Effect of Electro-acupuncture on Ovarian Function and Outcome of Ivf-et of Women With Diminished Ovarian Reserve: Study Protocol for a Randomized Controlled Trial.

Le Yang
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
https://orcid.org/0000-0003-3707-2537
Hanwang Zhang
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
Li Zhou
First Hospital of Wuhan
Ying Gao
Huazhong University of Science and Technology Tongji Medical College First Clinical College: Wuhan Union Hospital
Lijuan Yang
Reproductive Hospital affiliated to Jiangxi University of Traditional Chinese Medicine
Yajun Hu
First Hospital of Wuhan
Lu Xu
Reproductive Hospital affiliated to Jiangxi University of Traditional Chinese Medicine
Dongmei Huang (hdmjcr@qq.com)
Reproductive Hospital affiliated to Jiangxi University of Traditional Chinese Medicine

Study protocol

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Abstract

Background:

Diminished ovarian reserve (DOR) is the precursor state of ovarian failure, and can cause the decline of women's reproductive function. DOR also leads to poor outcome of in vitro fertilization and embryo transfer (IVF-ET) by affecting the oocytes, high qualified embryo rate, pregnancy rate, etc. Some studies have demonstrated that acupuncture can improve ovarian function. But to date, there is limited evidence indicating that acupuncture or electro-acupuncture is efficient to DOR. This trial aims to evaluate the efficiency and safety of electro-acupuncture for the ovarian function and the following outcome of IVF-ET in DOR patients.

Methods:

This will be a multicenter randomized controlled clinical trial. A total of more than 338 women with DOR will be randomly allocated to treatment and control groups in 1:1 ratio receiving acupuncture before undergoing IVF-ET. The primary outcome will be the clinical pregnancy rate per cycle of IVF-ET after acupuncture. The secondary outcomes will be ovarian reserve function, outcomes of IVT-ET, blood biochemical index, Massachusetts General Hospital Acupuncture Sensation Scale (MASS), scores from the self-rating anxiety and depression scale, quality of life, and pregnancy outcomes. The safety of acupuncture will also be assessed.

Discussion:

The results of this trial may provide high quality evidence regarding the effectiveness of electro-acupuncture in the treatment of DOR and following outcomes of IVF-ET. This will also help patients with DOR and their physicians by offering a new treatment option.

Trial registration:

ChiCTR1900024626. Registered on 19 July 2019.

Background

Diminished ovarian reserve (DOR) indicates a reduced number of retrieved follicles and the reduction of the quality of oocytes affected by non-biological reasons before the age of 40. Multiple studies consider that the cause of DOR is related to age, genetic factors, immune factors, social and environmental factors, infection, iatrogenic injuries, multiple pregnancies, etc. Normally, the ovarian reserve function declines at the age of 30, and fall off sharp after 35. The incidence rate of DOR is climbing and shows younger trend. However, there is no effective pharmacotherapy at present.

In-vitro fertilization-embryo transfer (IVF-ET) is a main therapy treating infertility, and controlled ovarian hyperstimulation (COH) is the key step. In IVF-ET, the success of COH depends on the ovarian reserve

function and ovarian response. A clinical trial finds that when undergoing IVF-ET, the DOR patients' initial dose of gonadotropin (Gn), total Gn, Gn days and abortion rate are obviously higher than patients with normal ovarian function, but the oocytes, M II oocytes rate, high qualified embryo rate, fertilization rate, implantation rate and pregnancy rate are all distinct inferior to patients with normal ovarian function [1]. In addition, DOR can cause reproductive endocrine dysfunction including less menstrual volume, menstrual loose, and amenorrhea, which further affects women's reproductive function. Besides, it also leads to perimenopause related symptoms, such as hot flashes, night sweats, irritability, etc. All these symptoms impact on patients' quality of life. And it will develop into premature ovarian failure (POF) within 1-6 years when without intervention at early stage. Hence, a new therapy for DOR is necessarily required.

In recent years, as an important part of traditional Chinese medicine, acupuncture has attracted tons of attention in the fields of reproductive endocrine and infertility worldwide [2-4]. Acupuncture can stimulate the neuro-endocrine system, regulate the overall endocrine level and ovarian microenvironment as a whole, improve the internal environment of follicles, improve ovarian hemodynamics, and thereby promote follicular development and ovulation. In addition, acupuncture can also enhance endometrial receptivity and relieve patients' tension and anxiety in assisted reproductive technology (ART) [5]. Acupuncture, which can improve ovarian function, is now widely used in the treatment of POF. Two Meta-analysis show that acupuncture is a relative effective intervention treating POF, which helps for recovering menstruation and boosting level of serum follicle-stimulating hormone(FSH) and estradiol(E2). The curative is sustainable till a month after the end of treatment [6-7]. Since DOR can develop to POF within 1-6 months, why don't we intervene it in the early DOR phase in order to delay or reverse the progress to POF?

Given its effect on ovarian function, acupuncture is expected to be a new option for the treatment of DOR. But to date, the available evidence of the efficacy of acupuncture or electro-acupuncture for DOR remains insufficient. Only a few small sample, non-randomized controlled clinical trials suggest that acupuncture or electro-acupuncture can improve ovarian reserve function of DOR patients [8-9]. Therefore, we aim to conduct a large sample, multicenter, randomized controlled trial to evaluate the efficiency and safety of electro-acupuncture for the ovarian function and the following outcome of IVF-ET in DOR patients.

Methods

Trial design

This will be a multicenter randomized controlled clinical trial. A total of more than 338 women with DOR will be randomly allocated to treatment and control groups in 1:1 ratio receiving acupuncture before undergoing IVF-ET. Patients will be recruited from the following 4 hospitals in China: Tongji Hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology, Union Hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology, Wuhan First Hospital, and Reproductive Hospital affiliated to Jiangxi University of Traditional Chinese Medicine. This

protocol is in accordance with the principles of the Declaration of Helsinki. Written informed consent will be obtained from patients prior to enrolment.

This trial has been registered at Chinese Clinical Trial Registry (ChiCTR1900024626). The flowchart and study design schedule are presented in Fig. 1 and Table. 1. The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist is provided as Additional file 1.

Participants

Eligible patients will be recruited from participating hospitals through following strategies. Posters will be placed on doctors' offices, bulletin boards or other places in the hospitals. Advertisements are also put via network, Wechat, etc. Eligible patients from the outpatient and inpatient clinics will be advised by doctors. In each hospital, specialized staff will contact with patients and make appointment of screening visit.

Inclusion criteria:

1. Age < 40, will undergo IVF-ET;
2. Low ovarian reserve: Antral follicle count (AFC)<7, or AMH<1.1ng/ml; or has a history of poor ovarian response: in the last hyper stimulation cycle, the number of retrieved oocytes<3;
3. Spouse' semen examination is normal, or after semen prewash can reach the standard of common IVF or Intracytoplasmic Sperm Injection(ICSI).

Exclusion criteria:

1. Male with azoospermia;
2. Male/female's chromosome is abnormal;
3. Adenomyosis, uterine fibroids, endometrial polyps, scar uterine, reproductive system tuberculosis, oviduct effusion, pelvic lesions such as ovarian endometriosis cyst or tumor;
4. Female has other endocrine disease: thyroid diseases, hyperprolactinemia, insulin resistance, diabetes, adrenal diseases, etc.
5. Definitively diagnosed autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, antiphospholipid syndrome;
6. Other pathogenesis that leads to recurrent miscarriage or agnogenic recurrent miscarriage;
7. A history of cancer and has received radiotherapy and chemotherapy;
8. Had acupuncture treatment in recent 3 months;
9. Unwilling to sign the informed consent of this study.

Randomization and masking

Internet-based central randomization system will be applied in this trial. 338 participants will be randomly allocated to treatment group and control group in 1:1 ratio. The Internet-based central randomization

system designs the random parameters and allocation. The appointed researchers in each hospital will apply for the group assignment allocated by the system through inputting specific code. The randomization protocol and the parameters set during it are collectively called blinding code, which are kept strictly by researchers who are not involved in the process of outcome evaluation and statistical analysis.

Interventions

The treatments of both two groups start after a spontaneous period or a withdrawal bleeding by progestin. For patients with amenorrhea or oligomenorrhea, the withdrawal bleeding is induced using progesterone by the outpatient doctors, and then the intervention starts after the withdrawal bleeding. The acupuncture protocols are formulated according to the theory of traditional Chinese medicine. The number of needles used, methods of stimulation (manipulation and electro-acupuncture), frequency and time of the treatment are detailed described following the CONSORT and STRICTA recommendations [10].

Fixed protocols are used for both the treatment group and the control group. All patients receive the treatment of active acupuncture or sham acupuncture, twice a week with a maximum of 24 times in 12 weeks. Treatments will not be performed during menstruation in both two groups. The treatment date, time, the name of the acupuncturist and the intensity of the electro-acupuncture stimulation which can vary between the different electrodes are recorded when the patient receives acupuncture treatment. The range of the intensity of the electro-acupuncture stimulation will be recorded: e.g. 1.2 - 3.0 mA.

1. Treatment group:

For treatment group, two groups of acupoints will be used alternatively.

The first group consists of governor vessel (GV) 20, conception vessel (CV) 6, CV 3, bilateral stomach (ST) 29, bilateral spleen (SP) 6, bilateral ST 36 and bilateral pericardium (PC) 4. The patients will be asked to stay in supine position and keep the whole body relaxed and comfortable. Disposable sterilized needles (Size: 0.25Í40/50 mm) will be inserted into a depth of 15~35 mm and stimulated manually to evoke needle sensation ("Deqi" in traditional Chinese medicine, TCM). And then CV 3 and CV 6, bilateral ST29, and SP6 and SP 9 bilaterally will be thereafter connected to electrical stimulators (Export Abteilung, Schwa-Medico GmbH, Wetzlarer Str. 41-43; 35630 Ehringshausen) and stimulated with low-frequency of 2Hz, 0.3 ms pulse length for 30 minutes. The intensity was adjusted to the maximum tolerated intensity of the patients. The other needles without electrical stimulators will be manually stimulated to evoke “Deqi” every 10 minutes.

The second group of acupoints consists of bladder (BL) 23 and 32 bilaterally, bilateral kidney (KI) 3 and SP 6, and GV 20. Patients will be asked to stay in prone position and keep the whole body relaxed and comfortable. GV 20 will be punctured obliquely, and other acupoints will be perpendicularly needled into a depth of 15~35 mm using disposable sterilized needles (Size: 0.25Í40/50 mm). All acupoints will be stimulated manually to evoke needle sensation ("Deqi" in TCM). And then BL 23 and 32, and KI 3 and SP

6 bilaterally will be stimulated with low-frequency in the same way as the first group. GV 20 will be stimulated manually to evoke “Deqi” every 10 minutes.

2. Control group:

For control group, four pseudo-acupoints are used, with two points on each shoulder and the two on each upper arm, which are not located on any meridians. The patients will be asked to stay in supine position and keep the whole body relaxed and comfortable. Disposable sterilized needles (Size: 0.18Í25 mm) will be inserted superficially to a depth of < 5 mm without any manual stimulus and the needle sensation ("Deqi" in TCM) should not be evoked. Electrodes are connected to the needles, but the stimulators should be turned on at an intensity of zero. Each intervention lasts for 30 minutes.

3. IVF-ET treatment:

After the acupuncture treatment, patients’ ovarian reserve function will be evaluated again, and then IVF-ET cycle treatment will be conducted. This will be performed by the reproductive medicine center according to patients’ situation and the standard procedure.

Concomitant treatments

Drugs and other treatments, which may interface the evaluation of electro-acupuncture effect, will be discouraged. Discouraged treatments include sexual hormones, contraceptives, and herbs. If treatment not recommended in this trial has already been performed, relevant information should be recorded in patient’s case report form.

Outcome measures

Primary outcome measure:

The primary outcome measure is the clinical pregnancy rate per cycle of IVF-ET after acupuncture.

Secondary outcome measures:

1. Assessment of ovarian reserve function

Assessing patients’ ovarian reserve function before and after acupuncture intervention, including:

(1) The serum AMH, inhibin, and FSH and E2 levels on the third day of menstruation;

(2) AFC.

2. Outcomes of IVF

(1) Gn dosage and usage days;

(2) E2 level and endometrial thickness on human chorionic gonadotropin (HCG) day;

(3) number of oocytes;

(4) MII oocytes;

(5) normal fertility rate;

(6) the number of available embryos;

(7) number of high-quality embryos;

(8) cycle cancellation rate (including cycle cancellation rate caused by various reasons);

(9) implantation rate: including fresh periodic implantation rate, per cycle implantation rate and cumulative implantation rate;

(10) fresh cycle clinical pregnancy rate and cycle cumulative clinical pregnancy rate;

(11) early, mid and late pregnancy abortion rate;

(12) risk of ovarian hypertrophy and incidence of obstetric complications;

(13) FSH, LH, E2 and AMH in follicular fluid; Oxidative stress related indicators such as reactive oxygen species (ROS), superoxide dismutase (SOD) level, etc.;

(14) live rate: including fresh cycle live rate, cycle live rate and cumulative live rate.

3. Blood biochemical index examination before and after acupuncture

Testing the levels of blood corticotrophin-releasing hormone (CRH), norepinephrine, adrenaline, 5-hydroxytryptamine, beta-aminobutyric acid (GABA), dopamine (DA) and neuro-endorphin of the patients before and after treatment.

4. De qi sensation scale of acupuncture

After each acupuncture treatment, patients will be asked to rate the MASS [11] independently by evaluating 12 acupuncture sensation degrees within 5 minutes. The higher the weighted total score is, the more obvious is the Deqi sensation degree.

5. Questionnaires

Evaluation of anxiety, depression and quality of life is performed before and after treatment.

(1) Baker anxiety self-rating scale (BAI) and baker depression self-rating scale (BDI-Ⅱ) : higher score indicates higher degree of depression or anxiety [12-13].

(2) Zung anxiety self-rating scale (Zung-SAS), Zung depression self-rating scale (Zung-SDS) [14-15].

(3) Quality of life measurement (QOL): Quality of life will be assessed by SF-36 and Chinese quality of life scale (CHQOL).

6. Follow-up detection

Pregnancy patients will be followed up to the end of pregnancy. Adverse pregnancy outcomes and live birth rates will be recorded. Non-pregnant patients will be followed up for 1 year after treatment, testing the ovarian reserve function, follow-up treatment and pregnancy status of the patients within 1 year.

Adverse events

Adverse events (AEs) will be classified. The percentage of AEs and severe adverse events occurred during treatment will be recorded in detail. Chi-square test is used to analyze the total proportion of adverse events in each treatment regimen and the differences between the classifications. Unless otherwise formally requested, each report from safety monitor board will report details and summaries of adverse events in a double-blind manner.

Data management and quality control

Researchers including acupuncturists, outcome assessors, and statisticians will receive training about data management. In this trial, online monitoring will be used. Data of participants will be inputted into the electronic case report form (eCRF) through clinical trial management public platform called ResMan. The clinical research associates are responsible for verifying the accuracy of data. All the research documents, which includes both the paper files and electronic documents, will be preserved for at least five years after publication. The private information of patients (name, telephone number, and ID number) will be anonymous to ensure participant confidentiality.

The quality controller of each hospital will complete self-inspection at least once every month. Remote or onsite monitoring will be performed for all centers once per 3 months by the principle investigator. The auditing will be done by Clinical Evaluation Center of Tongji hospital at the beginning, middle, and end of the trial. WeChat will be used for making appointments and regular reminders, in order to improve participant compliance. For patients who deviate from the trial, causes and relevant outcome data will be recorded in a case report form as much as possible.

Statistical methods

1. Sample size calculation:

According to studies conducted by Hong SB et al. and Roustan A et al., the clinical pregnancy rate of DOR patients is respectively 20.6% and 20.2% [16-17]. Due to the lack of effective randomized blinded controlled clinical trials that choose clinical pregnancy rate per cycle as the primary outcome measure in evaluating acupuncture efficacy, we presume 15% for the patient's clinical pregnancy rate per cycle. Assuming that the clinical pregnancy rate can be increased by 15% after acupuncture treatment, $\alpha=0.05$, $\beta=0.20$, power is

80%. The sample size is calculated according to the following formula [18], nA=κnB, nB=[pA(1-pA)/κ+pB(1- pB)](z1-α/2+z1-β)2/(pA-pB)2,κ=1, then we get nA=nB=135,which means the sample size of each group is 135. Allowing for a 20% dropout rate, the sample size of this trial is 338 participants.

2. Statistical analysis:

All analyses will be based on the intention-to-treat principle. Persenka square test is used to compare the clinical pregnancy rate of primary outcome measure. The secondary measures are analyzed by the corresponding statistical methods (Chi-Square test is used for count data, and t test is used for measurement data with equal variance).

Discussion

This trial aims to determine whether electro-acupuncture can improve ovarian function and clinical pregnancy rate during IVF cycles for DOR patients. Some clinical and experimental studies have found that acupuncture can promote the follicular development and ovulation, and further improve ovarian function. [6-9] A few clinical trials have shown effectiveness in treating DOR through acupuncture or electro-acupuncture. [8,9,19,20] And the pregnancy rate in IVF-ET after acupuncture is assessed by several studies. [21-22] However the population of these trials are all small sample and non-controlled. Therefore we will confirm the efficacy by a large sample, multi-center randomized controlled clinical trial. And this trial will assess the effect of acupuncture on the clinical pregnancy rate per IVF-ET cycle for DOR women as the primary outcome measure.

In this trial, the form of the electro-acupuncture is manual acupuncture. All acupoints will be stimulated manually to evoke needle sensation called Deqi. After that, the electrodes will be connected to the needles and start simulating. The intensity of Deqi sensation is critical for the effects of acupuncture. [22] The MASS will be used to quantify the intensity of de qi sensations after each treatment, which aims to explore the association between the intensity of Deqi sensations and the efficacy of electro-acupuncture.

There are some limitations in this trial. The acupuncture protocol consists of two groups of acupoints, which seems to be a little sophisticated for acupuncturists, and may increase the difficulty in implementing placebo acupuncture. Besides, the acupuncturists in this trial will not be blinded to the intervention. The solution of the situation is blinding the group assignment before electro-acupuncture.

In conclusion, the results of this trial may provide high quality evidence evaluating the effectiveness of electro-acupuncture in the treatment of DOR patients and following outcomes of IVF-ET. This study will contribute to offer a therapy option for DOR patients.

Trial status

This trial is currently recruiting participants. The protocol version number and date: V3.0, September 8, 2020. The recruitment began on August 1, 2019. The estimated completion date of recruitment is August

1, 2022.

Abbreviations

DOR: diminished ovarian reserve

IVF-ET: in vitro fertilization and embryo transfer

MASS: Massachusetts General Hospital Acupuncture Sensation Scale

COH: controlled ovarian hyperstimulation

Gn: gonadotropin

POF: premature ovarian failure

ART: assisted reproductive technology

FSH: follicle-stimulating hormone

E2: estradiol

AFC: antral follicle count

ICSI: intracytoplasmic sperm injection

GV: governor vessel

CV: conception vessel

ST: bilateral stomach

SP: bilateral spleen

PC: bilateral pericardium

TCM: traditional Chinese medicine,

BL: bladder

KI: bilateral kidney

HCG: human chorionic gonadotropin

ROS: reactive oxygen species

SOD: superoxide dismutase

CRH: corticotrophin-releasing hormone

GABA: beta-aminobutyric acid

BAI: baker anxiety self-rating scale

BDI-Ⅱ: baker depression self-rating scale

Zung-SAS: Zung anxiety self-rating scale

Zung-SDS: Zung depression self-rating scale

QOL: quality of life measurement

CHQOL: Chinese quality of life scale

AEs: adverse events

eCRF: electronic case report form

Declarations

Ethics approval and consent to participate

Central ethical approval has been confirmed from the Medical Ethics Committee of Tongji hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology (TJ-IRB20190620). 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.

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Authors' contributions

DMH and LY conceived and designed the study protocol. LY drafted the manuscript. LY completed the trial registration. DMH and HWZ substantively revised the manuscript. DMH and LY designed the statistical plan. LZ, YG, LJY, YJH, LX and HWZ participated in data acquisition and provided administrative, technical, or material support. All authors have read and approved the final manuscript and will take public responsibility for appropriate portions of the content.

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Not applicable.

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Table

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

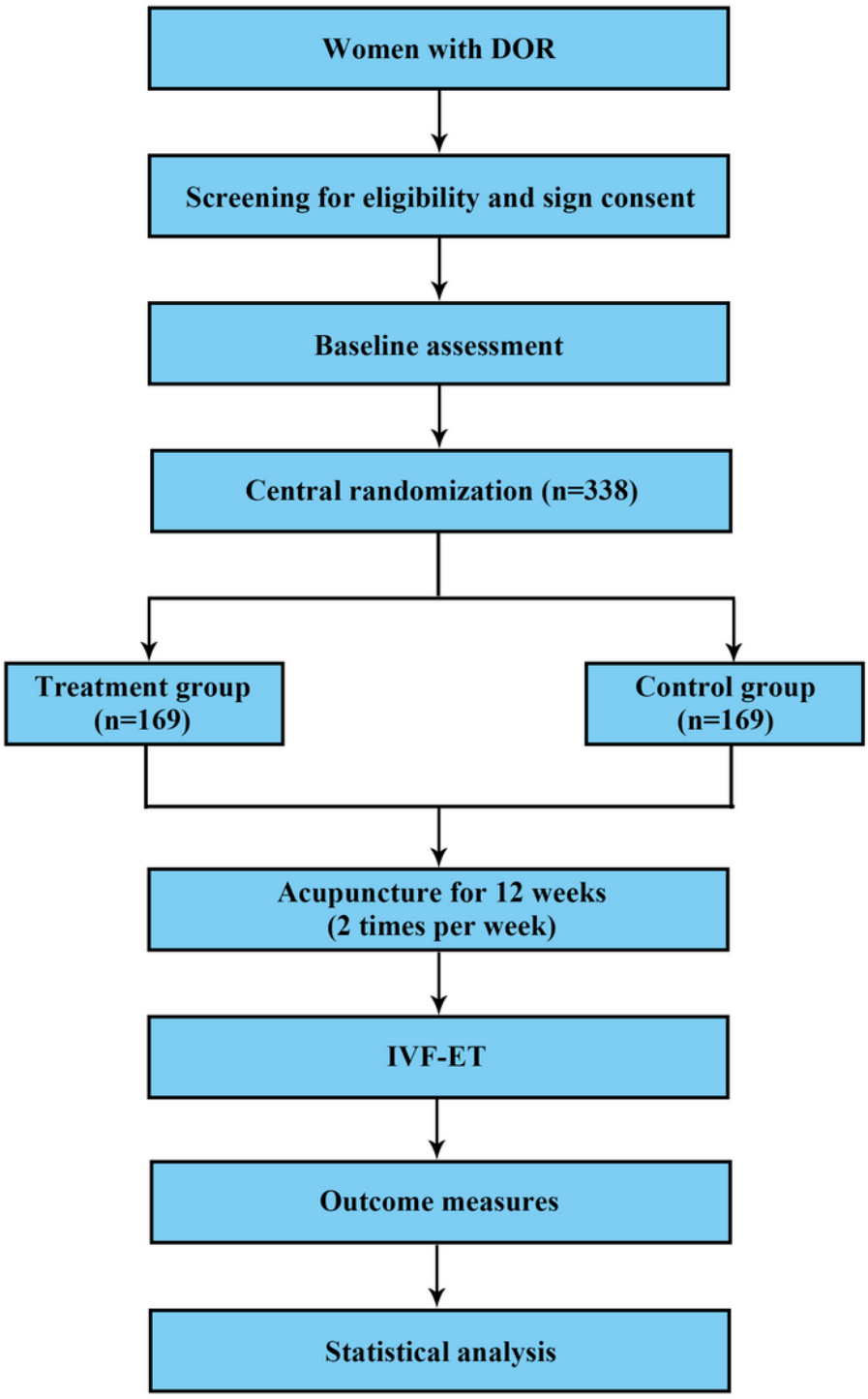

Figure 1

Flowchart of the trial procedures

Supplementary Files

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

- Aditionalfile1SPIRITchecklist.docx
- Table1Participantstimeline.doc