

**The Efficacy of Tung's Acupuncture for Sex Hormones in  
Polycystic Ovary Syndrome: A Randomized Controlled Trial**

**Study Protocol and Statistical Analysis Plan**

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# Study Protocol and Statistical Analysis Plan

## Study design

The study was a randomized, drug-controlled, parallel group trial to determine the efficacy and safety of Tung's acupuncture for sex hormones compared with the oral contraceptive (CPA/EE) in patients with PCOS. The trial was performed at the Acupuncture and Gynecology Department of Beijing Hospital of Integrated Traditional Chinese and Western Medicine. Participants were recruited through advertisements on local newspapers and posters and signed informed consent before study participation. The study lasted 28 weeks: a 1-4weeks baseline period, 12-week treatment period, and a 12-week follow-up period.

## Participants

PCOS was diagnosed according to the Rotterdam criteria<sup>1</sup>: ultrasound-verified polycystic ovaries ( $\geq 12$  follicles of 2–9 mm and/or ovarian volume  $\geq 10$  ml in one or both ovaries), oligo/amenorrhea, or clinical signs of hyperandrogenism (hirsutism or acne). Participants were considered eligible upon meeting the following conditions: 1) 18-45 years old; 2) met the diagnostic criteria for polycystic ovary syndrome, and no reproductive requirement in the six months; 3) volunteered to join this research and give informed consent prior to receiving treatment.

The exclusion criteria included: 1) hypertensive patients with blood pressure exceed 160/100 mmHg; 2) patients with a history of thrombosis, cerebrovascular disease, cancer, liver disease or liver dysfunction, hyperlipidemia, mental disorder and severe infection; 3) had taken any pharmacological treatments affecting reproductive endocrine system, or received acupuncture in the previous 3 months; 4) smoked more than 15 cigarettes a day; 5) patients with pacemakers, metal allergies or severe fear of acupuncture.

## Randomization and blinding

Eligible participants were randomly allocated into either acupuncture group or CPA/EE group in a ratio of 1:1 by a computer-generated, randomly location sequence (random list generated with SPSS 13.0). The random number and group assigned were sealed in the envelope. Randomization was performed by an independent administrator who had no other role in this study. All other individuals involved in the study including patients, practitioners, and outcome assessors were blinded to the randomization procedure. In this study, physicians could not be blinded due to the nature of the intervention. The acupuncturists had to know the group assignment because of manipulation. Data collection was performed by 2 blinded evaluators. Data entry and analysis was undertaken blind to the study group. Apart from the differences in treatment methods between the two groups, all subjects were treated as equally as possible.

## Treatment protocol

Acupuncture group: the acupuncture group received acupuncture treatments twice per week for 12 weeks with a total of 24 times. Huatuo brand needles (size 0.20mm $\times$ 25mm, 0.20mm $\times$ 40mm, manufactured by Suzhou Medical Appliance, Suzhou, Jiangsu Province, China) together with SDZ-V Electro-Acu Stimulators (Suzhou Medical Appliance, Suzhou, Jiangsu

Province, China) were used. The study took Tung's acupoints as main acupuncture points, which were Fuke, Huanchao, Tianhuang, Renhuang, as well as the traditional acupoints Guanyuan (CV4) and Zigong (EX-CA1). The location of these acupuncture points referred to *Tung's Acupuncture*<sup>12</sup>. Fuke point (Fig 1) is on the ulnar side of the proximal joint of thumb. There are two points up and down, 1/3 of the distance away from the interphalangeal joint. Huanchao point is on the middle of the central joint, on the ulnar side of ring finger. Tianhuang point is at the same location of Yin Lingquan (SP9), on the medial side of the shank, on the lower border of the medial condyle of the tibia. Renhuang point is at the same location of San Yinjiao (SP6), on the medial side of the shank, 3 cun directly above the tip of the medial malleolous, on the posterior border of the medial aspect of the tibia. The specific manipulation was as follows: Fuke were inserted vertically closed to the bone (depth, 7.5mm) with 0.20 mm×25mm needles, Huanchao was inserted vertically closed to the bone (depth, 5-7.5mm) with 0.20 mm×25mm needle, the bilateral two points alternately used (left Fuke with right Huanchao, right Fuke with left Huanchao). Bilateral Tianhuangs, Renhuangs were inserted vertically with a depth of 20-30mm, and stimulated manually by rotating the needle to evoke de qi (defined as numbness, heaviness, pressure, soreness, or tingling). The insertion of CV4 and EX-CA1 was straight to the abdominal muscle until the needle sensation was achieved. Bilateral EX-CA1s were connected to an electrical stimulator with a continuous wave of 20Hz, and slowly increased the current intensity to a slight tremor in the abdomen. At the same time a moxa box was administered via smokeless moxa stick on the lower abdomen for 30minutes.

CPA/EE group: CPA/EE (Diane-35, Bayer Healthcare Co. Ltd) was taken orally from the 8th day of menstrual cycle or any day for patients with amenorrhea, one tablet per day, and the pill was administered for 21 days consecutively. The patients then stopped taking the pill for seven days and on the eighth day, the patients began the pill again for three menstrual cycles (28 day cycles). Patients were asked to visit the doctor every menstrual cycle, in order to receive the medicine of next cycle and communicate with the doctor about their physical condition, or the patients were required to visit the doctors if they feel any discomfort.

## Outcome measurements

The change in LH/FSH ratio from baseline at end of treatment was considered as the primary outcome in this study, and secondary outcomes include the changes in BMI, FSH, LH, TT, ovarian volume, polycystic ovary number and menstrual frequency at week 12 and 24 from baseline. The blood samples were measured at the 2-3rd Day of menstrual cycle during the baseline period, after the last treatment and 12-week follow-up period. If no ovulation or bleeding had occurred during the period, the measurements were taken one week after the end of treatment and 12-week follow-up period. The sex hormones were analyzed by chemiluminescent microparticle immunoassay at the medical laboratory of Beijing Hospital of Integrated Traditional Chinese and Western Medicine.

The polycystic ovary number and volume were measured by ultrasound, which were together with BMI and menstrual frequency taken 3-7th days after menstruation during the baseline period, after the last treatment and 12-week follow-up period. The polycystic ovary number was defined as the number of ovaries with cysts/follicles (>12 cysts), the ovarian volume was measured in three dimensions by an experienced sonographer who was blinded to the group.

Safety evaluation included acupuncture related adversary events such as hematoma, fainting, severe pain, post-treatment soreness and local infection during and after acupuncture, as well as

CPA/EE related adversary events like vomiting, nausea, headache and skin reactions during taking pills.

### Sample size

This trial was a difference test design, the primary outcome measure was the change of the ratio of LH/FSH from baseline at the end of treatment. Based on the results of our pilot study<sup>2</sup> and previous studies<sup>3,4</sup>, we anticipated a mean difference in the ratio of LH/FSH was 1.2 (SD, 0.8) in CPA/EE group, and 1.68 (SD, 0.2) in Tung's acupuncture group. Sample size was determined with a 0.05-probability of a type I error and 80% power, 2 sided test with a 20% attrition rate. 30 patients will be needed for each group, which implied a total sample size of 60 participants for the entire trial.

### Statistical analysis

Statistical analysis performed using SPSS 13.0 statistics software (SPSS Inc, Chicago, IL, USA) and the statistician was blinded from group allocation. The Kolmogorov-Smirnov test was used to test the normal distribution of continuous variables. Continuous variables were presented by mean (standard deviation) if they were normally distributed or by median with interquartile range if they are not normally distributed. The modified intention-to-treat (ITT) approach was used to analyze the study group, all subjects with at least having one treatment data in randomized groups were included. A paired samples t test or Wilcoxon Matched-Pairs SignedRanks test was examined to compare the differences between the quantitative indices before and after treatment in one group, while an independent sample t test or Mann-Whiney U test was used for comparison between groups. The entire statistical test used bilateral examination and estimation of the median with 95% CI was calculated.

### References

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