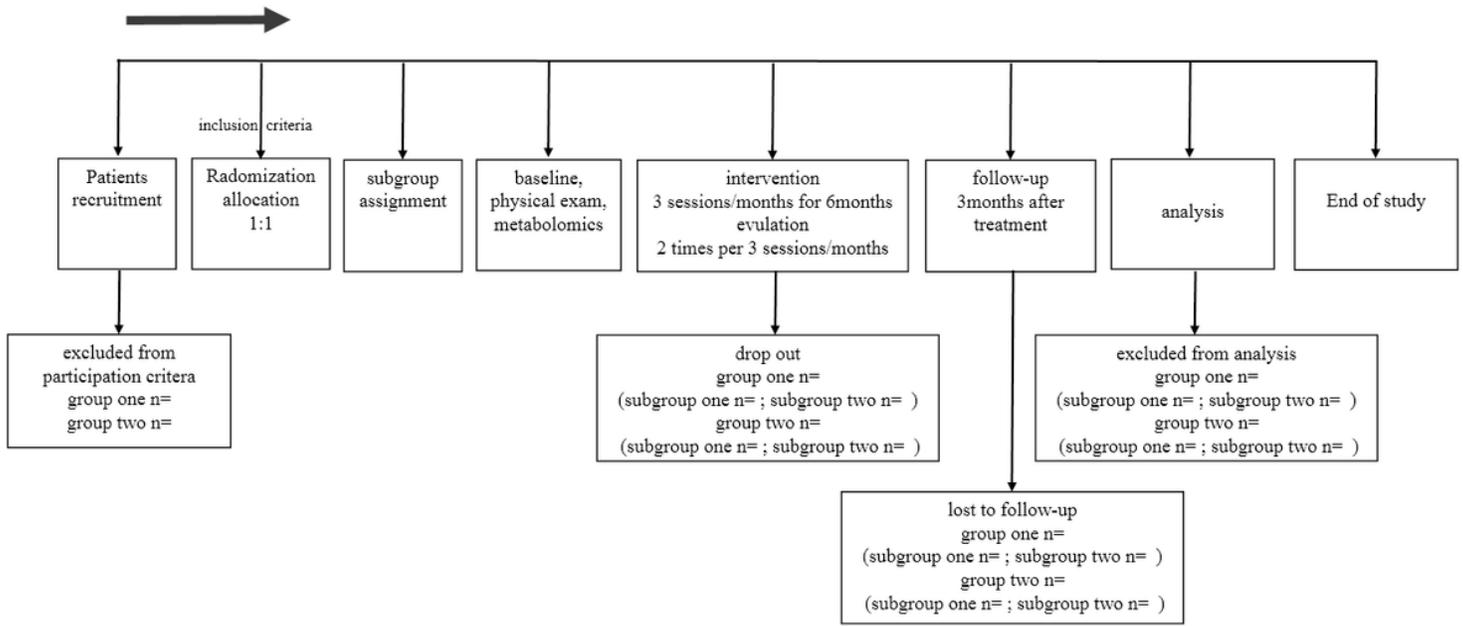


Figure 2

time	W-6-W0	W0	W04 +1	W15 +1	W21 +1	W26 +1	W30 +1	W34 +1	W38 +1	W41 +1	
period	Screening	Baseline	Intervention				Follow-up				
patients											
Eligibility	x										
Demography	x										
Informed consent	x										
Sign the informed consent		x									
Medical history	x										
Physical examination	x			x		x					
Metabolomics detection		x		x		x					
Randomization		x									
Inclusion criteria		x									
Intervention											
healthy control group(n=60)	x	x	Physical examination and Metabolomics detection								
Diagnosed based on metabolomics indicators (n=108)			Diane-35 po.qd (constant 21days, pause 7days)								
			Metformin po.2000-2500mg/d								
Diagnosed based on clinical indicators(n=108)			Diane-35 po.qd (constant 21days, pause 7days)								
			Metformin po.2000-2500mg/d								
Outcomes											
Menstrual period record	x	x		x		x	x	x	x	x	
Pregnancy registry	x	x		x		x	x	x	x	x	
Metabolism assessment	x	x		x		x	x	x	x	x	
Trial evaluation											
Patient's compliance			x	x	x	x	x	x	x	x	
Blinding test			x	x	x	x	x	x	x	x	
Credibility test			x	x	x	x	x	x	x	x	
Safety evaluation			x	x	x	x	x	x	x	x	
Adverse events			x	x	x	x	x	x	x	x	
Analysis	x	x	x	x	x	x	x	x	x	x	

Physical examination includes: ultrasonography of bilateral ovarian and clinical biochemical indicators.

Clinical biochemical indicators includes routine urine tests, routine blood tests, liver function, pregnancy tests, LH/FSH ratio, sex hormone related indicators, blood lipid related indicators and endocrine related indicators.

There were twenty-five clinical biochemical indexes including eight sex hormone-related indexes: FSH, LH, PRL, E2, P, Ts, AMH and VitD; nine blood lipid related indicators: TC, TG, HDL, LDL, ApoA1, Apo B, Lp(a), FFA, hsCRP; seven endocrine-related indicators: GLU (0h, 30min, 120min), INS (0h, 30min, 120min), BUA. The main evaluation index was LH/FSH ratio.

Note: ultrasonography examination is scheduled for the 14th day of menstruation, if the patient with rare ovulation has follicular diameter >10mm or luteal appearance, should be reviewed in the subsequent menstrual cycle. Unmarried women without sexual life suggested abdominal B ultra. All blood samples will be collected on an empty stomach within 72 hours from the first day of menstruation.

Figure 3

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Biologicalspecimens.pdf](#)

- [SPIRITChecklist.doc](#)
- [Informedconsentmaterials.docx](#)