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Study on biological characteristics of meridian points and efficacy of intradermal acupuncture treatment for diminished ovarian reserve: study protocol for a case-control experiment and a single-blinded randomized controlled trial

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Study protocol

Keywords: Diminished ovarian reserve, Intradermal acupuncture, Biological characteristics, Case-control, Randomized controlled trial, Study protocol

Posted Date: August 11th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4718880/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

Diminished ovarian reserve (DOR) is characterized by decreased female fertility, menstrual disorders and perimenopausal symptoms due to sex hormone deficiency or fluctuations. For now, there are no uniform diagnostic criteria or an ideal single detection index for DOR. Acupuncture has been extensively used to treat female infertility. This study is aim to discover the biological characteristics of the DOR-related acupoints and explore the efficacy of intradermal acupuncture treatment on DOR patients.

Methods/design:

To discover which acupoints are associated with DOR,there will be 50 DOR patients and 50 healthy participants included to detect the biological characteristics of certain acupoints. The primary outcome will be pain threshold and infrared thermal temperature. In the following randomized controlled trial, 106 patients with DOR will be randomly divided into intradermal acupuncture or sham intradermal acupuncture in a 1:1 ratio to achieve treatment for 12 weeks. The primary outcomes will be the changes in serum follicle-stimulating hormone(FSH) and Anti-Müllerian Hormone (AMH) level from baseline to the end of treatment(week 12). the secondary outcomes include the changes of biological characteristics of treated acupoints, the modified Kupperman Index (KI), Self-Rating Anxiety Scale (SAS) and Self-rating Depression Scale (SDS).

Discussion

The purpose of this study is to observe whether there is regularity in the biological characteristics of meridian points under the disease state, and consider these changes as an auxiliary detection means for DOR diagnosis. It also helps to screen out DOR-sensitive acupoints as a reference for treatment selection, and is expected to explore the efficacy of intradermal acupuncture and sham intradermal acupuncture in treating DOR.

Trial registration:

Registered in ClinicalTrials.gov NCT06395623(Registered on 3 may, 2024), NCT06223178(Registered on 28 January, 2024)

Background

Diminished ovarian reserve (DOR) is characterized by decreased female fertility, menstrual disorders and perimenopausal symptoms due to sex hormone deficiency or fluctuations. As an important cause of infertility, DOR accounts for 20% of ovarian disease[1, 2] and can be up to 50% among women aged 40

years and older[3]. In the long term, patients with DOR are exposed to multiple health risks, such as an increased incidence of cardiovascular diseases, genitourinary diseases, and psychological disorders[4].

For now, there are no uniform diagnostic criteria or an ideal single detection index for DOR because of its insidious etiology and pathogenesis, causing the lack of specific therapy to effective restoration of complete ovarian function. With the similar clinical manifestations to those of menopausal symptoms, the diagnosis of most patients with DOR may only be recognized when evaluating for infertility. Thus, we are seeking an effective and non-invasive adjunct to DOR detection.

The theory of traditional Chinese medicine suggests that there are specific changes in the location of associated acupoints with the disease[5–7], such as disorder of pain threshold and surface temperature[8–11]. A review[12] described that stimulating pain-sensitized acupoints shows better efficacy of treatment than routine acupoints by analyzing 3728 clinical trials of acupuncture.

Although many experiments and researches tried to prove that the biological characteristics of meridian points associated with specific disease[13–15], it is currently unclear whether or which acupoints related to DOR.

Hormone replacement therapy(HRT) is a common treatment to alleviate symptoms of low estrogen[16]. But its increasing risk of the prevalence of endometrial and breast cancers[17, 18] brings concerns. Thus, we are seeking an effective non-pharmacological therapy with better patient compliance to treat DOR. For instance, acupuncture has been extensively used to treat female infertility. Reports showed the efficacy of acupuncture in the treatment of DOR. A study[19] came to conclusion that electroacupuncture could improve the endometrial receptivity, clinical pregnancy rate, embryo implantation rate and live birth rate of DOR patients with higher HCG positive rate. Another research[20] proved acupuncture therapy is effective in improving the ovarian reserve function in DOR patients.

Intradermal acupuncture is a special type of acupuncture. Different from traditional acupuncture, intradermal acupuncture causes long-lasting and stable stimulation on acupoints with less invasive pain, which leads to better patient compliance. Although previous studies have demonstrated the efficacy of intradermal acupuncture in treating some diseases, such as dry eye[21] or knee joint pain[22]. It remains unknown whether intradermal acupuncture is also efficacy in DOR treatment.

Therefore, we designed a study which includes an observational case-control experiment to explore the biological characteristics of the DOR-related acupoints, and a randomized, single-blind, sham-controlled trial designed to explore the efficacy of intradermal acupuncture treatment on DOR patients.

Method for observation of biological characteristics of acupoints

Observation design

50 healthy participants and 50 patients with diminished ovarian reserve were included for cross-sectional study. The sample size is decided on the actual conditions of this study, for there is no uniform standard and available formula for calculation in this study. All participants will be strictly screened or diagnosed for eligibility. Modern instruments are used to regularly test the pain threshold, infrared thermal image at the relevant body surface areas and meridian acupoints. It's aimed to filter DOR-related acupoints, and what the characterization changes on these acupoints are. This part will be an observational experiment, which does not involve random method, and the researcher will not be told whether the observations are of healthy participants or patients.Flowchart and study design schedule are presented in Fig. 1.

This study is in accordance with the principles of the Declaration of Helsinki and has been approved by the ethics committee of the Third affiliated hospital of Zhejiang Chinese Medical University (approval number: ZSLL-KY-2023-060-01, date of issue: 2023.11.03, version 2.0), and registered on ClinicalTrial(ID:NCT06395623). Written informed consent will be obtained from each participant before enrolment, and details about this study will be infomed.

This study will be conducted from December 2023 to May 2025. All participants are recruited from the Third affiliated hospital of Zhejiang Chinese Medical University and Hangzhou TCM Hospital Affiliated to Zhejiang Chinese Medical University.

The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist is provided as Additional file 1.

Eligibility criteria

1.Eligibility criteria for patients with DOR

The diagnostic criteria for DOR referred to < Consensus on clinical diagnosis and management of diminished ovarian reserve>[23]. Participants will be included if they meet all those inclusion criteria: (1) female, aged between 18 to 40; (2) meet both items in twice tests with an interval of at least 4 weeks (tested between 2rd to 4th day of menstrual cycle): (a)10 IU/L < FSH < 20 IU/L, (b)Anti-Müllerian hormone (AMH) < 1.1ng/mL. (3) without any mind disease, willing to participate in the study and sign the informed consent.

2.Eligibility criteria for healthy participants

Participants meet all those inclusion criteria will be included: (1)Healthy participants who can provide medical examination reports within the past 1 year and have routine physical examination conducted by researchers to confirm that there are no serious underlying diseases of the reproductive system, urinary system, blood, endocrine, nervous system, etc; without symptoms of oligomenorrhea or amenorrhea at

present; Between the ages of 18 to 40, female; without any mind disease, volunteering to participate in the study and sign the informed consent.

Exclusion criteria

1.Exclusion criteria for patients with DOR

Participants will be excluded if they meet any of following criteria: (1)Low or no response to exogenous gonadotropins, or with congenital anomalies in the development of the reproductive tract;(2)Pregnant or breastfeeding; (3)Abnormal body temperatures at the moment; (4)Psychopath, alcoholic, patient with severe depression, history of substance abuse and severe primary diseases of cardiovascular, liver, kidney and hematopoietic system(5) Currently enrolled in other clinical trials or refuse to cooperate with the study protocol.

2.Exclusion criteria for healthy participants

Those meeting any of the above criteria are excluded: (1)Patient with mental illness, severe depression, alcohol dependence or a history of drug abuse; (2)In pregnant or breastfeeding; (3)abnormal body temperature at present; (4) Participants who are currently participating in other clinical trials or who do not cooperate with the study protocol.

Biological Characteristics Detection

All participants are asked to refrain from taking medications that affect microcirculation and vasoactivity for 1 week prior to testing, and no tea, alcohol or coffee for 1 day prior, as well as spicy food or smoking. Exercise and food intake were also prohibited for 1 hour prior to the test. After the participants were instructed to remain quiet, breathe naturally, and relax throughout the test after taking a rest for 30 min to adapt to the environment of the testing room before the formal testing. There will be a special text room in each site, where the room temperature is controlled at (26 ± 1) ℃ and relative humidity is controlled at 40%±5%. The room is required to be free from direct sunlight and obvious air convection, with good sound insulation conditions. The location of the selected acupoint complies with the national standard (GB/T 12346 − 2021) in Table 1 and Fig. 2. The following acupoints will be inspected on both sides except those in the Conception Vessel(CV): CV12(Zhongwan), CV13(Shangwan), ST21(Liangmen), KI14 (Siman), CV4(Guanyuan), KI13(Qixue), EX-CA1(Zigong), ST36(Zusanli), SP9(Yinlingquan), SP8(Diji), SP6(Sanyinjiao), ST25(Tianshu), BL31(Shangliao), BL32(Ciliao), ST42(Chongyang), SP3(Taibai).

Table 1
Location of acupoints

ACUPOINTS	LOCATION
CV12(Zhongwan)	On the anterior median line of the upper abdomen, 4 cun above the navel.
CV13(Shangwan)	On the anterior median line, 5 cun above the navel (1 cun above CV12).
ST21(Liangmen)	On the upper abdomen, 2 cun lateral to CV12.
CV4(Guanyuan)	On the anterior median line of the lower abdomen, 3 cun below the navel.
KI14 (Siman)	On the lower abdomen, 2 cun below the navel and 0.5cun lateral to the anterior median line.
KI13Qixue)	On the lower abdomen, 3 cun below the navel and 0.5cun lateral to the anterior median line (0.5 cun lateral to CV4).
EX-CA1(Zigong)	On the lower abdomen, 4 cun below the navel and 3 cun lateral to the anterior median line.
ST36(Zusanli)	On the anterior lateral side of the tibial, 3 cun below ST35 (Dubi), on the connecting line between ST35 and ST41.
SP9(Yinlingquan)	On the medial side of the calf, at the pit where edial tibial condyle converges the posterior border of the medial tibia.
SP8(Diji)	On the medial side of the calf, 3 cun below SP9, by the posterior border of the medial tibia.
SP6(Sanyinjiao)	On the medial side of the calf, 3 cun above the medial malleolus, by the posterior border of the medial tibia.
BL31(Shangliao)	At the first post-sacral foramen.
BL32(Ciliao)	At the second post-sacral foramen.
ST42(Chongyang)	On the dorsum of the foot, at the base of the second metatarsal and the middle cuneiform segment joint where the pulse of dorsalis pedis artery can be touched).
SP3(Taibai)	At the proximal end of the first metatarsophalangeal joint.

Outcome

Pressure pain threshold, infrared thermal imaging, laser speckle imaging, resistance and biological ultra-weak luminescence of the each detected acupoints area will be measured as the primary outcome.

Questionnaires of the modified Kupperman Index (KI), Traditional Chinese Medicine physique test, Self-Rating Anxiety Scale(SAS) and Self-rating Depression Scale(SDS) are also applied to assess the subject's condition, and be correlated with above test values. The results of these questionnaires will be the secondary outcomes.

Statistical analysis

An independent statistician who are blinded of the group assignments will use SPSS V.25.0 statistical software (IBM, Armonk, New York, USA) for data analysis. Continuous variables will be described as mean and standard deviation($\bar{x}$±s) with a 95% CI in a normal distribution, and median or interquartile spacing [M (P25, P75)] in abnormal distribution. Categorical data such as Binary or ordinal data were presented as numbers and percentages. Comparisons between groups of data that met normality will be analyzed using t-test or Welch's t-test. Wilcoxon rank sum test applied for groups of data that non-normality or rank variable. All statistical tests will be two-sided, and $p < 0.05$ will be considered statistically significant.

Method for RCT

Trial design

This will be a multi-center, randomized, sham-controlled trial. All of 106 patients with DOR will be randomly divided into intradermal acupuncture or sham intradermal acupuncture in 1:1 ratio. It's designed to validate whether the intradermal acupuncture has superior efficacy in treating DOR. The whole observation period of RCT will be 20 weeks, including a 12-week intervention period (weeks 0–12), and a 8 weeks follow-up period (week 13–20). Assessments will be conducted at baseline, week 4, week 12, and week 20. Flowchart and study design schedule are presented in Figure. 3 and Table 2, respectively.

This study is in accordance with the principles of the Declaration of Helsinki and has been approved by the ethics committee of the Third affiliated hospital of Zhejiang Chinese Medical University (approval number: ZSLL-KY-2023-060-01, date of issue: 2023.11.03, version 2.0), and registered on ClinicalTrial(ID:NCT06223178). Written informed consent will be obtained from each participant before enrolment. The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist is provided as Additional file 1.

Table 2
Assessment time points and flow chart.

	STUDY PERIOD			
Phase	Screening	Intervention		Follow-up
Time(week)	Baseline	Week 4	Week 12	Week 20
ENROLMENT:				
Sociodemographic data	×			
Medical history of DOR	×			
Physical condition	×			
Inclusion / exclusion	×			
Informed consent	×			
Allocation	×			
INTERVENTIONS:				
Intradermal acupuncture				
Sham acupuncture				
ASSESSMENTS:				
FSH	×	×	×	×
Biological characteristics	×	×	×	×
AMH	×		×	
KI	×	×	×	×
SAS, SDS	×	×	×	×
SAFETY:	×	×	×	×
Adverse events		×	×	×
Medicine alliance	×	×	×	×

Eligibility criteria

Participants will be included if they meet all those inclusion criteria: (1) female, aged between 18 to 40; (2) meet both items in twice tests with an interval of at least 4 weeks (tested between 2rd to 4th day of menstrual cycle): (a)10 IU/L < FSH < 20 IU/L, (b)Anti-Müllerian hormone (AMH) < 1.1ng/mL. (3) without any mind disease, willing to participate in the study and sign the informed consent.

Exclusion criteria

Participants will be excluded if they meet any of following criteria: (1) Low or no response to exogenous gonadotropins, or with congenital anomalies in the development of the reproductive tract;(2) Pregnant or breastfeeding; (3) Abnormal body temperatures at the moment; (4) Psychopath, alcoholic, Patient with severe depression, history of substance abuse and severe primary diseases of cardiovascular, liver, kidney and hematopoietic system; (5) Currently enrolled in other clinical trials or refuse to cooperate with the study protocol.

Randomization and Blinding

Eligible patients with DOR will be randomly assigned to intradermal acupuncture group or sham intradermal acupuncture group in 1:1 ratio and all participants will not be told which group they are allocated to. The randomization sequence will be generated by SPSS V.21.0 statistical software (IBM, Armonk, New York, USA) with a completely randomized design. Randomization was stratified by site and defined with a block size of 6, which was masked to the investigators. There will be a type of sham intradermal acupuncture applied to mask verum and sham intradermal acupuncture while each patient will be treated in a separate room. A blinding assessment will be performed between patients in acupuncture groups. However, because of the nature of acupuncture, it is difficult to achieve masking for acupuncturists. Thus, the outcome assessors and statisticians will be masked with respect of group assignments in the process of performing this study and data analysis. Emergency unblinding will be allowed if there is any serious adverse event caused by intradermal acupuncture or other inducement of this trial.

Interventions

To ensure the efficacy and safety of intradermal acupuncture, operations are performed by acupuncturists who have been trained in the clinical standards of practice for this study and registered practitioners of traditional Chinese medicine (TCM). All participants will achieve 24 sessions of treatments in 12 weeks (2 sessions per week) and follow-up for 8 weeks. The sterile disposable intradermal acupuncture (Seirin, Shizuoka, Japan) the trial use for verum acupuncture treatments is 15mm in length and 0.25 mm in diameter. And the sham needle is produced by same company in same appearance but needleless. The needling acupoints will be the sensitized acupoints that filtered by the previous observational experiment. All patients will be asked to stop any other treatment, especially using hormonal drugs. Specialist gynecological construction for basic treatment will be provided to promote participant retention. If patients need to take any medicine to improve menstrual symptoms, a qualified gynecologist will judge whether this patient is retained or eliminated, and the details of medication use should be documented in the case report form (CRF).

Intradermal acupuncture group

Patients in the intradermal acupuncture group will achieve 24 sessions treatment of intradermal acupuncture over 12 consecutive weeks beside basic gynecological treatment. Sterilizing skin and avoiding vessels, the intradermal acupuncture are directly stock to the surface of acupoints by a band-aid, kneading and pressing for 5 minutes. The intradermal acupuncture will be embedded in the skin and keep staying for 72 hours once. The participants will be taught to press them 3 to 4 times per day. As tolerated by them, each pressing will lasts 1 minutes with an interval of about 4 hours.

Sham intradermal acupuncture group

The sham intradermal acupuncture, which has no difference in appearance with the verum intradermal acupuncture but is lack of needle, are used for sham acupuncture. Times and frequency of treatment that the patients insham intradermal acupuncture group will achieve are equivalent to patients in intradermal acupuncture group, as well as the acupoints and manipulation in those operations.

Outcome measures

1.Primary outcome

The changes in serum hormone will be the primary outcome.

1.The changes in follicle-stimulating hormone (FSH)

The increasing of FSH level in follicular phase, which is a common sex hormone indicator that widely clinical accepted for measuring ovarian function, is one of the main symptoms of Diminished Ovarian Reserve. The normal value of FSH is 1.37-9.9U/L in follicular phase, and higher the FSH value, the worse ovarian function. Patients in this study will be asked to take the serum FSH text between day2 to day4 of menstrual period in whole observation. Patients with amenorrhea take every 28 days as a supposed menstrual cycle instead. The schedule of measurement and visit points are shown in Table1. The change in FSH from baseline to the end of treatment (week 12) will be the final primary outcome.

2.The change in Anti-Müllerian Hormone (AMH) level

AMH is another recommend index to measure ovarian function. Compared to FSH, Anti-Müllerian Hormone has less fluctuation and more accuracy. The normal value of serum AMH is 2-6.8ng/ml in follicular phase, and lower the value, the worse condition. The serum AMH will be measured on days 2–4 of the menstrual cycle at the basement and the end of the treatment.

2.Secondary outcome

The secondary outcome includes the changes of biological characteristics of treated acupoints, the modified Kupperman Index (KI), Self-Rating Anxiety Scale (SAS) and Self-rating Depression Scale (SDS). Every participant is required to finish all these measurements in every visit.

(1)Biological characteristics of sensitized acupoint

We design the observational experiment to explore the biological characteristics of the responses of the DOR-related meridian acupoints and their specific changes in pathological condition, including pain threshold, infrared thermal imaging, laser speckle imaging, resistance and biological ultra-weak luminescence. All of these changes of biological characteristics will be valued at basement, week 4, week 12(the end of treatment) and week 20(week 8 of follow-up phase).

(2)the modified Kupperman Index (KI)

Menopausal symptoms can occur in patients with DOR. The modified Kupperman Index (KI) is widely used for female self-evaluation of the severity of menopausal symptoms in clinical practice. It values 13 items of menopausal symptoms on a scale of 0 to 3. The final score of KI is the sum of each score multiplied with the base score respectively, ranging from 0–63. The final KI score is less than 6 means normal, between 6 to 15 are classified as mild, 16 to 30 as moderate, and greater than 30 as severe.KI will be measured at basement, week 4, week 12 and week 20.

(3)Psychological condition

Self-Rating Anxiety Scale (SAS) and Self-rating Depression Scale (SDS) are used in this study. According to the frequency of symptom occurrence, the SAS contains 20 items reflecting subjective feelings of anxiety, and each item is rated on a four-point scale of 1 to 4, including 15 positive and 5 negative items. 1 means never, 2 is for sometimes, 3 is for frequently, 4 is always. Positive items are rating from 1–4, while negative items is from 4 to 1. Once the evaluation is complete, the scores for each of the 20 items are added together as score X. Score X will multiply by 1.25 and then round to the nearest whole number to obtain a standardized score Y, which is the final result of SAS. According to the Chinese normative results, the cut-off value of the SAS standardized score is 50, where 50–59 is mild anxiety, 60–69 is moderate anxiety, and 69 or more is severe anxiety. The SDS also contains 20 items reflecting subjective feelings of depression, with 10 positive and 10 negative ratings. The way to rate every items and to calculate score Y of SDS is the same way as SAS, The only difference is that the cut-off value of SDS standardized score is 53, where 53 to 62 is considered as mild depression, 63 to 72 is moderate depression, and 72 or more is severe depression. SAS and SDS will be measured at basement, week 4, week 12 and week 20.

Adverse events and concomitant medication use

During the whole study, adverse events (AEs) coursed by intradermal acupuncture treatment or aggravation of symptoms caused by sham intradermal acupuncture treatment are recorded. Any other

symptoms or occurrence of undesirable signs that not related to the treatment are also recorded. All the reports of adverse events are recorded in details, including the date, type, severity and possible reasons and the researchers will measured whether or not this patient should be withdrawn from the study. Any cost that necessary to treat these injury or AEs will be beared.

It is recommended that patients try not to apply any other medication during the clinical trial. If other medicine is needed and allowed to taken by a qualified gynecologist's judgement, there should be a detailed record of the name, specifications, dosage, time of taking, and time of relief of the medicine after taking.

Criteria for discontinuing interventions

Patients with following condition should discontinue intervention:(1)Serious adverse reactions occurred during the study, assessed by a specialist to determine whether to continue or terminate the study. (2)Patients who develop serious complications or other serious illnesses during the study requiring emergency measures. (3)Other reasons that for the patient cannot continue the study.

Sample size

Referring to the results of previous similar studies, we expect the mean FSH of intradermal acupuncture group and sham intradermal acupuncture group after 12 weeks' treatment will be 11.5 IU/L and 15 IU/L, respectively. The difference of scores between two groups was 5.Calculated by PASS(version15.0),43 patients per group are needed at a 2-sided significance level of 5% and power of 90% in a 1:1 ratio. Estimating a dropout rate of 20%, 53 patients were proposed to be enrolled in each group (106 patients total) ultimately.

Statistical analysis

SPSS V.25.0 statistical software (IBM, Armonk, New York, USA) are used for data analysis by an independent statistician who are blinded from group assignments. All the statistical analysis will be conducted according to the intention-to-treat (ITT) principle. Continuous variables will be described as mean and standard deviation($\bar{X}$±s) with a 95% CI in a normal distribution, and median or interquartile spacing [M (P25, P75)] in abnormal distribution. Categorical data such as Binary or ordinal data were presented as numbers and percentages. Missing values were imputed using multiple imputation by chained equations. Comparisons between groups of data that met normality will be analyzed using t-test or Welch’s t-test. Wilcoxon rank sum test applied for groups of data that non-normality or rank variable. All statistical tests will be two-sided, and $p < 0.05$ will be considered statistically significant.

Recruitment

Eligible participants will be recruited from the Third affiliated hospital of Zhejiang Chinese Medical University, Hangzhou TCM Hospital Affiliated to Zhejiang Chinese Medical University and the community. Each participant will get detailed information about the purpose, possible risks, benefits and any other information that participants want to know of this study. Researchers will avoid using terminology to participants.

Data management and quality control

In order to ensure the unity and standardisation of the operation process of this study, there will be a consistent training for all the researchers(including acupuncturists, outcome assessors and statisticians) one month before the clinical trial officially started. Especially focusing on implementation of the program and the various standard operating procedures (SOPs) to ensure the reliability of this clinical research findings, such as acupoint positioning, instruments operating for measuring, CRF filling, etc. All researchers should passed the post-training examination before participating in clinical study.

All observations in clinical studies should be verified and repeatedly confirmed to ensure the reliability and originality of the data, and to ensure that the results and conclusions in clinical studies are derived from the original data. Professional data management companies are commissioned for clinical data management and specialized personnel for trial data collection and statistics are adopted to control trial bias. Strict implement clinical study quality inspection monthly and multi-center clinical study quality monitoring once a quarter to control inter-center bias.

Discussion

This trail aimed to examine whether the intradermal acupuncture is effective in relieving symptoms or improving ovarian function for patients with DOR. A review of the literature[24] suggested that acupuncture improves in vitro fertilization outcomes by four possible mechanisms: modulation of neuroendocrine factors, increased blood flow to the uterus and ovaries, modulation of cytokines and remission of stress, anxiety, and depression. Although there were some clinical researches and studies[25, 26] proved that acupuncture is useful to promote ovulation function and adjusting hormone levels, but generalization of acupuncture scheme is still hindered. intradermal acupuncture is a new morph of traditional acupuncture. It's a special made mini-needle painlessly inserted and fixed into intradermal or subcutaneous acupoint, for an longer retention of mild stimulating. Compared to traditional needle that provide heavy stimulation in a short period, intradermal acupuncture only penetrates into superficial tissue, helping to eliminate more considers and scares of patients, as well as bias of subjective Deqi sensation.

In this study, non-penetrating needles are designed as the control for real acupuncture stimulation in accordance with TCM theory. A placebo set is used to refrain all participants from distinguishing. Though it's hard to blind the operators of group assignment because of the nature of acupuncture, outcome assessors and statisticians are masked from group assignment to ensure the accuracy of the data. This study chose FSH, a widely recommended serum sex hormone indicator, as the primary outcome to assess the efficacy of intradermal acupuncture treatment for DOR. According to clinical experiment, although majority of patients seek medical treatment for infertility, there's a certain number of patients not considering about pregnancy. So the modified Kupperman Index, Traditional Chinese Medicine physique test, Self-Rating Anxiety Scale and Self-rating Depression Scale are also involved to measure the efficacy of intradermal acupuncture in improving symptoms of perimenopausal caused by lack or fluctuation of sex hormones.

There are still some limitations in this study. First of all, double blindness remains a challenge for acupuncture or acupuncture-like experiment. It's impossible to keep acupuncturists blinded of group assignment during their operations. Besides, although the sham intradermal acupuncture has same appearance with verum intradermal acupuncture, patients in sham intradermal acupuncture group still have better chance to identify their group. The population of participants who are only recruited in Hangzhou is also a limitation. It may fail to present other patients with DOR in other areas of China and patients abroad.

In conclusion, we expect this randomized, single-blind, sham-controlled trial can provide high high-quality evidence to validate the effectiveness of intradermal acupuncture in the treatment of DOR patients and following outcomes of sex hormone levels. And this study may contribute to offer a therapy option for DOR patients.

Trial status

The trial recruiting starts on 3 November, 2023 and is still ongoing. The whole trial is expect to be completed on 30 December, 2025.

Abbreviations

DOR:Diminished ovarian reserve; FSH:follicle-stimulating hormone; AMH: Anti-Müllerian Hormone; KI:the modified Kupperman Index; SAS:Self-Rating Anxiety Scale; SDS:Self-rating Depression Scale; HRT:Hormone replacement therapy; CV: Conception Vessel; ST: Stomach; SP: Spleen; KI:Kidney; EX-CA:Extra point-Che ST and Abdomen; BL:Bladder; TCM: Traditional Chinese medicine; RCT: Randomized controlled trial; CRF:case report form; AEs:adverse events; ITT: intention-to-treat; SOPs:standard operating procedures;

Declarations

Authors’ contributions

This study protocol was designed and conceived by XMS and YJZ. And the manuscript was drafted and revised substantially by HYL, so as the inform consent. Specialist gynaecological guidance provided by JM and QC. Ethical approval and trail registration are completed by XMS and HYL. Participant recruitment and data acquisition were provided by SQC. HM will take participants allocation. HMS will be in charge of data management, and statistical analysis will be conducted by HYL. Acupuncture treatments were administered by SQC, MH and HYL. All authors have read and approved the submitted version of this manuscript and will take public responsibility for appropriate portions of the content.

Funding

This study is supported by Ministry of Science and Technology of the People's Republic of China (grant number:2022YFC3500401. The sponsor has no role in any part of work in the whole study.

Ethics approval and consent to participate

This study is in accordance with the principles of the Declaration of Helsinki and has been approved by the ethics committee of the Third affiliated hospital of Zhejiang Chinese Medical University (approval number: ZSLL-KY-2023-060-01,date of issue: 2023.11.03, version 2.0), and registered on ClinicalTrial (ID:NCT06395623 on on 3 may, 2024; NCT06223178 on 28 January, 2024). Written informed consent will be obtained from patients prior to enrolment. There will be no private information about patients will be exposed during the dissemination. All data and information will not be used for any other purpose except this study.

Dissemination

The results of this trial will be published in its entirety in peer-reviewed journals. Authorship of future trial publications will be determined based on the contributions of the authors.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, XMS, upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Figures

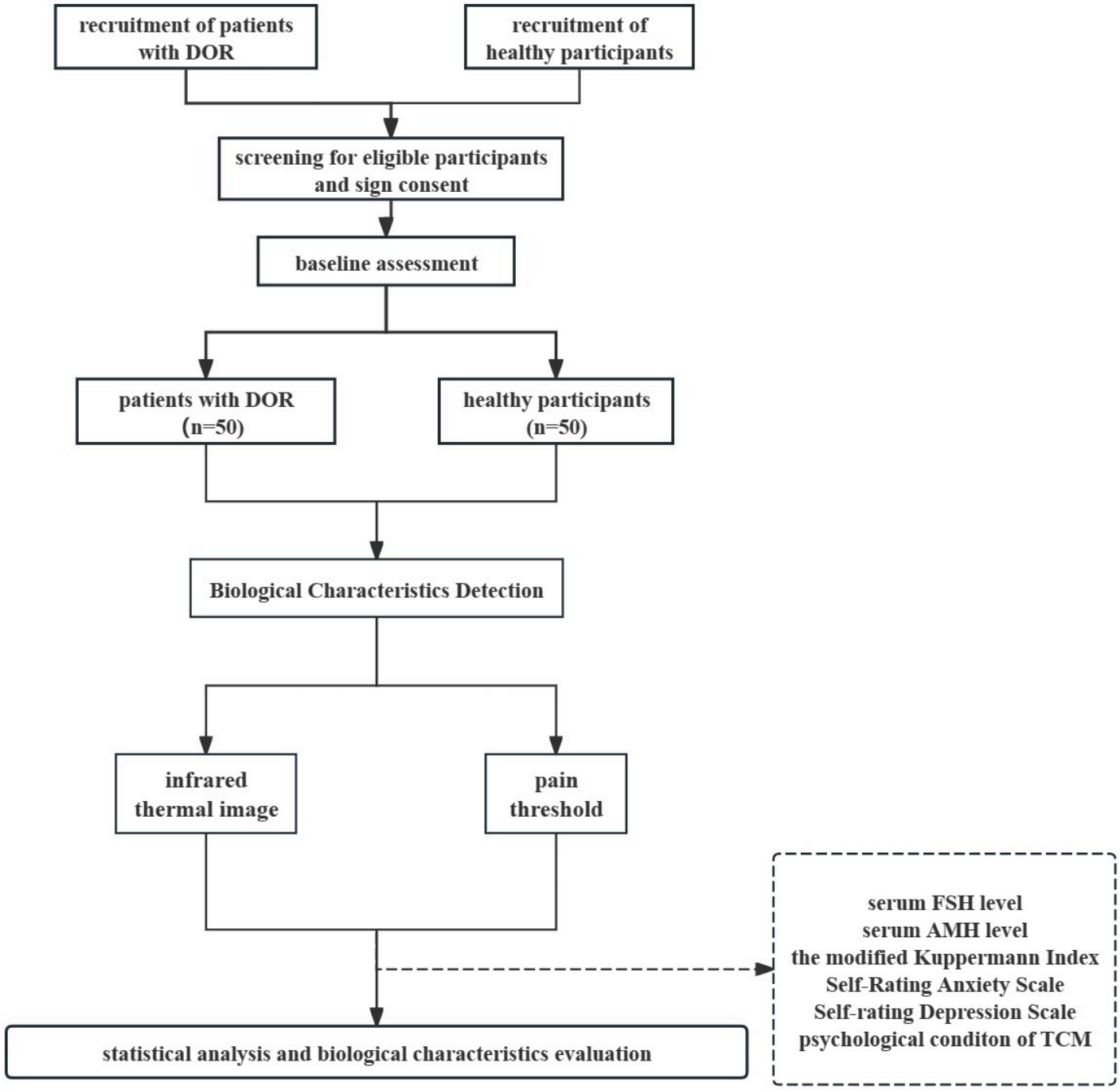


Figure 1

Detection flow chart

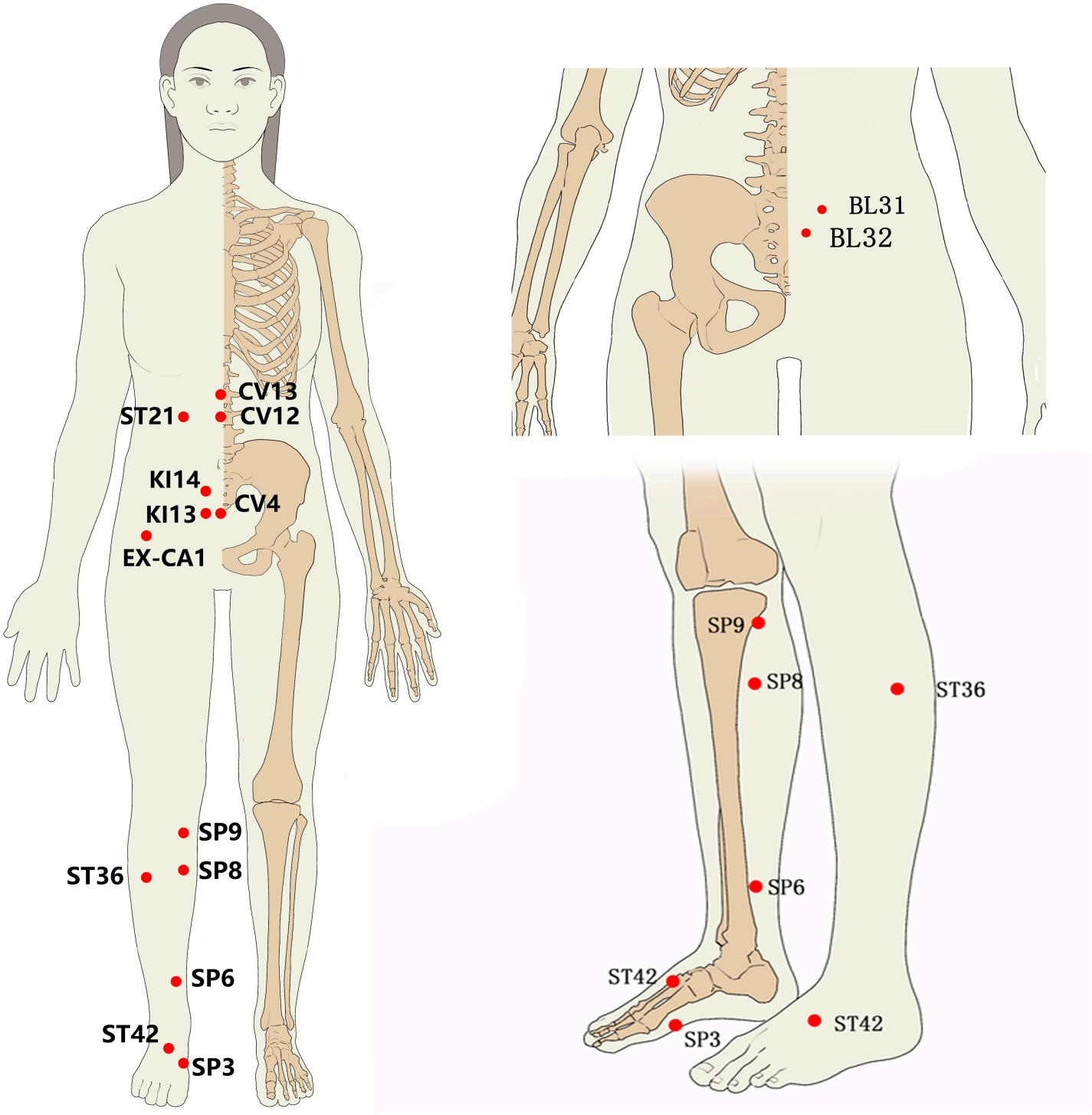


Figure 2

Location of acupoints

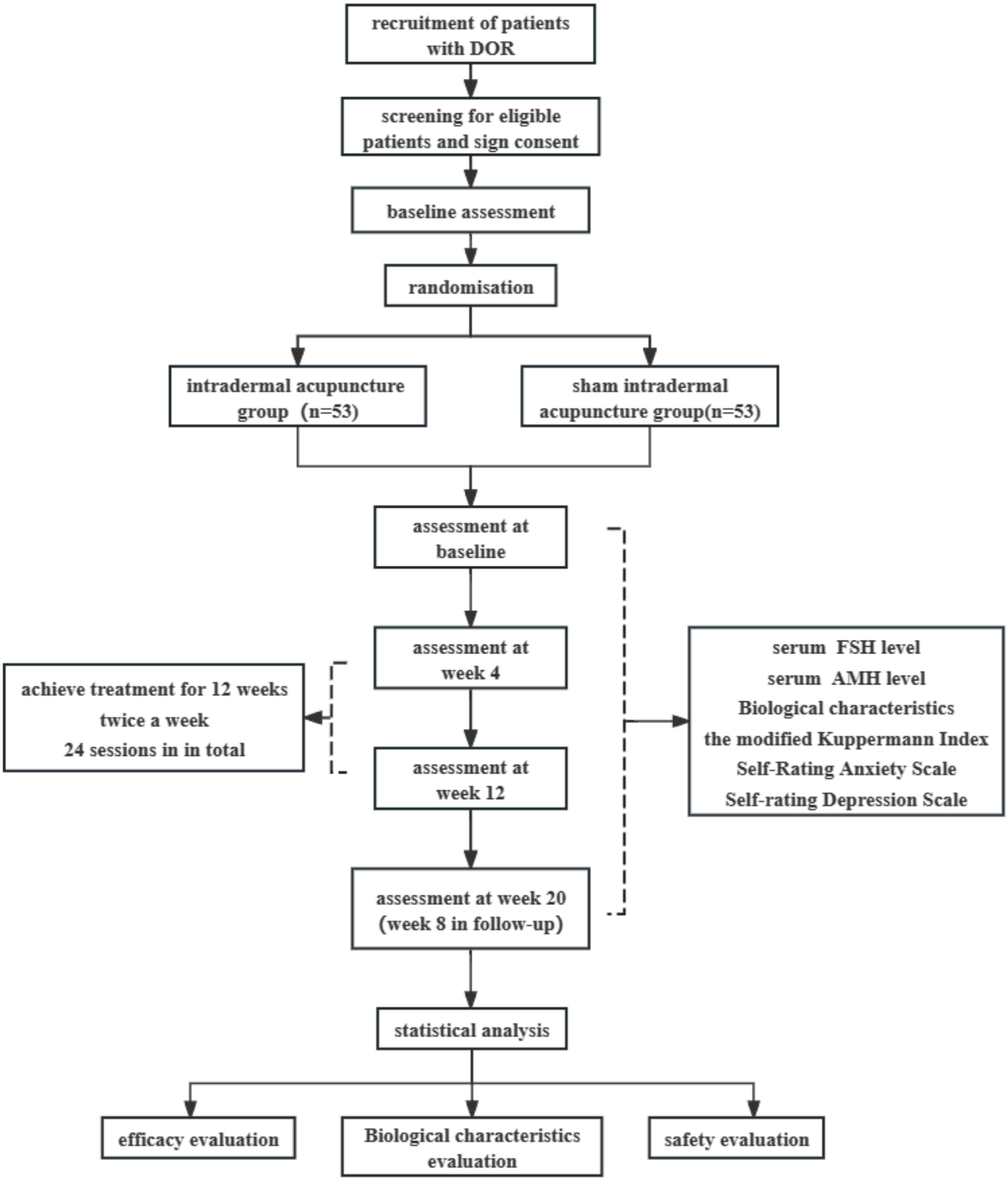


Figure 3

Intradermal acupuncture trial flow chart