BMC Complementary Medicine and Therapies

STUDY PROTOCOL Open Access

# Efficacy and safety of HYYK formula for residual follicle revival in premature ovarian insufficiency: a multicenter, randomized, double-blind, placebo-controlled trial protocol

Yin Peng[1], Jian Liu[2], Yang Li[1], Guangning Nie[2], Fangping Cheng[2], Daning Feng[2], Qiaolin Du[2], Jian Huang[2] and Hongyan Yang[2,3*]

## Abstract

**Background** Premature ovarian insufficiency (POI), a condition impacting women under 40, is rising globally, posing significant risks to fertility, bone health, and cardiovascular function. Conventional hormone replacement therapy (HRT) alleviates symptoms but fails to restore ovarian function. Our prior studies have demonstrated that the Huyang Yangkun (HYYK) formula effectively supports menstrual cycle frequency and alleviates menopause-related symptoms in POI patients. This study aims to evaluate the efficacy and safety of the HYYK formula, a traditional Chinese medicine (TCM) approach, in enhancing residual follicle activity in POI patients.

**Methods** This multicenter, randomized, double-blind, placebo-controlled trial will enroll 102 women with POI, who will be randomly assigned to receive either the HYYK formula or a placebo for 24 weeks. Primary outcomes will be assessed through Hoogland and Skouby scores (indicative of residual follicle activity) and menstrual cycle regularity. Secondary outcomes include hormone levels (AMH, FSH, LH, E2), ovarian volume, antral follicle count, and clinical symptom scales, such as the Greene Scale and the Female Sexual Function Index. Safety assessments will involve routine physical exams and adverse event monitoring.

**Discussion** This is the first multicenter, randomized, double-blind, placebo-controlled study to investigate the efficacy of TCM in stimulating residual follicle recovery in POI patients. The trial rigorously investigates the potential of the HYYK formula as a therapeutic alternative for POI, aiming to deliver high-quality evidence that supports the use of TCM in POI management. This study will concentrate on stimulating residual follicle development in POI patients, offering a viable approach to delaying ovarian decline and generating compelling clinical evidence.

**Trial registration** Chinese Clinical Trials.gov ChiCTR2100049604 Registered on August 6, 2021 (http://www.chictr.org.cn).

*Correspondence:
Hongyan Yang
hongyanyang@gzucm.edu.cn

Full list of author information is available at the end of the article

**Keywords** Premature ovarian insufficiency (POI), Traditional Chinese medicine (TCM), Residual follicle, Randomized controlled trial

## Background

Premature ovarian insufficiency (POI) is characterized by the onset of ovarian dysfunction prior to the age of 40, primarily manifested through irregular menstrual cycles (amenorrhea, oligomenorrhea), elevated gonadotropin levels, and diminished fluctuations in estrogen levels [1]. Initial studies suggested that the prevalence of POI in the general population is approximately 1%. However, recent trends indicate a rising incidence, with reports from 2019 revealing that the global prevalence of POI has escalated to as high as 3.7% [2].

The majority of POI patients are women of reproductive age, and the consequent loss of ovarian function severely impacts their fertility. Current theories posit that ovarian function is irreversible. The conventional treatment for POI is hormone replacement therapy (HRT), which alleviates symptoms associated with estrogen deficiency, playing a vital role in relieving vasomotor symptoms, mitigating bone mass loss, and reducing cardiovascular and cerebrovascular risks associated with POI. However, HRT does not enable women with POI to promote the development of residual follicles or restore ovarian function. Currently, guidelines addressing fertility concerns in POI recommend the use of donor eggs for in vitro fertilization and embryo transfer [1]. Due to the scarcity of eggs, many women at risk of POI are now pursuing medical options to preserve their fertility [3].

The decline of ovarian function is a gradual process, leaving residual follicles in atrophic ovaries that retain the potential for development and even fertilization [4–6]. Over time, the lack of inhibitory feedback from functional follicles contributes to the depletion of all remaining follicles. Consequently, strategies that promote predictable ovarian resumption and enable manipulable follicle activation show promise for the treatment of POI. Thus, finding effective methods to stimulate the development of residual follicles and delay ovarian decline remains an urgent clinical challenge in the management of POI [7]. In recent years, innovative technologies such as in vitro activation of follicles (IVA) [8, 9] stem cell transplantation [10], and platelet-rich plasma (PRP) [11–13] injection have emerged to activate dormant residual follicles, offering women with POI the possibility of obtaining mature eggs. While these approaches hold significant promise, current findings primarily stem from basic research and a limited number of clinical case studies, lacking robust safety and efficacy data.

In recent years, clinical practice has highlighted the promising potential of traditional Chinese medicine (TCM) in the treatment of POI [14, 15]. Based on our clinical experience, the Huyang Yangkun (HYYK) formula has been employed for POI treatment over the past decade. Results from our previous placebo-controlled randomized controlled trials (RCTs) demonstrate that the HYYK formula effectively promotes menstrual recovery in women with POI (unpublished). Fundamental pharmacodynamic research suggests that the HYYK formula promotes the recovery of follicles in the atrophic ovaries of VCD-induced POI mice and increases the number of primordial and preantral follicles in naturally aging rats. The mechanism of action appears to be closely linked to pathways associated with follicular development and apoptosis, as well as genes related to the quality of follicular cells [16–18]. These findings indicate that the HYYK formula enhances ovarian function, supports the recovery of follicles in atrophic ovaries.

Therefore, we aim to conduct a double-blind, randomized, placebo-controlled trial to evaluate the effectiveness and safety of the HYYK formula in reactivating follicles in women with POI. This study seeks to provide an effective treatment protocol for residual follicle activity in POI patients, along with high-quality clinical evidence.

## Methods

### Study design

This is a multicenter, randomized, double-blind, placebo-controlled trial designed to evaluate the effectiveness of Chinese herbal medicine in promoting residual follicle development in patients with premature ovarian insufficiency. Eligible participants will be recruited and randomly assigned in a 1:1 ratio to either the Chinese herbal medicine group or the placebo group. The intervention lasts for 24 weeks. The overall study design and timeline are presented in Fig. 1.

The trial has been approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (approval document No. BF2020-155-01) and is registered with the Chinese Clinical Trial Registry (ChiCTR2100049604). Participant assessments will be conducted at the Second Affiliated Hospital of Guangzhou University of Chinese Medicine, which includes four locations: Dade Road Headquarters, Ersha Island Branch, Fangcun Branch, and University City Hospital.

### Sample size

The sample size calculation was based on the primary outcome using Hoogland and Skouby scores [19, 20]. Based on our previous unpublished data, we estimate that the average number of active follicles, including

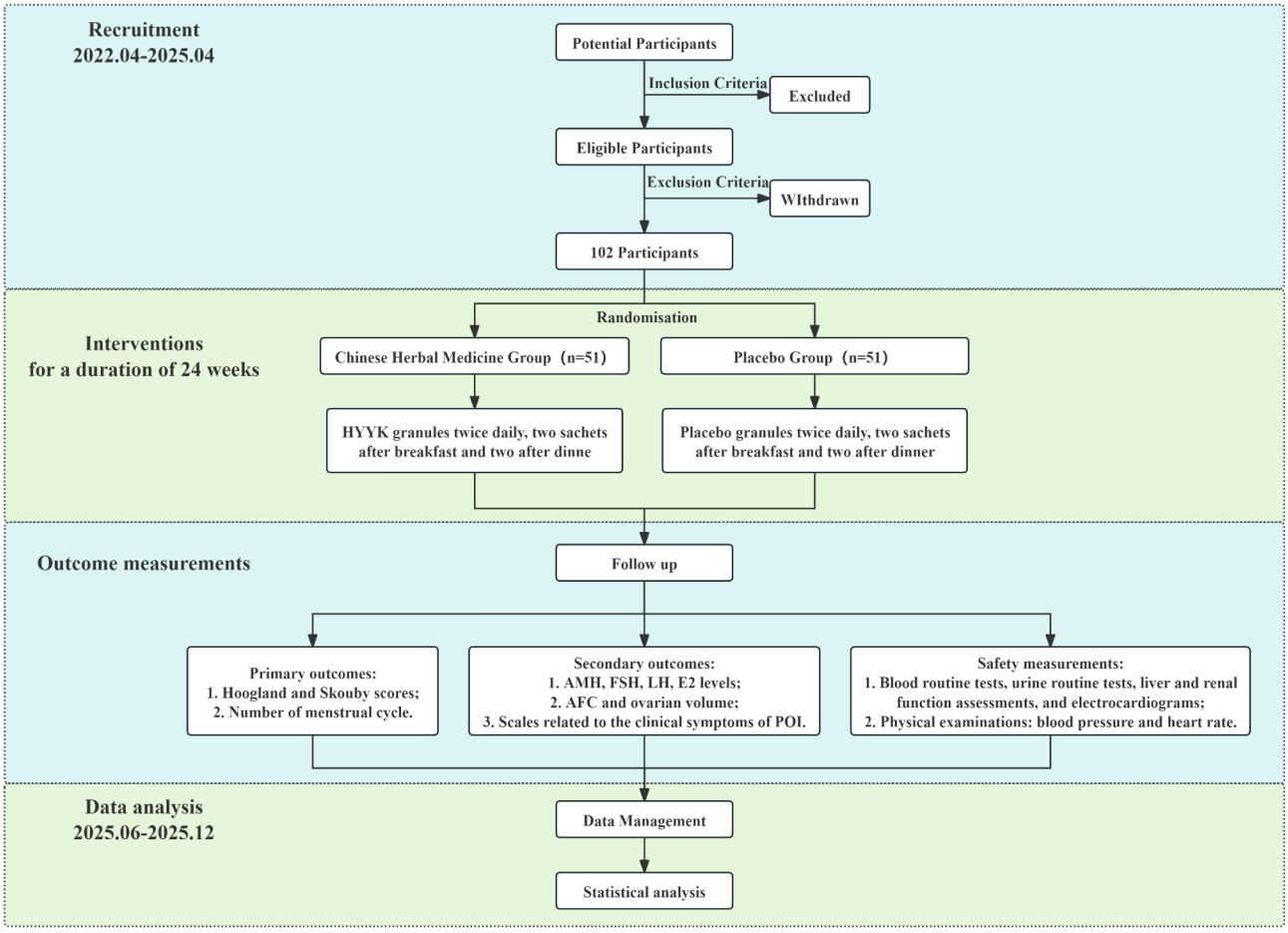


**Fig. 1** Flowchart of the double-blind randomized placebo-controlled trial

luteinized unruptured follicles (LUF) and ovulation, will be 4 in the treatment group and 2.6 in the control group. A two-sided, independent-sample t-test will be used for analysis, assuming a standard deviation of 2, an alpha level of 0.05, a beta level of 0.20, and a statistical power of 90%. According to calculations performed using PASS 11.0(Power Analysis and Sample Size, NCSS, LLC.), the required sample size for each group is 43, leading to a total of 86 participants. Considering an anticipated drop-out rate of 15%, a minimum total of 102 participants will be required for the study.

### Randomisation and blinding

The method of randomization was carried out by researchers from the Clinical Methodology Team of Guangdong Provincial Hospital of Traditional Chinese Medicine, using the PROC PLAN procedure of SAS 9.4 statistical software to generate random numbers. Subjects were randomly assigned to either the treatment group or the placebo control group in a 1:1 ratio. The PROC PLAN procedure in SAS statistical software was executed to produce the random allocation results, which were then entered into a database for use in the central randomization system. The random number table generated by the program was saved in duplicate, with a detailed description and documentation of the method, process, group settings, and randomization results.

The study employs a rigorous double-blind and secondary blinding design, ensuring that all researchers, participants, and statistical analysts remain blinded throughout the trial. Both the placebo and experimental drug are indistinguishable in form, color, and taste. At the first level of blinding, participants are assigned to Group A or Group B, while the second level of blinding conceals the assignment of the actual treatment or placebo. Randomization and blinding are overseen by the statistical team, with blind codes securely sealed and documented according to protocol. In case of emergencies, sealed envelopes containing the blinding codes are held by the clinical trial unit's director.

### Participants

Eligible participants meet the following criteria:

1. Age: 18–42 years, diagnosed with POI before 40;
2. oligomenorrhea or amenorrhoea for at least 4 months;
3. The duration of amenorrhea is less than 6 months;
4. bFSH > 25 U/L (repeated twice 4 weeks apart).
5. Participant written informed consent and agreement to sign medical records release forms.

The criteria for exclusion in this study are listed as follows:

1. Menstrual disorders caused by congenital hypoplasia of the gonads or acquired organic diseases;
2. Menstrual disorders caused by endocrine diseases, including polycystic ovary syndrome (PCOS), hyperprolactinemia, dysfunctional uterine bleeding (DUB), hypogonadotropinism, hyperthyroidism, and other related conditions;
3. Patients who are receiving assisted reproductive technology treatment for infertility;
4. Individuals with a history of abnormal vaginal bleeding, as indicated by clinically relevant abnormalities on endometrial biopsy, or undiagnosed irregular vaginal bleeding in the previous 12 months;
5. Patients with suspected cervical cancer or precancerous lesions, suspected breast malignancy, or known or suspected history of hormone-dependent tumors or malignancies;
6. Individuals with severe mental illness or obvious suicidal tendencies during clinical consideration;
7. Patients with internal medical conditions that are beyond control and/or diagnosis and may interfere with or affect the research and treatment, such as severe liver, kidney, heart, and brain diseases, uncontrolled or untreated hypertension (systolic blood pressure > 160 mmHg and/or diastolic pressure > 100 mmHg), or abnormal thyrotropin that can cause symptoms similar to menopause;
8. Pregnant or lactating women;
9. Individuals who have enrolled in other trials within the past 3 months.

#### Recruitment and screening visit

Potential participants are primarily identified and invited to join the trial through physician referrals. Within one week of referral, a trial investigator contacts them by phone and provides a clear and concise explanation of the trial in easily understandable language. Once the participants have given their consent, the investigators collect detailed clinical information, including medical history, symptoms, and laboratory test results. Based on this information, the participants are then arranged for a screening appointment.

After written informed consent is obtained, participants provide demographic information and medical history, undergo reproductive endocrine-related assessments and safety examinations. Patients with normal safety examinations are randomized into groups, while those who screen positive may be rescreened once in 2 weeks.

#### Interventions

In the Chinese herbal medicine group, participants received HYYK formula granules produced by Jiangsu Jiangyin Tianjiang Pharmaceutical Co., Ltd. (Jiangsu Province, China). The ingredients of the HYYK formula have been reported in our previous study [16]. Each dose contained 110 g of raw herbal materials, which were extracted to yield 12.2 g of granules. These granules were packaged in aluminum foil sachets lined with a medicinal composite membrane. Participants took the HYYK granules twice daily, two sachets after breakfast and two after dinner, for a duration of 24 weeks. A 4-week supply was dispensed at each treatment visit.

In the placebo group, participants received inert placebo granules designed to match the taste, dosage, and packaging of the HYYK granules. The placebo granules, containing only inactive ingredients, were also manufactured by Jiangsu Jiangyin Tianjiang Pharmaceutical Co., Ltd., ensuring consistency in production and quality control across both groups.

It's important to note that in both treatment groups, if patients do not ovulate by the 40th day of their menstrual cycle, progesterone treatment will be required. This will involve taking progesterone for 10–14 days per month, with options including microgranular progesterone at a dose of 200-300 mg/day, dydrogesterone at a dose of 10-20 mg/day.

#### Outcome measurements

The assessment timeline of enrollment, interventions, outcome measurements is visually depicted in Table 1.

#### Primary outcomes

##### *Hoogland and Skouby scores(residual follicle activity)*

Residual follicle activity is assessed focusing on the presence, size, and persistence of follicle-like structures within the ovaries. The Hoogland and Skouby scoring system (H/S score), initially introduced by Hoogland and Skouby in 1993 [21], provides a standardized approach for evaluating ovarian activity. A modified H/S score [20] is determined for each 28-day treatment period in this study.

The participants underwent a transvaginal ultrasound examination approximately every two weeks. Once a follicle with a diameter greater than 10 mm was detected, continuous monitoring was initiated. Follicular outcomes

**Table 1** The schedule of trial enrolment, interventions and outcome measurements

| **Study Period** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Enrollment** | **Allocation** | **24-week Treatment Period** | | | | | | **Follow-up** |
| **Timepoint** | **-4 week** | **Baseline** | **4w** | **8w** | **12w** | **16w** | **20w** | **24w** | **28 week** |
| **Enrollment** | | | | | | | | | |
| Eligibility screen | × | | | | | | | | |
| Informed consent | × | | | | | | | | |
| Baseline assessment | × | | | | | | | | |
| **Allocation** | | × | | | | | | | |
| **Interventions** | | | × | × | × | × | × | × | |
| **Primary Outcomes Measurements** | | | | | | | | | |
| H/Skouby scores | | × | × | × | × | × | × | × | × |
| Menstrual Cycle | | × | × | × | × | × | × | × | × |
| **Secondary Outcomes Measurements** | | | | | | | | | |
| AMH, FSH, LH, E2 | × | | | | | | | | × |
| AFC, ovarian volume | | × | × | × | × | × | × | × | × |
| Greene Scale | | × | | | | × | | | × |
| Female Sexual Function Index | | × | | | | × | | | × |
| **Safety measurements** | | | | | | | | | |
| Blood routine tests | | × | | | | | | | × |
| Urine routine tests | | × | | | | | | | × |
| Liver function assessments | | × | | | | | | | × |
| Renal function assessments | | × | | | | | | | × |
| Electrocardiograms | | × | | | | | | | × |
| Physical examinations | | × | × | × | × | × | × | × | × |
| **Adverse events** | | | × | × | × | × | × | × | × |

were classified as follows: regression after achieving diameters of 10–13 mm; regression after surpassing 13 mm; anovulatory follicular cysts; hemorrhagic anovulatory follicles; ovulation, or concurrent growth of two follicles measuring 10 mm. Anovulatory follicular cysts were characterized as follicles that reached diameters of 25 mm yet failed to ovulate and subsequently regressed [22].

#### *Number of menstrual cycle*

The resumption of the menstrual cycle is a key clinical indicator of ovarian function recovery in patients with premature ovarian insufficiency. Only natural menstruation, influenced by endogenous progesterone, is acknowledged as a valid outcome marker. Monitoring basal body temperature or conducting serum progesterone tests can help confirm the presence of endogenous progesterone.

### Secondary outcomes

The assessment of ovarian function, including evaluations of anti-Müllerian hormone (AMH), follicle-stimulating hormone(FSH), luteinising hormone (LH), estradiol(E2) levels, antral follicle count(AFC) and ovarian volume, was regarded as a secondary outcome. Hormonal analysis—measuring FSH, LH, E2, and AMH levels—was conducted on the third day of the menstrual cycle at both the beginning and conclusion of treatment. AFC and ovarian volume were measured via transvaginal ultrasound between days 2 and 5 of menstruation each month. Menstruation could occur naturally or be induced by progesterone. In cases of persistent amenorrhea, ovarian function assessment can be conducted irrespective of menstruation.

Secondary outcome measures also include the assessment of scales related to the clinical symptoms of POI, such as the Greene Scale and the Female Sexual Function Index. These scales will be distributed as electronic questionnaires to all participants before treatment, three months after treatment, and upon completion of treatment, serving as supplementary evaluation indicators.

### Safety measurements

To ensure the safety of the study, security measures such as blood routine tests, urine routine tests, liver and renal function assessments, and electrocardiograms will be routinely monitored. Physical examinations, including measurements of blood pressure and heart rate, will be conducted at the start and end of the treatment, and as necessary throughout the treatment period.

Adverse events are defined as any unfavorable experience occurring in participants during the trial, irrespective of their connection to the intervention. All observed or reported adverse events, including newly developed conditions or exacerbation of pre-existing symptoms, will be thoroughly documented in the case report form (CRF). In the event of a serious adverse event (AE), the

lead researcher will be notified within 24 h. This information will be recorded in the original files, and any serious adverse events related to the intervention will be reported to the Ethics Review Committee.

## Data management

Upon enrollment, each participant will be assigned a unique identifier to maintain confidentiality across all data records, and no identifying information will be disclosed. Data quality control will occur at two levels: initial verification by investigators during paper case report forms (CRFs) entry, followed by monitoring and validation by the Data Coordinating Center (DCC). CRFs, informed consent forms, and medical reports will be securely stored in a locked cabinet at the Second Affiliated Hospital of Guangzhou University of Chinese Medicine. After the study's completion, all related information will be securely retained at the sites for a period of 10 years.

## Statistical analysis

### *Data processing*

Before conducting statistical analysis, the research data undergoes cleaning and processing to ensure it is transformed into an appropriate format. Additionally, it is necessary to define the population for efficacy and safety analysis and divide it into the following three data analysis sets. ITT (Intent-to-Treat) data set: Includes participants who meet the inclusion criteria, do not meet exclusion criteria, receive treatment for more than four weeks, and have at least one efficacy evaluation metric. PPS (Per-Protocol Set) data set: Consists of participants who complete the medication plan outlined in the protocol and have at least one efficacy evaluation metric. FAS (Full Analysis Set) data set: Comprises participants with at least one safety evaluation metric for safety assessment.

### *Statistical methods*

Statistical analyses will follow a predefined statistical analysis plan and will be conducted by an independent statistician blinded to the allocation and intervention processes, utilizing SPSS version 27 (SPSS Inc., Chicago, IL, USA). All statistical tests will be two-tailed, with significance established at $p < 0.05$.

Descriptive statistics for demographic and baseline characteristics were reported as mean (SD) and frequency (%). Differences between two groups for continuous variables were assessed by Student's t-test for normally distributed variables or Mann-Whitney U test for nonnormally distributed variables. For categorical variables, $\chi 2$ test or Fisher's exact test was used. Adverse events are presented if they were predefined or occurred in at least 5% of participants; Fisher's exact tests were used to compare adverse events when appropriate.

The residual follicle activity rate is defined as the ratio of ovulatory to anovulatory follicles, as well as dominant to subordinate follicles, in women exhibiting varying numbers of follicle waves based on the H/S score. The residual follicle activity rate during cycles of the herbal medicine group was compared using independent samples t-tests and general linear model one-way analyses of variance (GLM 1-Way ANOVA). Scheffe's post hoc tests were conducted when an overall significant difference was identified. To compare the residual follicle activity rates between the two groups, a GLM 2-way ANOVA was employed. Additionally, univariate logistic regression analysis was utilized to assess the differences in residual follicle activity rates between the two groups, focusing on risk difference (RD) and the 95% confidence interval (CI). Cumulative residual follicle activity was analyzed using Kaplan-Meier survival estimation alongside the log-rank test to evaluate the equality of survival functions.

The generalized linear mixed model (GLMM) was employed to estimate the differences in scale scores between the intervention and placebo groups, adjusting for baseline scores and hospital site. For further analysis, a GLMM was utilized with the treatment group as the between-subject factor, time as the repeated within-subject variable, and a group-by-time interaction as the fixed-effect covariate. The variance-covariance matrix was specified as unstructured. In this model, the coefficient associated with the interaction term represents the difference in the slopes of scale scores between the intervention and control groups.

### *Imputation Procedure for Missing Data*

The reasons for withdrawal from both randomization groups will be documented and qualitatively compared. The impact of missing data on study outcomes will be evaluated through sensitivity analyses using augmented datasets. To address missing baseline data, the multiple imputation method will be applied, assuming that the data are missing at random.

## Quality assurance

A third-party organization will be responsible for quality monitoring throughout the study, ensuring adherence to standardized practices, including study processes, inclusion criteria, measurement standards for experimental indicators, data collection methods, and referral procedures for patients with high-risk cardiovascular conditions. The production, storage, distribution, and recovery of investigational products will also follow strict quality control measures.

To ensure the study's quality, pretrial training will be provided to all researchers, covering the trial protocol,

central randomization, medication distribution, follow-up procedures, and data management. The principal investigator will conduct periodic inspections of randomization, blinding, adverse events, and collected data to ensure compliance with the trial protocol, standard operating procedures, and applicable laws and regulations.

## Discussion

To our knowledge, this is the first multicenter, randomized, double-blind, placebo-controlled study investigating the use of traditional Chinese medicine (TCM) to stimulate the recovery of residual follicles in patients with POI. The protocol has been developed in accordance with SPIRIT(Standard Protocol Items: Recommendations for Interventional Trials) reporting guidelines [23] and formatted following TIDieR(Template for Intervention Description and Replication) specifications [24]. Trial outcomes will be reported in line with the revised CONSORT (Consolidated Standards of Reporting Trials) guidelines [25]. This clinical trial protocol adheres to rigorous methodological standards and implements strict quality control measures.

Studies reveal that approximately one-third of women diagnosed with POI maintain a reserve of resting follicles despite impaired ovarian function. This finding emphasizes the importance of tailored treatment strategies, particularly in cases where POI patients display hormonal profiles or ultrasound evidence indicative of antral or resting follicles. In vitro activation (IVA) techniques aim to reactivate dormant follicles, offering a promising fertility option for POI patients. Encouraging results have been observed in certain cases [26]. Stem cell therapy, notably through mesenchymal stem cells (MSCs), holds promise for regenerating ovarian tissue and fostering follicle development in POI patients [27]. Similarly, platelet-rich plasma (PRP) therapy involves administering concentrated plasma derived from a patient's own blood, which may rejuvenate the ovaries by releasing growth factors that promote tissue repair and cellular proliferation [28]. Despite successful pregnancy outcomes in some cases, broader clinical validation remains limited. Additional clinical trials are necessary to develop standardized protocols, evaluate long-term effectiveness, and confirm safety in human applications [29, 30].

TCM has long been a vital pillar of healthcare in China and has garnered global interest for its potential in addressing age-related disorders, including menopause, POI and other hormone-related conditions. Zishen Yutai Pills (ZYP) is commonly used in reproductive treatments and has shown potential in enhancing ovarian function and improving fertility rates through hormonal regulation [31]. Erxian Decoction, administered alone or in combination with other treatments, has demonstrated effectiveness in alleviating POI symptoms, improving serum hormone levels, and preserving ovarian reserve. When paired with acupuncture, it has also proven beneficial in enhancing ovarian response [32]. Research highlights TCM's impact on key pathways, such as PI3K/Akt/mTOR and TGF-β/Smads, which contribute to follicle activation and reduce follicular atresia [33]. While TCM interventions show promise in modulating ovarian function and mitigating POI symptoms, the evidence supporting their efficacy often derives from studies with inconsistent methodological standards. More robust, large-scale clinical trials are necessary to validate these findings and elucidate the underlying mechanisms. Currently, most studies display low methodological quality, and there remains a shortage of clinical trials confirming TCM's effectiveness in reactivating residual ovarian follicles.

The HYYK formula, a widely practiced treatment with more than a decade of clinical application, originates from the renowned traditional Chinese prescription, Danggui Buxue Tang. Composed of seven herbs—Astragali Radix, Herba Epimedii, Dioscoreae Rhizoma, Semen Cuscutae, Rehmanniae Radix, Angelicae Sinensis Radix, and Glehniae Radix—at a 5:1:1:1:1:1:1 ratio, its effectiveness in alleviating symptoms and restoring menstrual cycles in women with POI has been validated through prior clinical trials. Animal pharmacological studies [15, 16] have further shown that HYYK can increase primordial, antral, and mature follicle counts in VCD-induced premature ovarian failure rat models, improve irregular estrous cycles, and elevate AMH levels. Drawing on clinical and experimental evidence, we propose that HYYF can preserve residual follicular function in patients with POI, promote follicular development, restore menstrual cycles, and enhance fertility for POI patients. This study aims to evaluate HYYF's efficacy in stimulating residual follicle development, with a focus on follicular growth and maturation as primary outcome measures, ultimately offering robust evidence to substantiate the role of traditional Chinese medicine in POI treatment.

This study includes several key considerations. The POI patients enrolled had experienced amenorrhea for less than six months, indicating that they were in the late stage of menopausal transition. At this stage, patients retain a certain level of endogenous estrogen but lack sufficient progesterone, which increases the risk of endometrial hyperplasia. To safeguard the endometrium and prevent hyperplasia, we will promptly administer an appropriate dose of progesterone if the amenorrhea duration exceeds 30 days and endometrial thickness surpasses 8 mm. We chose dydrogesterone for this purpose because, at standard doses, it has a neutral effect on the female reproductive endocrine axis. Furthermore, patients with fertility aspirations were not excluded, even if they opted against assisted reproductive technologies.

Since HYYF contains no substances that could harm pregnancy, patients who conceive during the follow-up period are allowed to leave the trial, with their data analyzed according to ITT or PP (Per-Protocol) datasets.

## Trial status

This study is ongoing. The protocol has been peer-reviewed by the funding body(Guangdong Provincial Hospital of Traditional Chinese Medicine) and independent reviewers on March 30, 2019. The trial has been approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (approval document No. BF2020-155-01) on August 21, 2020, and registered on Chinese Clinical Trials.gov with number ChiCTR2100049604 on August 6, 2021 (http://www.chictr.org.cn). However, due to the impact of the COVID-19 epidemic, the research was not carried out immediately. Based on the discussion among experts and project team members, we have made two revisions to the protocol. In April 2022, we received the ethical approval of revised protocol. Recruitment for the trial started in April 2022, the first patient was included on May 13, 2022, and the study is expected to be completed in December 2025. The ethical approval of the final version was approved in April 2023, and the protocol was updated on Chinese Clinical Trials.gov synchronously.

### Abbreviations

| | |
|---|---|
| POI | Premature ovarian insufficiency |
| HRT | Hormone replacement therapy |
| HYYK | Huyang yangkun |
| TCM | Traditional chinese medicine |
| IVA | In vitro activation |
| RCT | Randomized controlled trial |
| LUF | Luteinized unruptured follicles |
| PCOS | Polycystic ovary syndrome |
| DUB | Dysfunctional uterine bleeding |
| H/S score | Hoogland and Skouby scoring system |
| AMH | Anti-Müllerian hormone |
| FSH | Follicle-stimulating hormone |
| LH | Luteinising hormone |
| E2 | Estradiol |
| AFC | Antral follicle count |
| CRF | Case report form |
| AE | Adverse event |
| DCC | Data coordinating center |
| ITT | Intent-to-treat |
| PPS | Per-protocol set |
| FAS | Full analysis set |
| GLM 1-Way ANOVA | General linear model one-way analyses of variance |
| RD | Risk difference |
| CI | Confidence interval |
| GLMM | Generalized linear mixed model |
| MSC | Mesenchymal stem cell |
| SPIRIT | Standard protocol items: recommendations for interventional trials |
| TIDieR | Template for intervention description and replication |
| CONSORT | Consolidated standards of reporting trials |
| PRP | Platelet-rich plasma |
| ZYP | Zishen Yutai Pills |
| AMH | Anti-Müllerian hormone |

### Acknowledgements

The authors are very grateful to all the researchers involved in this study.

### Author contributions

YHY designed the study; PY, LY, CFP, FDN, DQL and HJ participated in the statistical and study design; YHY and LJ obtained ethical approval; YHY, PY and LJ will draft recruitment advertisements; YHY, LJ, NGN, CFP, FDN, DQL, HJ will help recruit participants; PY drafted the manuscript; YHY, LJ and LY provided technical and methodological support. YHY revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

### Funding

This study was supported by the Clinical Research Special Project Fund from Guangdong Provincial Hospital of Chinese Medicine (YN10101912), the National Natural Science Foundation of China (82274568), Guangdong Provincial Basic and Applied Research Fund Project(2022A1515220058), and Special Research Project for Traditional Chinese Medicine Science and Technology of Guangdong Provincial Hospital of Traditional Chinese Medicine (YN2023ZH09).

### Data availability

No datasets were generated or analysed during the current study.

## Declarations

### Ethics approval and consent to participate

The trial has been approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (approval document No. BF2020-155-01). All participants will sign written informed consent.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

### Author details

[1]The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510006, Guangdong Province, People's Republic of China
[2]Department of Gynecology, Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangzhou 510120, Guangdong Province, People's Republic of China
[3]Guangdong Provincial Key Laboratory of Clinical Research on Traditional Chinese Medicine Syndrome, Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangzhou 510120, Guangdong Province, People's Republic of China

Received: 13 November 2024 / Accepted: 29 January 2025
Published online: 11 February 2025

### References

1. ESHRE Guideline. Management of women with premature ovarian insufficiency. Hum Reprod. 2016;31:926–37.
2. Li M, Zhu Y, Wei J, Chen L, Chen S, Lai D. The global prevalence of premature ovarian insufficiency: a systematic review and meta-analysis. Climacteric. 2023;26:95–102.
3. The ESHRE Guideline Group on Female Fertility Preservation, Anderson RA, Amant F, Braat D, D'Angelo A et al. Chuva De Sousa Lopes SM,. ESHRE guideline: female fertility preservation†. Hum Reprod Open. 2020;2020:hoaa052.
4. Massin N, Gougeon A, Meduri G, Thibaud E, Laborde K, Matuchansky C, et al. Significance of ovarian histology in the management of patients presenting a premature ovarian failure. Hum Reprod. 2004;19:2555–60.
5. Bachelot A, Rouxel A, Massin N, Dulon J, Courtillot C, Matuchansky C, et al. Phenotyping and genetic studies of 357 consecutive patients presenting with premature ovarian failure. Eur J Endocrinol. 2009. https://doi.org/10.1530/EJE-09-0231

6. Zhang X, Han T, Yan L, Jiao X, Qin Y, Chen Z-J. Resumption of ovarian function after ovarian Biopsy/Scratch in patients with premature ovarian insufficiency. Reprod Sci. 2019;26:207–13.
7. Huang Q, Chen S, Chen J, Shi Q, Lin S. Therapeutic options for premature ovarian insufficiency: an updated review. Reprod Biol Endocrinol. 2022;20:28.
8. Fàbregues F, Ferreri J, Méndez M, Calafell JM, Otero J, Farré R. In Vitro Follicular activation and stem cell therapy as a Novel treatment strategies in diminished Ovarian Reserve and primary ovarian insufficiency. Front Endocrinol. 2021;11:617704.
9. Wu S, Wang Y, Ding H, Sun N, Liu Y, Wang L, et al. The efficacy and safety of the mTOR Signaling Pathway Activator, MHY1485, for in vitro activation of human ovarian tissue. Front Genet. 2021;11:603683.
10. Cacciottola L, Vitale F, Donnez J, Dolmans MM. Use of mesenchymal stem cells to enhance or restore fertility potential: a systematic review of available experimental strategies. Hum Reprod Open. 2023;2023:hoad040.
11. Sfakianoudis K, Simopoulou M, Grigoriadis S, Pantou A, Tsioulou P, Maziotis E, et al. Reactivating ovarian function through autologous platelet-rich plasma intraovarian infusion: Pilot Data on premature ovarian insufficiency, Perimenopausal, Menopausal, and poor Responder women. J Clin Med. 2020;9:1809.
12. Ahmadian S. Intra-ovarian injection of platelet-rich plasma into ovarian tissue promoted rejuvenation in the rat model of premature ovarian insufficiency and restored ovulation rate via angiogenesis modulation. 2020. https://doi.org/10.1186/s12958-020-00638-4
13. Barrenetxea G, Celis R, Barrenetxea J, Martínez E, De Las Heras M, Gómez O, et al. Intraovarian platelet-rich plasma injection and IVF outcomes in patients with poor ovarian response: a double-blind randomized controlled trial. Hum Reprod. 2024;39:760–9.
14. Li H-F, Shen Q-H, Chen W-J, Chen W-M, Feng Z-F, Yu L-Y. Efficacy of traditional Chinese medicine tonifying kidney (Bushen) and activating blood (Huoxue) prescription for premature ovarian insufficiency: a systematic review and Meta-analysis. Evid Based Complement Alternat Med. 2020;2020:1789304.
15. Ju S, He J, Wang H, Yang L, Guo A, Guo Y, et al. Potential therapeutic drug targets and pathways prediction for premature ovarian insufficiency —based on network pharmacologic method. J Ethnopharmacol. 2023;304:116054.
16. Wang L, Li M, Liu J, Nie G, Li Y, Yang H. Protective effect of Huyang Yangkun Formula on ovarian function in premature ovarian insufficiency rats based on apoptotic mechanism. J Ethnopharmacol. 2021;280:114477.
17. Li M, Xie L, Li Y, Liu J, Nie G, Yang H. Synergistic effect of Huyang Yangkun Formula and embryonic stem cells on 4-vinylcyclohexene diepoxide induced premature ovarian insufficiency in mice. Chin Med. 2020;15:83.
18. Xie L, Wu S, Cao D, Li M, Liu J, Nie G et al. Induced premature ovarian insufficiency in rats via the Hippo–JAK2/STAT3 signaling pathway. 2019.
19. Spona J, Binder N, Höschen K, Feichtinger W. Suppression of ovarian function by a combined oral contraceptive containing 0.02 mg ethinyl estradiol and 2 mg chlormadinone acetate given in a 24/4-day intake regimen over three cycles. Fertil Steril. 2010;94:1195–201.
20. Duijkers IJM, Klipping C, Rautenberg T, Schug BS, Kochhar PS, Osterwald H, et al. Effect on ovarian activity and ovulation inhibition of different oral dosages of levonorgestrel. Contraception. 2022;110:6–15.
21. Hoogland HJ, Skouby SO. Ultrasound evaluation of ovarian activity under oral contraceptives. Contraception. 1993;47:583–90.
22. Baerwald AR, Olatunbosun OA, Pierson RA. Effects of oral contraceptives administered at defined stages of ovarian follicular development. Fertil Steril. 2006;86:27–35.
23. Chan A-W, Tetzlaff JM, Gotzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. BMJ. 2013;346:jan08.
24. Hoffmann TC, Glasziou PP, Boutron I, Milne R, Perera R, Moher D, et al. Better reporting of interventions: template for intervention description and replication (TIDieR) checklist and guide. BMJ. 2014;348(mar07 3):g1687–1687.
25. Butcher NJ, Monsour A, Mew EJ, Chan A-W, Moher D, Mayo-Wilson E, et al. Guidelines for reporting outcomes in Trial Reports: the CONSORT-Outcomes 2022 extension. JAMA. 2022;328:2252.
26. Xu S, Gong Z, Wang F, Cao M, Liu J, Chen C, et al. Intervention of muscle-building and antifrailty exercise combined with Baduanjin for frailty of different functional levels: study protocol for a randomised controlled trial. BMJ Open. 2023;13:e074827.
27. Polonio AM, García-Velasco JA, Herraiz S. Stem cell Paracrine Signaling for treatment of premature ovarian insufficiency. Front Endocrinol. 2021;11:626322.
28. Maged AM, Mohsen RA, Salah N, Ragab WS. The value of intraovarian autologous platelet rich plasma in women with poor ovarian reserve or ovarian insufficiency: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2024;24:85.
29. Vo KCT, Kawamura K. Vitro Activation Early follicles: from the Basic Science to the clinical perspectives. Int J Mol Sci. 2021;22:3785.
30. Ali I, Padhiar AA, Wang T, He L, Chen M, Wu S, et al. Stem cell-based therapeutic strategies for premature ovarian insufficiency and infertility: a Focus on Aging. Cells. 2022;11:3713.
31. Maharajan K, Xia Q, Duan X, Tu P, Zhang Y, Liu K. Therapeutic importance of Zishen Yutai Pill on the female reproductive health: a review. J Ethnopharmacol. 2021;281:114523.
32. Efficacy of supplemented. Er-xian decoction combined with acupoint application for poor ovarian response. J Physiol Pharmacol. 2020. https://doi.org/10.26402/jpp.2020.2.09
33. Wang Y, Teng X, Liu J. Research Progress on the Effect of Traditional Chinese Medicine on Signal Pathway Related to premature ovarian insufficiency. Evid Based Complement Alternat Med. 2022;2022:1–13.


## Publisher's note