

# Effect of acupuncture for diminished ovarian reserve: study protocol for a randomized controlled trial

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## Study protocol

**Keywords:** Diminished ovarian reserve, Acupuncture, Randomized controlled trial, Study protocol

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# Abstract

**Background:** Diminished ovarian reserve (DOR) is described as reduced fecundity. A satisfactory treatment is lacking despite the upward trend of the prevalence. Acupuncture has been reported as an alternative therapy for female infertility. The purpose of this study is to investigate the effect of acupuncture for women with DOR.

**Methods/Design:** In this randomized controlled trial, a total of 120 women with DOR will be randomized to receive either acupuncture or sham acupuncture for 12 weeks. The primary outcome is the antral follicle counting (AFC).

**Discussion:** This study is expected to investigate the effect of acupuncture versus superficial needling in improving ovarian reserve for women with DOR.

**Trial registration:** Acupuncture-Moxibustion Clinical Trial Registry (ChiCTR1800014988).

## 1. Background

Decreased or diminished ovarian reserve (DOR) describes women of reproductive age having regular menses whose response to ovarian stimulation is reduced compared with women of comparable age[1]. Currently, there is no uniformly accepted definition of DOR. The term DOR may refer to three related but distinctly different outcomes: oocyte quality, oocyte quantity, and reproductive potential. Ten percent of women (totaling 275,000) in an infertility clinic of USA were diagnosed with DOR[2,3]. Data from the US-based national Society for Assisted Reproductive Technology system showed 32% of in vitro fertilization (IVF) cycles (approximately 66,000 cycles) carried a diagnosis of DOR[4]. Worse yet, a significant upward trend in the prevalence of DOR was noted in patients up to 40 years of age[5,6]. Without timely and effective interventions, women of DOR may witness their ovarian gradually atrophy within 1 to 6 years, and their disease progress to primary ovarian insufficiency (POI) simultaneously[7].

However, only few modalities of treatment have been reported for DOR. The commonly used hormone replacement therapy (HRT)[8] is mainly for DOR women without birth demand. HRT is effective for relieving low estrogen symptoms but with a high recurrence rate after drug discontinuation and does not improve ovarian function. An international survey[9] indicated DOR patients respond poorly to controlled ovarian stimulation (COS) resulting in retrieval of fewer oocytes, producing poorer quality embryos, and reduced implantation rates and pregnancy rates. Some studies suggested the addition of growth hormone (GH)[10] during ovarian stimulation could enhance the response of granulosa cells to gonadotropins, and dehydroepiandrosterone (DHEA)[11] could improve ovarian function. However, there is insufficient evidence to support their effect. Thus, effective methods for DOR are still needed to improve female reproductive health and the quality of their life.

Acupuncture has been used for treating female infertility for thousands of years in China. A prospective observational study[12] reported significant decrease of follicle-stimulating hormone (FSH) and

Luteinizing hormone (LH) levels, and increase of Estradiol (E2) level among DOR patients treated with acupuncture. Similar results were observed in randomized controlled trials (RCTs) for women with POI[13], a condition that is more serious decline of ovarian function than DOR. Nowadays, acupuncture has gained worldwide popularity as an adjunct to assisted reproductive technology. A higher clinical pregnancy rate was reported in patients receiving acupuncture prior to and post-embryo transfer during IVF-ET cycles[14,15]. In this trial, a prospective randomized controlled study will be conducted to investigate the effect of acupuncture for women with DOR.

## 2. Study Design And Methods

### 2.1. Study design

This is a prospective, single-center, randomized, sham-controlled trial. Participants will be assigned to receive acupuncture or sham acupuncture in a 1:1 ratio. This study is in accordance with the principles of the Declaration of Helsinki and has been approved by the ethics committee of the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medicine Sciences (CACMS) (Approval number: SC2017-12-22-1-1). Written informed consent will be obtained from each participant before enrolment.

Total observation period will be 32 weeks, including a 8-week baseline period (week -8-0), a 12-week treatment period (weeks 0-12), and a 12-week follow-up period (weeks 13-24). Assessments will be conducted at baseline, week 12, and week 24. Flowchart and study design schedule are presented in Figure 1 and Table 1, respectively.

**Table 1. Study schedule of enrolment, intervention and assessments.**

Time point	Screening period (data collection) Weeks-4 to 0	Intervention period (weeks 1-12 )	Follow-up period (weeks 13-24)
Enrolment			
Informed consent	×		
Eligibility screen	×		
Randomization	×		
Allocation	×		
Interventions			
Acupuncture Group		×	
Control Group		×	
Assessments			
AFC	×	×	×
serum levels of FSH, LH, and E2	×	×	×
AMH	×	×	×
length of menstrual cycle	×	×	×
SAS score	×	×	×
Safety			
Adverse events	×	×	×

**Figure legends** AFC, antral follicle count; AMH, anti-müllerian hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estradiol; SAS, self-rating anxiety scale.

## 2.2. Participants

A total of 120 patients with DOR will be recruited from outpatients Acupuncture and moxibustion hospital of CACMS via hospital poster, Wechat, or network advertisement. ALL patients will be informed of details of the study such as the objectives, interventions, potential benefits and risks, etc.

## 2.3. Inclusion criteria

Participants who meet the following inclusion criteria will be included: (1) female, aged 18-40 years; (2) meet any of the following three items in both tests with an interval of at least 4 weeks: a,  $10 \text{ IU/L} < \text{FSH} < 20 \text{ IU/L}$ , tested on day 2-4 of menstrual cycle; b, Antimüllerian hormone (AMH)  $< 1.1 \text{ ng/mL}$ ; c, antral

follicle count (AFC) < 5–7 , tested on day 2-4 of menstrual cycle; (4) voluntarily join the research and sign the informed consent.

## 2.4. Exclusion criteria

Participants meeting any of the following criteria will be excluded: (1) polycystic ovary syndrome, hyperprolactinemia, hyperandrogen, hypothyroidism, and other endocrine diseases which may inhibit ovulation; (2) DOR caused by iatrogenic factors (such as pelvic surgery, radiotherapy or chemotherapy, uterine artery embolism, drugs such as hormones or immunosuppressants, etc.); (3) participants who have taken hormone drugs in recent three months, such as estrogen, contraceptive, glucocorticoids, etc.; (4) the score of self-rating anxiety scale (SAS) greater than 70; (5) serious cardiovascular, cerebral, liver or kidney diseases, cancer or psychiatric disease.

## 2.5. Randomization and blinding

This trial will use the central randomization system provided by the Institute of Basic Research in Clinical Medicine of CACMS. The randomization scheme and its related parameters protected by a strict viewing permission will be kept as blind code by a member of staff who is not involved in this study. Acupuncturists will be responsible for randomization. They will obtain a random number and group assignment after entering gender and birthday of eligible participants into the central randomization system over cellphone or web. Participants, outcome assessors, and statisticians will be blind to treatment allocation during the whole study. Acupuncturists will not take part in the outcome assessment.

# 3. Interventions

In this trial, *Hwato* disposable needles of different specifications (0.25×25 mm, 0.25×40 mm, and 0.30×75 mm) and SDZ-Ⅱ electroacupuncture (EA) apparatus (Suzhou Medical Appliance, Jiangsu, China) will be used. Interventions in both groups will be administered by three acupuncturists who are registered practitioners of traditional Chinese medicine (TCM) and have at least 3 years' clinical experience in acupuncture practice. Acupuncturists will receive training on study protocol and standard acupuncture manipulation before study initiation.

## 3.1. Acupuncture group

The protocol for this group is based on clinical experience of the corresponding author[16]. It consists of two groups of acupoints. Group 1 (in supine position): GV20 (Baihui), GV24 (Shenting), GB13 (Benshen), CV12 (Zhongwan), ST25 (Tianshu), CV4 (Guanyuan), KI12 (Dahe), EX-CA1 (Zigong), SP6 (Sanyinjiao), and LR3 (Taichong); and group 2 (in prone position): BL23 (Shenshu), BL33 (Zhongliao) and KI3 (Taixi). All the acupoints will be located according to the "WHO Standard Acupuncture Point Locations in the Western Pacific Region"[17]. These two groups of acupoints will be used alternatively as initial treatment. GV20, GV24, and GB13 will be needled with transverse insertion for 10 to 20 mm. LR3 will be inserted towards the direction of KI1 (Yongquan) for 10 to 20 mm. BL33 will be needled deeply into the third

posterior sacral foramina for 60 to 70 mm. Other acupoints will be needled perpendicularly for 30 to 40 mm. Each acupoint is required to obtain deqi sensation (soreness, numbness, distention, heaviness, etc), and if needed, needle manipulation techniques (like lifting, thrusting, or twirling the needle, etc) can be applied to promote qi arrival. For acupoints of group 2, paired alligator clips from the EA apparatus will be connected to homolateral BL23 and BL33 with a dilatational wave and a current intensity that patients can tolerate. Participants will receive 3 sessions of acupuncture treatment per week (ideally every other day) for 12 weeks. Each session will last for 20 minutes.

### **3.2. Control group**

In this trial, we will use superficial acupuncture in the same acupoints with the acupuncture group as sham control. All acupoints in this group will be needled into skin for approximately 1 to 3 mm with transverse insertion for GV20, GV24, and GB13 and perpendicular insertion for other acupoints. No manipulation will be administered after needle insertion to avoid deqi. When acupuncture on the acupoints of group 2, participants will be informed to receive minor EA stimulation. The EA parameters will be the same as in acupuncture group, but electricity output will be the smallest possible one that participant agrees, and the final output should not induce visible needle shivering. The duration and frequency of the control group will be the same as in the acupuncture group.

### **3.3. Permitted and prohibited concomitant treatments**

All participants will be treated separately to prevent communication between groups. Participants will be prohibited from taking any other medicine for DOR, such as sexual hormones, contraceptives, Chinese herbs, Chinese patent medicine, and so on. They will also be discouraged to start an IVF cycle during acupuncture treatment. Nonetheless, after completion of 4-week treatment, participants are allowed to terminate this trial for the initiation of an IVF cycle if required. For treatment or IVF cycles that participants have already taken, relevant information will be documented in detail.

## **4. Measurements**

### **4.1 Outcome measures**

The primary outcome is the change of AFC from baseline to week 12. Secondary outcomes are listed as follows.

- (1) Changes in serum basal FSH, FSH/LH, and E2 at week12 and week24 compared with baseline.
- (2) Change from baseline of AMH at week 12 and 24.
- (3) Change from baseline of AFC at week 24.
- (4) Change in the length of menstrual cycle at weeks 12 and 24. The length of menstrual cycle is divided into normal, antedated, or delayed menorrhoea.

(5) Change from baseline in the score of self-rating Anxiety Scale (SAS) at weeks 12 and 24.

SAS is a self-rating scale for measuring the presence and severity of anxiety[18].It consists of 20 items with 4 possible responses: (1) never; (2) rarely/sometimes; (3) frequently and (4) always. The standard score is the integer part of the raw score (the sum of the 20 items) multiplied by 1.25. A higher score denotes more serious anxiety symptoms. In this trial, we will use a validated Chinese version of SAS[19].

## **4.2 Adverse events**

All participants will be requested to report adverse events (AEs), i.e., any occurrence or worsening of an undesirable sign, symptom, or disease, whether or not related to acupuncture treatment.Acupuncturists will record AEs in detail including date, type, severity, measures taken by researchers, and causal relationship with the treatment, etc.Common acupuncture-related AEs include intolerable needling pain, hematoma, local infection, etc.

# **5. Statistical Considerations**

## **5.1. Sample size**

Results of our previous study on acupuncture for DOR[20] showed that the increase in mean AFC from baseline to week 12 was 2.25 in acupuncture group and 0.30 in wait list control. Based on these results, we assumed an increase of 0.8 in AFC for superficial acupuncture after 12-week treatment. A sample size of 50 participants per group will be needed to provide 90% power and a two-sided significance level of 5%, assuming a standard deviation of 2.22. Allowing for a 20% dropout rate, 120 participants will be recruited with 60 participants per group.

## **5.2. Statistical analysis**

SPSS version 20.0 (SPSS Inc., Chicago, USA) will be used for statistical analysis. According to the intention-to-treat (ITT) principle, statistical analysis will be conducted on all randomized subjects. Missing data will be imputed using multiple imputation method. Continuous data will be presented with mean and standard deviation, or median and interquartile range, while categorical data will be presented with number and percentage. For comparison with the baseline, a paired t-test or non-parametric test will be used for continuous data, and nonparametric test for categorical data. Comparisons between groups will be analyzed using the independent t-test or non-parametric test for continuous data, chi-square test or Fisher exact test for categorical data, and nonparametric test for ranked data. All statistical tests will be two-sided, and  $p < 0.05$  will be considered statistically significant.

## **5.3. Quality control**

The trial protocol has been repeatedly discussed by experts on acupuncture, gynecology, and reproductive medicine. Before the start of this study, all staff will be required to attend a series of training sessions. These sessions will ensure that all staff involved in this trial fully understand the study protocol, study

flow, individual responsibilities, standard operating procedures for acupuncture manipulation, central randomization, data management system, etc. Staff in charge of quality control will check on study progress (including participant recruitment, randomization, acupuncture manipulation, data entry, treatment compliance, document of adverse events, etc) once per month during the trial. Regular reminders via WeChat will be used to improve participant compliance.

## 6. Discussion

This trial is to reveal whether acupuncture is effective in improving ovarian reserve for women with DOR. Though observational studies[21] showed promising hormone changes and improvement of TCM symptoms, RCTs focusing on women of DOR is scarce. Available RCTs on DOR are limited by small sample sizes, heterogeneity among acupuncture protocols and control methods (usually drug control or wait list) and single ovarian reserve tests (usually sex hormones)[22,23]. In this trial, we will use superficial acupuncture as sham control. Although placebo is a better control to investigate the effect of acupuncture than superficial acupuncture, it is hard to conduct owing to the high recognition of acupuncture by patients in China and the complexity of acupuncture protocol in this trial. Given patients' strong desire and urgent need for treatment, superficial acupuncture seems to be a better control method than placebo or wait list, nevertheless its effect can't be ignored.

In assessment of ovarian reserve, basal levels of FSH, LH, and E2 are the most commonly used outcomes for DOR. According to the committee opinion of the American Society for Reproductive Medicine, there is emerging evidence to support the use of AMH and AFC as a screening test for poor ovarian response[24]. AMH, produced by granulosa cells of early follicles, reflects the size of the primordial oocyte pool. It is gonadotropin-independent, and thus remains relatively consistent within and between menstrual cycles. AFC correlates with both number of remaining follicles and ovarian response during COS. Compared with basal serum levels of FSH, LH, and E2, AMH and AFC are more significant predictors of poor ovarian response. Since no single measure of ovarian reserve has 100% sensitivity and specificity to detect DOR, biochemical and imaging measures (including FSH, LH and E2, AMH, and AFC) will be combined in this trial to assess the change of ovarian reserve.

This trial has several limitations. Superficial acupuncture instead of placebo is used as control, which may increase the probability of false negative results. Needling method of BL33 (a deep needling into the third posterior sacral foramina) used in acupuncture group is difficult to grasp for acupuncturists without technical training. Although most of the participants enter the trial for fertility, this trial will only focus on the evaluation of ovarian reserve. Clinical pregnancy rate or live birth rate will not be assessed due to limitation of short observation period.

In conclusion, results of this trial will show the effect of acupuncture versus superficial needling in improving ovarian reserve for women with DOR.

### **Trial status**

Inclusion of the first patient was on 3 November, 2017. Data collection is in progress. Trial is open for further inclusion. The trial ends at 3 years after inclusion of the last patient.(Protocol version V1.0-20170413)

## Abbreviations

DOR:Diminished ovarian reserve; COH: controlled ovarian hyperstimulation; IVF: in vitro fertilization; ART: assisted reproductive technology; IVF-ET: in vitro fertilization-embryo transfer; POI: premature ovarian insufficiency; FSH: follicle stimulating hormone; LH: luteinizing hormone; AMH: anti-müllerian hormone; E2: estradiol; AFC: antral follicle count;RCT: randomized controlled trial; SAS: self-rating anxiety scale; AEs: adverse events.

## Declarations

### *Ethics approval and consent to participate*

This trial will be performed in accordance with the Declaration of Helsinki and has been approved by Institute of acupuncture and moxibustion, China Academy of Chinese Medical Science (Ethics approval number 京SC2017-12-22-1-1). The findings of this trial will be submitted to a peer-reviewed journal. Written informed consent will be obtained from patients prior to enrolment.

### *Consent for publication*

Not applicable.

### *Availability of data and materials*

Not applicable.

### *Competing interests*

The authors declare that they have no competing interests.

### *Funding*

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### *Authors' contributions*

YGF conceived and designed the study. HFX and MZH drafted and substantially revised the manuscript. HSY prepared the informed consent and finished trial registration. YGF, HFX and LY will in charge of acupuncture treatment. YQY is in charge of participant recruitment and data entry. CSZ is responsible for outcome assessment. All authors have read and approved the submitted version of this manuscript.

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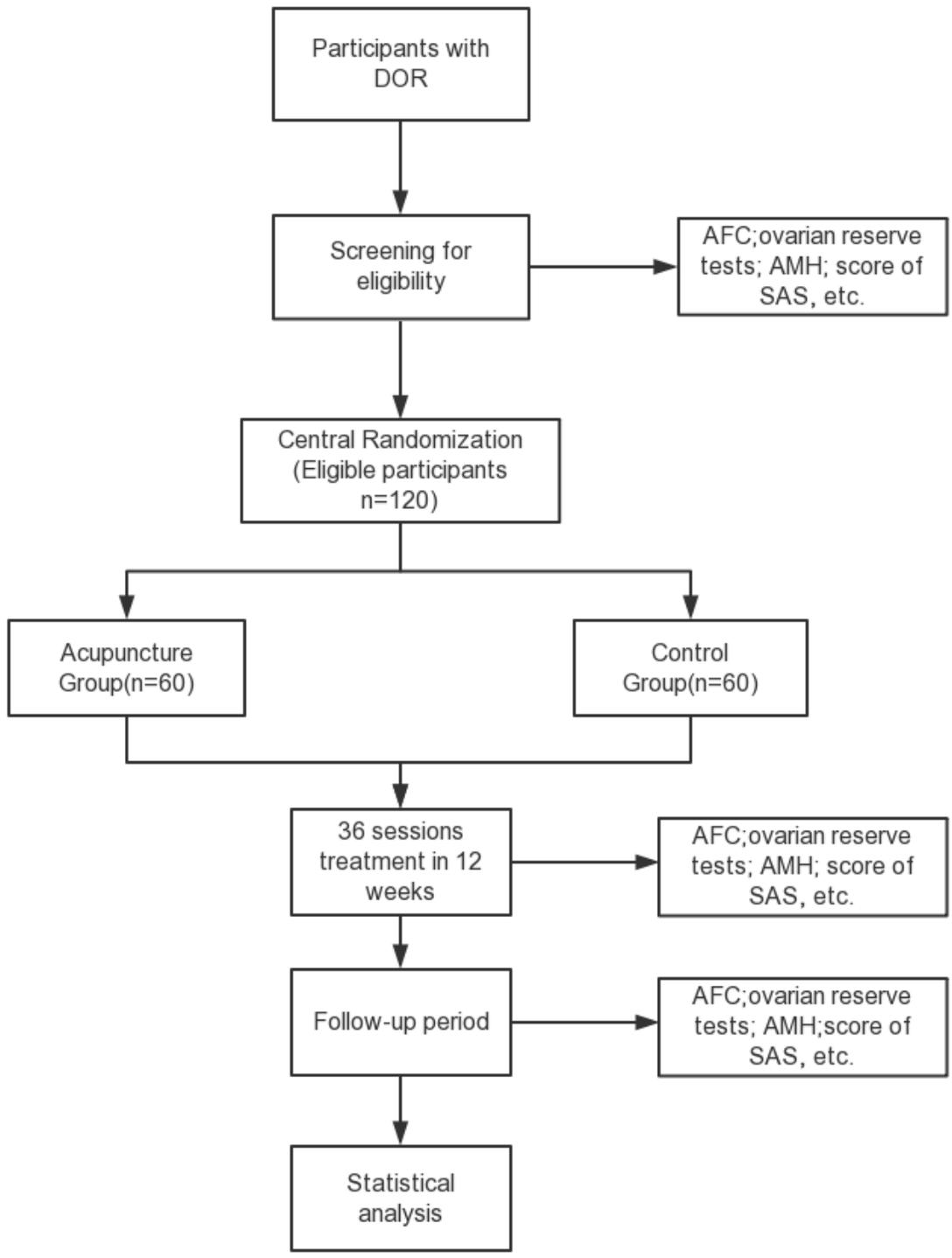
Not applicable.

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## Figures



**Figure 1**

Flowchart and study design. AFC, antral follicle count; AMH, anti-müllerian hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estradiol; SAS, self-rating anxiety scale.