Effect of acupuncture on women with poor ovarian response: study protocol for a multicenter randomized controlled trial

yigong Fang (fangyigong@163.com)
China Academy of Chinese Medical Sciences Institute of Acupuncture and Moxibustion
huanfang Xu
China Academy of Chinese Medical Sciences Institute of Acupuncture and Moxibustion
chensi Zheng
China Academy of Chinese Medical Sciences Institute of Acupuncture and Moxibustion
liyun He
Institute of Basic Research in Clinical Medicine China Academy of Chinese Medical Sciences
tongsheng Su
Shanxi Provincial Hospital of Chinese Medicine
huidan Wang
Shandong University Reproductive Hospital
yu Li
Sun Yat-sen Memorial Hospital of the Sun Yet-sen University
cui Zhao
Lanzhou University First Hospital
cuilian Zhang
Henan Provincial People's Hospital
yang Bai
Huazhong University of Science and Technology Reproductive Medicine Center of Tongji Medical College
guoqing Tong
Shanghai Shuguang Hospital
li Chen
East Region Military Command General Hospital
fang Zhao
Luoyang Women and Children Health Care Center
huisheng Yang
China Academy of Chinese Medical Sciences Institute of Acupuncture and Moxibustion
mingzhao Hao
China Academy of Chinese Medical Sciences Institute of Acupuncture and Moxibustion

yaqian Yin
China Academy of Chinese Medical Sciences Institute of Acupuncture and Moxibustion
li Yang
China Academy of Chinese Medical Sciences Institute of Acupuncture and Moxibustion
baoyan Liu
Institute of Basic Research in Clinical Medicine China Academy of Chinese Medical Sciences

Study protocol

Keywords: Acupuncture; Poor ovarian response; Number of retrieved oocytes; In vitro fertilization-embryo transfer; Randomized controlled trial

Posted Date: May 18th, 2020

DOI: https://doi.org/10.21203/rs.2.16359/v2

Version of Record: A version of this preprint was published on September 10th, 2020. See the published version at https://doi.org/10.1186/s13063-020-04690-8.

Abstract

Background: Poor ovarian response (POR), a manifestation of low ovarian reserve and ovarian aging, leads to a significantly reduced pregnancy rate after in vitro fertilization-embryo transfer. Acupuncture has been increasingly used for improving ovarian reserve. The purpose of this study is to evaluate the effect of acupuncture on increasing the number of retrieved oocytes after controlled ovarian hyperstimulation in women with POR.

Methods: This is a multicenter randomized controlled trial. A total of 140 women with POR will be randomly assigned to receive acupuncture or non-treatment for 12 weeks before controlled ovarian hyperstimulation. The primary outcome will be the number of retrieved oocytes. The secondary outcomes include antral follicle count , serum level of anti-müllerian hormone, basal serum levels of follicle stimulating hormone, luteinizing hormone and estradiol, the score of the self-rating anxiety scale, the fertilization rate, the cleavage rate, the available embryo rate and the high-quality embryo rate. The safety of acupuncture will also be assessed.

Discussion: The results of this trial will help to identify the effectiveness of acupuncture in the treatment of POR. This may provide a new treatment option for POR patients and their physicians.

Trial registration: AMCTR-IPR-18000198. Registered on 10 August 2018, *http://www.acmctr.org/showproj.aspx?proj=289*

Background

Poor ovarian response (POR) indicates a reduction in follicular response to the controlled ovarian hyperstimulation (COH) during in vitro fertilization (IVF) cycles, resulting in a reduced number of retrieved oocytes [1]. It is believed that approximately 9-24% of women who undergo IVF show POR during COH [2]. Women with POR usually experience less follicular development in COH cycles, low blood estrogen levels, high dosage of gonadotrophine, high cycle cancellation rates, decreased numbers of retrieved follicles, and low rates of clinical pregnancy [3]. Therefore, POR often leads to an unsatisfactory outcome in assisted reproductive technology (ART), which seriously affects patients' physical and mental health and their quality of life.

An individualized COH protocol designed according to the assessment of ovarian reserve is the key to success of POR treatment in the first and multiple cycles. Various protocols of ovarian stimulation, including gonadotrophine-releasing hormone (GnRH) agonist protocols [4], GnRH antagonist protocols [5] and some nontraditional protocols (micro-stimulation, natural cycles, luteal ovulation, etc.), have been proposed for optimizing ART outcomes; however, it remains challenging for poorly responding women to obtain good responses to COH. Many pretreatment modalities (including growth hormone, estrogen, dehydroepiandrosterone, oral contraceptives, etc) have been used prior to ovulation induction to increase the success rate for women undergoing ART [6-8], and yet evidences for the effectiveness of these pretreatments are limited [9].

Acupuncture, an important part of traditional Chinese medicine, has gained worldwide popularity on improving the reproductive outcomes for women undergoing ART. Although high quality research is still needed, increasing evidence has shown that acupuncture is effective at increasing the number of retrieved oocytes [10], improving clinical pregnancy rate and live birth rate [11, 12] and relieving anxiety [13] for women who undergo in vitro fertilization-embryo transfer (IVF-ET). Low ovarian reserve often indicates a poor ovarian response in COH. Although acupuncture has not been studied for POR, its effectiveness in improving ovarian reserve for women with diminished ovarian reserve (DOR) or premature ovarian insufficiency (POI) has been reported. Results of our previous studies showed that acupuncture could significantly lower serum levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH), raise serum levels of anti-müllerian hormone (AMH) and estradiol (E2), and increase antral follicle count (AFC) for patients with DOR [14, 15] or POI [16]. Acupuncture can also regulate the menstrual cycle and improve perimenopausal symptoms [14, 15].

Given its effect on the improvement of ovarian reserve and ART outcomes, acupuncture is expected to be a new choice for the treatment of POR. Besides, POR patients treated by transcutaneous electrical acupoint stimulation (TEAS), a noninvasive acupuncture-like therapy, showed significant improvement on basal serum sex hormones, AMH, the number of retrieved oocytes and fertilization rate [17, 18], which indirectly indicated the effect of acupuncture for POR. Therefore, we aim to conduct a multicenter, randomized, controlled trial (RCT) to evaluate the effectiveness of acupuncture on women with POR.

Methods

Study design

This is an exploratory multicenter RCT comparing acupuncture with non-treatment. A total of 140 women with POR will be recruited from the following 9 hospitals in China: Shanxi Provincial Hospital of Chinese Medicine, Shandong University Reproductive Hospital, Sun Yat-sen Memorial Hospital of the Sun Yat-sen University, Lanzhou University First Hospital, People's Hospital of Henan Province, Huazhong University of Science and Technology Reproductive Medicine Center of Tongji Medical College, Shanghai Shuguang Hospital, Nanjing General Hospital of Nanjing Military Command, and Luoyang Women and Children Health Care Center. This protocol is in accordance with the principles of the Declaration of Helsinki. It has been approved by the ethics committee of each participating hospital. Written informed consent will be obtained from patients prior to enrolment.

Eligible patients will be randomly assigned to receive acupuncture or non-treatment for 12 weeks, followed by an IVF cycle. Outcomes will be assessed at baseline, after acupuncture or non-treatment intervention, and after the IVF cycle. The flowchart and study design schedule are presented in Figure 1 and Table 1, respectively. This protocol has been registered at Acupuncture-Moxibustion Clinical Trial Registry (AMCTR-IPR-18000198).

Participants

This trial plans to recruit 140 women with POR from participating hospitals via poster, network or weChat. The poster will be placed on the bulletin board or other assigned place of hospital. In each participating hospital, at least one staff (usually postgraduate or doctor) will specialize in patient recruitment. Their contact information and clinic for screening visit will be detailed described in the recruitment advertisement.

Participants will be included if they meet the following criteria: (1) diagnosed as POR according to the Bologna criteria published in 2011 by European Society of Human Reproduction and Embryology [1]; (2) age<40 years; (3) a previous POR (≤3 oocytes retrieved in a conventional stimulation protocol); (4) an abnormal ovarian reserve test (i.e., AFC<5–7 follicles or AMH<0.5–1.1 ng/ml) or two episodes of POR after maximal stimulation; (5) suitable for GnRH antagonist protocol judged by physicians of reproductive medicine; (6) voluntarily join the research and sign the informed consent.

Participants will be excluded with any of the following criteria: (1) history of ovarian surgery, radiotherapy or chemotherapy; (2) repeated miscarriages (including biochemical pregnancy) of more than 2 sessions; (3) repeated implantation failure (experienced at least 3 embryo transfer cycles, or not get clinical pregnancy after cumulatively transferring 10 good quality embryos); (4) uterine malformations (unicornate uterus, bicornuate uterus, uterus didelphys, or untreated uterus septus) and other untreated diseases that can damage endometrial cavity such as adenomyosis, submucous myoma, intrauterine adhesions and scared uterus; (5) abnormal chromosomes of the patient or her husband (except chromosomal polymorphism); (6) untreated hydrosalpinx; (7) patients with contraindications for pregnancy or ART; (8) untreated diseases that can negatively affect pregnancy such as high blood pressure, symptomatic heart disease, diabetes, liver or kidney disease, severe anemia, venous thrombosis, cerebral vascular diseases, and cancer; (9) patients who received acupuncture or moxibustion treatment for POR in the latest 3 months.

Randomization and masking

A block randomization stratified by center will be applied in this trial. Eligible participants will be randomly assigned to acupuncture or non-treatment groups in a 1:1 ratio. The scheme of randomization including any planned restriction (e.g., blocking) will be designed and archived by a statistician who will not be involved in the statistical work of this trial. The allocation sequence will be computer-generated. If a participant is eligible for the trial, the appointed researcher (acupuncturist or reproductive physician) in each center will apply for the random number and group assignment via a telephone- and internet- based central randomization system of the Clinical Evaluation Center of the China Academy of Chinese Medical Sciences. A strict view permission will be set for the central randomization system, which allows no one except the top system administrator to view its randomization scheme. In this study, outcome assessors and data analysts will be blinded to group allocation.

Interventions

Acupuncture protocol

Patients in the acupuncture group will receive a 12-week acupuncture treatment before COH. Acupuncture will be performed by registered acupuncturists with over 2 years' experience. Hwato brand disposable acupuncture needles (size 0.25 × 25 mm, size 0.25 × 40 mm, and size 0.30×75 mm) and SDZ-Ⅱ electroacupuncture apparatuses (Suzhou Medical Appliance Factory, Suzhou, China) will be used.

The acupuncture protocol designed for this trial is based on our clinical practice for improving ovarian function for women with DOR [14]. 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), KI3 (Taixi), LR3 (Taichong); and group 2 (in prone position): BL23 (Shenshu) and BL33(Zhongliao). These two groups of acupoints will be used alternatively with group 1 as the initial treatment. All acupoints will be located according to the World Health Organization Standardized Acupuncture Points Location [19]. A needle depth of 10 to 20 mm will be applied for GV20, GV24 and GB13 with transverse insertion, and for LR3 with oblique insertion to the direction of KI1 (Yongquan). BL33 will be needled obliquely downward for approximately 60 to 70 mm into the third posterior sacral foramina. The remaining acupoints will be perpendicularly needled for approximately 30 to 40 mm. After insertion, small, equal manipulations of lifting, thrusting or twirling will be performed on all needles (for GV20, GV24 and GB13, twirling only) to reach deqi, a composite of sensations (including soreness, numbness, distention, heaviness, etc) recognized as an essential component for acupuncture effect. In acupuncture with acupoints of group 2, paired electrodes from the electroacupuncture apparatus will be attached transversely to the needle handles at bilateral BL23 and BL33 with a dilatational wave and a current intensity which patients can tolerate (preferably with the skin around the acupoints shivering mildly without pain). Each acupuncture session will last for 20 minutes. Participants will receive 3 sessions of acupuncture per week (ideally every other day) for 12 consecutive weeks, 36 sessions in total.

Non-treatment control

Participants in the control group will receive non-treatment for 12 weeks before COH.

COH regimen

Participant will start COH with a GnRH antagonist protocol on day 2 or 3 of the menstrual cycle after acupuncture or non-treatment period. GnRH antagonists are commonly used to prevent the premature LH surge which often occurs in poor responders [20,21]. It induces pituitary suppression within a few hours without a flare-up effect, and the suppression is released immediately after their discontinuation [22]. Compared with GnRH agonist, GnRH antagonist protocol shows a shorter treatment time, a lower dosage of Gn and a similar clinical pregnancy rate [5]. In this trial, individualized GnRH antagonist protocol will be administered by physicians of reproductive medicine in each center. Given that participants may become suitable for COH before they complete the 12-week acupuncture or non-treatment, an early termination of acupuncture or non-treatment aiming to seize the opportunity to initiate a COH cycle will be allowed on condition that they complete at least 4-week acupuncture treatment (12 sessions of acupuncture) or non-treatment period.

Permitted and prohibited concomitant treatments

Participants will be discouraged from taking drugs that may interfere with the assessment of acupuncture effect, including Chinese herbs, Chinese patent medicine, sexual hormones, contraceptives, etc. If treatment not recommended in this trial has already been used, relevant information should be recorded in patient's case report form.

Outcome measures

The primary outcome is the number of retrieved oocytes. The secondary outcomes include the assessment of ovarian reserve (AFC and serum levels of AMH, FSH, LH, and E2), other IVF outcomes (fertilization rate, cleavage rate, available embryo rate and high-quality embryo rate) and the score of the self-rating anxiety scale (SAS). Ovarian reserve will be assessed at baseline and after acupuncture or non-treatment period. AFC (via transvaginal ultrasound) and serum levels of FSH, LH and E2 are required to be measured on day 2 to 5 of the menstrual cycle. AMH can be measured on any day of the menstrual cycle. SAS is a self-rating scale for measuring the presence and severity of anxiety [23]. A validated Chinese version of SAS will be used in this trial [24]. It consists of 20 items rated on a 4-point likert scale. When using the scale, participants will be asked to rate each item from 1 to 4 points according to how it applies to them within the past week. The standard score is the sum of the integer part of 1.25 times the raw score (the sum of the 20 items ranging from 20 to 80). The presence of anxiety symptoms is defined as a SAS standard score of greater than 50. A higher score indicates a more serious case of anxiety. SAS will be assessed at baseline and after acupuncture or non-treatment period.

Assessment of safety

Adverse events (AEs) will be obtained by researchers via inquiring participants at each treatment or follow-up visit, or voluntarily reported by participants. Information of AEs will be appropriately documented throughout the trial. Acupuncture-related AEs mainly include intolerable needling pain, bleeding after needle withdrawal, hematoma, local infection, etc. AEs related to COH include pain, organ injury, colporrhagia and infection caused by egg retrieval, ovarian hyperstimulation syndrome, thrombosis, allergy, etc.

Data management and quality control

A pre-trial training will be done for all participating staff on trial protocol, acupuncture manipulation, usage of the central randomization and data management systems, etc. Double data entry will be applied in this trial to ensure the accuracy of data entering. A three-level quality control system (including self-inspection of each center, monitoring by project management group, and auditing by a third party) will be applied to ensure the implementation, record and report of clinical trials conform to the trial protocol, standard operation specifications and relevant laws and regulations. Self-inspection will be done at least once every month by the quality controller of each center. On site or remote monitoring will be done for all centers once every three months by the monitors appointed by the principle investigator. The auditing will

be done by Clinical Evaluation Center of the China Academy of Chinese Medical Sciences at the beginning, middle, and end of the trial. Ethics committee will review conduct especially on safety, rights and wellbeing of the participant at the middle and the end of the trial. Regular reminders via WeChat will be used to improve participant compliance. For cases which discontinue or deviate from the protocol, causes and relevant outcome data will be recorded in case report form as much as possible.

Statistical methods

Sample size calculation

There are no available clinical studies on the effect of acupuncture for POR. Base on results of a TEAS study on POR [18], we assumed the mean (SD) number of retrieved oocytes is 4.88 (1.84) in the acupuncture group and 3.95 (1.66) in the control group. A sample size of 57 participants per group will be needed to provide 80% power and a two-sided significance level of 5%. Allowing for a 20% dropout rate, 140 participants will be recruited with 70 participants per group.

Statistical analysis

Statistical analysis will be performed by a statistician blinded to group assignments using SAS version 9.4 (SAS Institute Inc). All analysis will be based on the intention-to-treat principle. Missing data will be imputed using the multiple imputation method. Continuous data will be presented with mean and standard deviation, or median and interquartile range; categorical data will be presented with number and percentage. Comparisons between groups will be analyzed using the independent t test or wilcoxon rank-sum test for continuous variables, and chi-square test or Fisher exact test for categorical variables. All statistical tests will be two-sided, and $p < 0.05$ will be considered statistically significant.

Discussion

This trial aims to find out whether acupuncture can improve follicular response to the COH during IVF cycles for women with POR. Increasing studies have shown that acupuncture could improve ovarian reserve and reproductive outcomes in women undergoing IVF. However, the study population of these trials generally did not focus on women with POR. Up to date, there is only one literature (a study protocol published in 2018) [25] aiming to assess the effect of acupuncture for POR. This trial is the second study using acupuncture treating POR. Compared with the published POR research [25], this trial will use the same control (non-treatment) and primary outcome (the number of retrieved oocytes), but different acupuncture protocol and more secondary outcomes to better assess the acupuncture effect for POR. In this trial, non-treatment rather than placebo will be used as control due to the following reasons: placebo acupuncture is difficult to implement [26], and women are usually suggested to rest for 2-3 months between the two IVF cycles in clinical practice. It is worth mentioning that the acupuncture protocol of this trial is a mature protocol which has been used in our clinical practice for more than 8 years, and it is validated to be effective for improving ovarian function [14, 15].

There are some limitations in this trial. We will only include POR women aged under 40 and suitable for GnRH antagonist protocol, which may weaken the representative of the sample. Based on clinical practice, the acupuncture protocol comprising two series of acupoints seems a little sophisticated for a RCT, and it increases the difficulty in implementing a placebo acupuncture. The results of this trial can not expel the placebo effect and thus may exaggerate the effect of acupuncture by using non-treatment as control. This trial will not assess the effect of acupuncture on the end points of living birth rate or clinical pregnancy rate for POR women due to limitation of short observation period and too much confounding factor during IVF-ET.

In conclusion, results of this trial will show the effect of acupuncture versus non-treatment in increasing number of retrieved oocytes and improving ovarian reserve for women with POR. This study will contribute to the research of acupuncture for POR worldwide via publishing results in a peer-reviewed journal.

Trial status

This trial is currently recruiting participants. The protocol version number and date: V1.0, May 9, 2018. Date of recruitment began: October 1, 2018. Estimated completion date of recruitment: February 29,2021. Estimated study completion date: October 30, 2021.

List Of Abbreviations

POR: poor ovarian response; COH: controlled ovarian hyperstimulation; IVF: in vitro fertilization; ART: assisted reproductive technology; GnRH: gonadotrophine-releasing hormone; IVF-ET: in vitro fertilization-embryo transfer; DOR: diminished ovarian reserve; POI: premature ovarian insufficiency; FSH: follicle stimulating hormone; LH: luteinizing hormone; AMH: anti-müllerian hormone; E2: estradiol; AFC: antral follicle count; TEAS: transcutaneous electrical acupoint stimulation; 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 ethics committees of all the participating centers. Written informed consent will be obtained from patients prior to enrolment. The name of the ethics committee and the reference number were listed as follows:

Ethics committee (EC) of Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences (2018-05-21-1);

EC of Shanxi Provincial Hospital of Chinese Medicine (AF/SC-04/01);

EC of Reproductive Hospital Affiliated to Shandong University (2018-05-21-1);

EC of Sun Yat-sen Memorial Hospital of the Sun Yat-sen University (2019-28);

EC of the first Hospital Affiliated to Lanzhou University (2018-05-21-1);

EC of reproductive medicine of Henan Provincial People's Hospital (SYZ-LL-2019012411);

EC of Reproductive Medicine Center of Tongji Medical College Affiliated to Huazhong University of Science and Technology (2018-05);

EC of Shanghai Shuguang Hospital (2018-05-21-1);

EC of Nanjing General Hospital of Nanjing Military Command (2018-05-21-1);

EC of Luoyang Women and Children Health Care Center (2018-05-21-1).

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the National key research and development program (NO. 2017YFC1703600) by the Ministry of Science and Technology of the People's Republic of China. The funder has no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

YGF and BYL conceived and designed the study protocol. HFX and CSZ drafted the manuscript. HFX completed the trial registration. HFX and CSZ and substantively revised the manuscript. LYH and HSY designed statistical plan. TSS, HDW, YL, YB, GQT, LC, FZ, CZ, CLZ, YQY, MZH and LY participated in data acquisition and provided administrative, technical, or material support. All authors have read and approved the submitted version of this manuscript and agreed both to be personally accountable for their own contributions and to ensure that questions related to the accuracy or integrity of any part of the work.

Acknowledgements

Not applicable.

References

1. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L, et al. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod. 2011;26(7):1616-24.
2. Polyzos NP, Devroey P. A systematic review of randomized trials for the treatment of poor ovarian responders: is there any light at the end of the tunnel? Fertil Steril. 2011;96(5):1058-61 e7.
3. Jaiswar SP, Natu SM, Sujata, Sankhwar PL, Manjari G. Prediction of Poor Ovarian response by Biochemical and Biophysical Markers: A Logistic Regression Model. J Obstet Gynaecol India. 2015;65(6):411-6.
4. Feldberg D, Farhi J, Ashkenazi J, Dicker D, Shalev J, Ben-Rafael Z. Minidose gonadotropin-releasing hormone agonist is the treatment of choice in poor responders with high follicle-stimulating hormone levels. Fertil Steril. 1994;62(2):343-6.
5. Xiao J, Chang S, Chen S. The effectiveness of gonadotropin-releasing hormone antagonist in poor ovarian responders undergoing in vitro fertilization: a systematic review and meta-analysis. Fertil Steril. 2013;100(6):1594-601 e1-9.
6. Sonmezer M, Ozmen B, Cil AP, Ozkavukcu S, Tasci T, Olmus H, et al. Dehydroepiandrosterone supplementation improves ovarian response and cycle outcome in poor responders. Reprod Biomed Online. 2009;19(4):508-13.
7. Chang EM, Han JE, Won HJ, Kim YS, Yoon TK, Lee WS. Effect of estrogen priming through luteal phase and stimulation phase in poor responders in in-vitro fertilization. J Assist Reprod Genet. 2012;29(3):225-30.
8. Fanchin R, Mendez Lozano DH, Schonauer LM, Cunha-Filho JS, Frydman R. Hormonal manipulations in the luteal phase to coordinate subsequent antral follicle growth during ovarian stimulation. Reprod Biomed Online. 2005;10(6):721-8.
9. Jeve YB, Bhandari HM. Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis. J Hum Reprod Sci. 2016;9(2):70-81.
10. Zhou L, Xia Y, Ma X, Tang L, Lu J, Tang Q, et al. Effects of "menstrual cycle-based acupuncture therapy" on IVF-ET in patients with decline in ovarian reserve. Zhongguo Zhen Jiu. 2016;36(1):25-8.
11. Manheimer E, Zhang G, Udoff L, Haramati A, Langenberg P, Berman BM, et al. Effects of acupuncture on rates of pregnancy and live birth among women undergoing in vitro fertilisation: systematic review and meta-analysis. BMJ. 2008;336(7643):545-9.
12. Xie ZY, Peng ZH, Yao B, Chen L, Mu YY, Cheng J, et al. The effects of acupuncture on pregnancy outcomes of in vitro fertilization: a systematic review and meta-analysis. BMC Complement Altern Med. 2019;19(1):131.

13. Isoyama D, Cordts EB, de Souza van Niewegen AM, de Almeida Pereira de Carvalho W, Matsumura ST, Barbosa CP. Effect of acupuncture on symptoms of anxiety in women undergoing in vitro fertilisation: a prospective randomised controlled study. Acupunct Med. 2012;30(2):85-8.
14. Li X, Xu H, Fang Y, Shang J, Yang H, Zhou X, et al. Acupuncture with regulating menstruation to promote pregnancy for diminished ovarian reverse: a prospective case series study (Article in Chinese). China Journal of Traditonal Chinese Medicine and Pharmacy. 2017;37(10):1061-5.
15. Chen Y, Fang Y, Yang J, Wang F, Wang Y, Yang L. Effect of acupuncture on premature ovarian failure: a pilot study. Evid Based Complement Alternat Med. 2014;2014:718675.
16. Yang H, Fang Y, Xu H, Li X, Shang J, Yin Y. Systematic evaluation on the clinical efficacy of acupoint stimulation therapy for treatment of premature ovarian insufficiency on the basis of network Meta-analysis. World Journal of Acupuncture-Moxibustion. 2017;27(3):26-39.
17. Jiao J, Jia T, Feng X, Sun W. Observation the effects and the changes of the TEAS in serum sex hormone sinus Follicular number and AMH of the patients with ovarian poor response in 90 cases (Article in Chinese). Chinese Journal of Birth Health & Heredity. 2017;8(25):114-6.
18. Lian F, Li R. Treatment of Poor Ovarian Response with kidney Deficiency by Transcutaneous Acupoint Electrical Stimulation Combined Intracavitary Physiotherapy (Article in Chinese). Chinese Journal of Integrated Traditional and Western Medicine. 2017;37(5):522-5.
19. Pacific WROftW. WHO Standard Acupuncture Point Locations in the Western Pacific Region. Manila: World Health Organization; 2008.
20. Al-Inany H, Aboulghar M. GnRH antagonist in assisted reproduction: a Cochrane review. Hum Reprod. 2002;17(4):874-85.
21. Nikolettos N, Al-Hasani S, Felberbaum R, Demirel LC, Kupker W, Montzka P, et al. Gonadotropin-releasing hormone antagonist protocol: a novel method of ovarian stimulation in poor responders. Eur J Obstet Gynecol Reprod Biol. 2001;97(2):202-7.
22. Pu D, Wu J, Liu J. Comparisons of GnRH antagonist versus GnRH agonist protocol in poor ovarian responders undergoing IVF. Hum Reprod. 2011;26(10):2742-9.
23. Zung WW. A rating instrument for anxiety disorders. Psychosomatics. 1971;12(6):371-9.
24. Liu L, Pang R, Sun W, Wu M, Qu P, Lu C, et al. Functional social support, psychological capital, and depressive and anxiety symptoms among people living with HIV/AIDS employed full-time. BMC Psychiatry. 2013;13:324.
25. Lee H, Choi TY, Shim EH, Choi J, Joo JK, Joo BS, et al. A randomized, open phase IV exploratory clinical trial to evaluate the efficacy and safety of acupuncture on the outcome of induction of ovulation in women with poor ovarian response: A study protocol for a randomized controlled trial. Medicine (Baltimore). 2018;97(34):e11813.
26. Chae Y. The dilemma of placebo needles in acupuncture research. Acupunct Med. 2017;35(5):382-3.

Tables

Table 1. Study design schedule

Period	Screening	Baseline	Intervention	COH
Week (*W*)		*W*0	*W*12	*W*14
Informed consent	×			
Eligibility	×			
Demography and medical history	×			
AFC		×	×	
Serum level of AMH		×	×	
Basal serum levels of FSH, LH and E2		×	×	
Score of SAS		×	×	
Number of retrieved oocytes				×
Number of fertilized oocytes				×
Number of cleavage				×
Number of available embryos				×
Number of high-quality embryos				×
AEs		×	×	×
Compliance		×	×	×

AFC, antral follicle count; AMH, anti-müllerian hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estradiol; SAS, self-rating anxiety scale; AEs, adverse events.

Figures

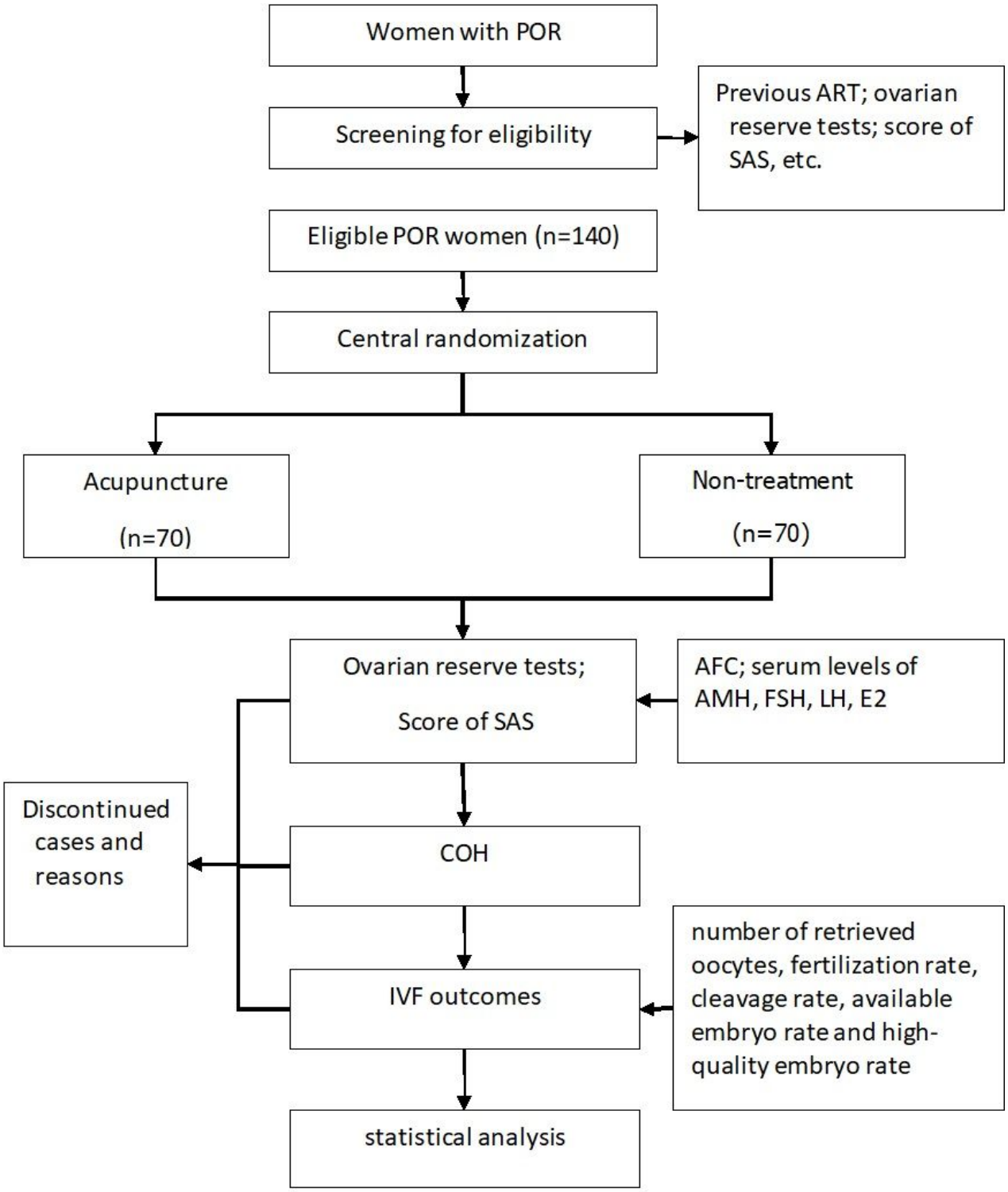

Figure 1

Trial flow chart

Supplementary Files

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

- SPIRITchecklist20200509.docx