

Kidney-Tonifying Chinese Herbal Therapy Before and During Letrozole Ovarian Stimulation for Infertility Associated With Diminished Ovarian Reserve: A Multicenter Randomized Trial Protocol

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Background: Diminished ovarian reserve (DOR) is a leading cause of female infertility, with an increasing prevalence and a younger age at onset. Traditional Chinese medicine (TCM), based on the theory that ‘the kidney governs reproduction’, shows potential for improving ovarian function, but evidence remains limited by small sample sizes and inadequate blinding.

Methods: This randomized, double-blind, placebo-controlled superiority trial will be conducted at 18 centers in China and will enroll 320 eligible women aged 22–39 years. Participants will be randomized in a 1:1 ratio to receive either Xiehe DOR Kidney-Tonifying Formula granules or matched placebo granules. During the first 3 months, participants will receive only the assigned granules. Participants who remain nonpregnant after the first 3 months will continue to receive the assigned granules and will additionally receive letrozole for three cycles.

Results: The primary outcome is the cumulative clinical pregnancy rate within 6 months after randomization, as confirmed by transvaginal ultrasonography. Secondary outcomes include ovulation, biochemical pregnancy, live birth, and miscarriage rates; ovarian reserve markers (anti-Müllerian hormone, follicle-stimulating hormone, and antral follicle count); TCM syndrome and quality-of-life scores; and safety outcomes. The primary analysis will follow the intention-to-treat principle.

Conclusions: This trial is expected to provide rigorous evidence on the efficacy and safety of TCM for DOR-associated infertility and to inform the development of an integrative treatment strategy.

Clinical Trial Registration: ClinicalTrials.gov (NCT07296614) and the International Traditional Medicine Clinical Trial Registry (ITMCTR2026000661).

Keywords: DOR; infertility; TCM; clinical pregnancy; randomized controlled trial

Introduction

Diminished ovarian reserve (DOR) refers to a decline in oocyte quantity and quality within the ovaries, leading to reduced reproductive potential [1]. Clinically, DOR commonly manifests as shortened menstrual cycles and decreased menstrual volume, together with laboratory and sonographic indicators such as elevated basal follicle-stimulating hormone (FSH), decreased anti-Müllerian hormone (AMH), and reduced antral follicle count (AFC) [2,3]. In recent decades, the incidence of DOR has increased worldwide, and the condition increasingly affects younger women, a trend driven by delayed childbearing,

environmental factors, and the rising prevalence of iatrogenic ovarian injury [4]. As a major cause of infertility, DOR poses a significant challenge in contemporary reproductive medicine.

Current management strategies for women with DOR primarily include pharmacological ovulation induction and assisted reproductive technology (ART) [5]. However, women with DOR typically exhibit poorer ovarian responses to stimulation, higher cycle cancellation rates, and lower clinical pregnancy rates than women with normal ovarian reserve [6]. Even when pregnancy is achieved, this population has an increased risk of early pregnancy loss [7,8,9]. These challenges highlight the unmet need for safe,

effective, and affordable interventions to improve ovarian reserve and increase the likelihood of conception and successful ART outcomes in women with DOR.

Traditional Chinese medicine (TCM) is commonly used in some Chinese clinical settings as an adjunctive approach for reproductive disorders. Within the TCM framework, kidney deficiency (shen xu) is generally regarded as a fundamental pattern underlying DOR-associated infertility, and accompanying patterns such as blood stasis and liver-qi stagnation have also been described [10]. Kidney-tonifying herbal formulas have been associated with changes in menstrual symptoms and ovarian reserve markers in observational studies and small trials [11], but the existing evidence is limited by heterogeneous diagnostic criteria, small sample sizes, and a lack of credible placebo controls.

The Xiehe DOR Kidney-Tonifying Formula (XD-KTF) is a multi-herb formula developed by the study team through the modification and integration of several classical TCM prescriptions, with its therapeutic principle centered on tonifying the kidney and replenishing essence. Its use in the present trial was further informed by our previously proposed two-stage treatment framework of “simultaneous conditioning of follicular development and ovulation” [12]. We hypothesize that residual dormant primordial follicles in the ovaries of women with DOR may be preserved and supported in their development through modulation of the local ovarian microenvironment. Accordingly, kidney-tonifying therapy is administered initially to enhance the ovarian environment and support follicular development, followed by pharmacologic ovarian stimulation to promote follicular growth and ovulation. Letrozole was selected as a standardized oral ovarian-stimulation agent for the second stage [13]. As women with DOR may retain spontaneous ovulation, its role in the present study is not limited to the treatment of anovulation but is intended to provide mild ovarian stimulation during attempts at conception.

In this context, this multicenter, double-blind, randomized controlled trial will compare the cumulative clinical pregnancy rate, ovulation rate, ovarian reserve biomarkers, and other indicators throughout the treatment period in women with DOR-associated infertility attempting conception without ART. The goal is to address the key limitations of previous research and provide high-quality evidence on the role of integrative TCM and Western approaches in the management of DOR.

Methods

Trial Design and Setting

This is a multicenter, randomized, double-blind, placebo-controlled superiority trial. A total of 320 participants will be allocated in a 1:1 ratio to an intervention or control group. This study was registered with ClinicalTrials.gov (NCT07296614, <https://clinicaltrials.gov/study/NCT07296614>).

<https://clinicaltrials.gov/study/NCT07296614>) and the International Traditional Medicine Clinical Trial Registry (ITMCTR2026000661, <https://itmctr.ccebtcn.org.cn/mgt/project/view/2034933752748179456>). The trial will be conducted at 18 hospitals and research institutions across China, with participating centers listed in **Supplementary File 1**. The protocol was developed in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines (see **Supplementary File 2** for the Standard Protocol Items: Recommendations for Interventional Trials [SPIRIT] checklist) [14]. The study flow is shown in Fig. 1.

Study Objectives

Primary Objective

To determine whether the integrative regimen increases the cumulative clinical pregnancy rate compared with the same letrozole-based ovarian stimulation strategy plus placebo.

Secondary Objectives

- To compare changes in AMH, basal FSH, and AFC from baseline to months 3 and 6.
- To compare biochemical pregnancy, ovulation, live birth, and miscarriage outcomes between groups.
- To compare changes in TCM kidney-deficiency syndrome and quality-of-life scores.
- To evaluate adverse events, laboratory abnormalities, vital signs, and treatment tolerability.

Participants and Recruitment

Potential participants will be recruited at the participating centers. Eligible participants will be women aged 22–39 years with a clinical diagnosis of DOR and infertility. As part of the baseline infertility assessment, evidence of at least one patent fallopian tube is required, based on hysterosalpingography, hysterosalpingo-contrast sonography, laparoscopy with chromopertubation, or a documented intrauterine pregnancy within the last three years in the absence of subsequent tubal risk events. Additional tubal evaluation will be conducted if prior evidence is lacking or if clinical history or pelvic ultrasonography indicates potential tubal abnormalities. A TCM kidney-deficiency pattern will be diagnosed according to the Guidelines for Clinical Research of New Chinese Medicines and will require all major symptoms (menstrual irregularity with scanty or light-colored menses and lumbosacral soreness), at least one minor symptom (e.g., dizziness, tinnitus, or low libido), and supportive tongue and pulse findings [15,16].

Eligibility Criteria

Inclusion Criteria

- (1) Women aged 22–39 years, inclusive.
- (2) Infertility, defined as failure to achieve preg-

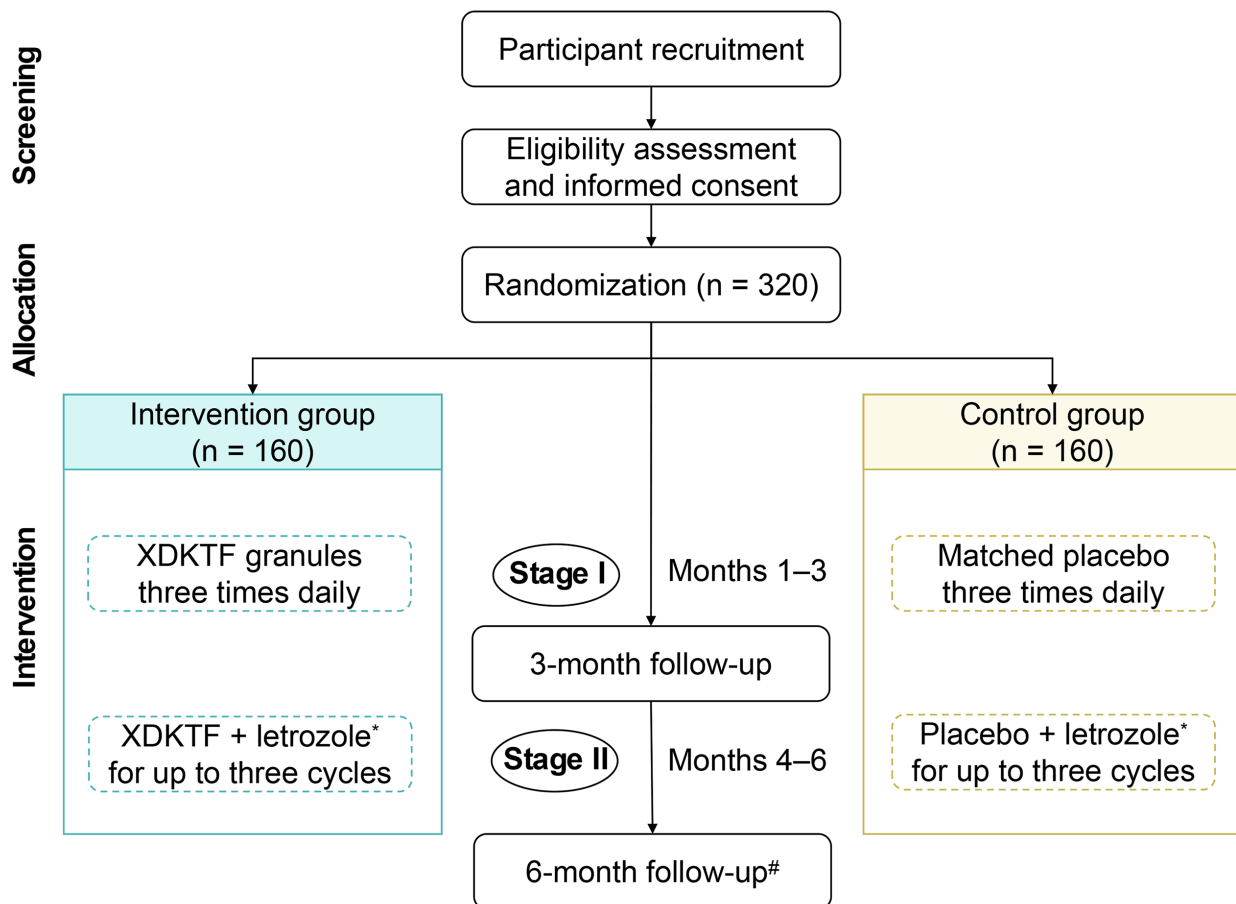


Fig. 1. Study flow chart. *Letrozole 2.5 mg once daily on menstrual-cycle days 3–7. #Pregnancies identified during the trial will be followed to pregnancy outcome. XDKTF, Xiehe DOR Kidney-Tonifying Formula; DOR, diminished ovarian reserve.

nancy after 12 months of regular unprotected intercourse in women aged <35 years, or after 6 months in women aged ≥35 years [17].

(3) DOR defined by at least two of the following: basal FSH 10–25 IU/L, AMH <1.1 ng/mL, or AFC <7 [18].

(4) A TCM kidney-deficiency pattern as defined above.

(5) Ability and willingness to provide written informed consent and comply with trial procedures.

Exclusion Criteria

(1) Premature ovarian insufficiency or ovarian failure, including basal FSH >25 IU/L, or organic ovarian, uterine, endometrial, cervical, or tubal disease considered sufficient to explain infertility, including ovarian tumors, endometrial tuberculosis, intrauterine adhesions, or clinically important endometrial injury.

(2) Infertility attributable to a male factor based on semen analysis according to World Health Organization criteria [19].

(3) DOR attributed to previous pelvic surgery, radiotherapy, chemotherapy, uterine artery embolization, or another specified iatrogenic cause.

(4) Congenital adrenal hyperplasia, Cushing syndrome, or another relevant endocrine disorder.

(5) Clinically important hepatic, renal, cardiovascular, cerebrovascular, hematopoietic, or psychiatric disease.

(6) Known allergy to any component of the investigational product, placebo, or letrozole.

(7) Use of hormonal medications, including estrogen or hormonal contraceptives, within 1 month before enrollment, or use of kidney-tonifying Chinese herbal medicines or proprietary Chinese medicines within 1 month before enrollment.

(8) Current participation or planned participation in another interventional clinical trial.

Interventions

Each participant will be followed for up to 6 months after randomization in two consecutive stages.

Stage 1: Initial Treatment Phase (First 3 Months)

Participants will receive either XDKTF or matched placebo granules according to their randomized allocation and take one 15-g sachet dissolved in warm water three times daily.

XDKTF was formulated through the modification and integration of components from several classical TCM prescriptions, including Guizhi Jia Longgu Muli Decoction from *Jin Gui Yao Lue* (Synopsis of Prescriptions of the Golden Chamber), Huanglian Ejiao Decoction from *Shang Han Lun* (Treatise on Cold Damage), and Zuogui Pill from *Jing Yue Quan Shu* (The Complete Works of Jingyue). The formulation adheres to the TCM principle of tonifying the kidney and replenishing essence, while also incorporating complementary actions of nourishing blood and activating blood circulation. XDKTF granules were produced by Fushoutang Pharmaceutical Henan Co., Ltd. (Yima, China; internal manufacturing batch numbers: 260801–260806) using a standardized extraction and granulation process. The daily prescription contained a total crude-herb equivalent of 196 g, formulated into three 15-g sachets of finished granules. All medicinal materials were required to comply with Chinese Pharmacopoeia standards and were subject to batch qualification and traceability.

The matched placebo was prepared from approximately 5% of the starting herbal-material quantity used for the corresponding XDKTF preparation, with the same relative proportions of the 19 medicinal materials. The herbal materials underwent the same extraction, concentration, drying, and pulverization procedures, after which pharmaceutical-grade dextrin and caramel color were added for granulation. The finished placebo was packaged as 15-g sachets and administered according to the same dosing schedule as XDKTF. Detailed information on XDKTF and the matched placebo is provided in **Supplementary File 3**.

During Stage 1, pregnancy monitoring will follow the same procedures and timing as in Stage 2. If menstruation is delayed, participants will perform a home urine pregnancy test. A positive result will lead to serum beta-human chorionic gonadotropin (β -hCG) testing and transvaginal ultrasound at about 5–7 weeks of gestation to confirm an intrauterine gestational sac.

Stage 2: Letrozole Ovarian Stimulation Phase (Subsequent 3 Months)

Participants who do not achieve pregnancy within the first three months will continue to receive their assigned granules and undergo up to three cycles of letrozole treatment with timed intercourse. Pregnancy will be ruled out before each cycle. Letrozole will be administered orally at a dose of 2.5 mg once daily for five consecutive days, starting on menstrual cycle day 3 [20].

After letrozole treatment, participants will perform daily urinary luteinizing hormone (LH) testing using standardized test kits provided by the study team. Testing will continue until the first positive result or the onset of menstruation. Participants will be advised to have timed intercourse on the day of the positive LH test and during the following 1–2 days. Ovulation will be confirmed by a serum progesterone concentration >5 ng/mL measured 5–7

days after the positive LH test or by transvaginal ultrasonographic evidence of ovulation when clinically indicated. If no urinary LH surge is detected, serum progesterone measurement will be conducted approximately 7 days before the expected onset of the next menstruation.

Routine transvaginal ultrasonography will not be required during every treatment cycle. If ultrasonography performed for clinical indications identifies an excessive ovarian response, the cycle may be cancelled, and participants will be advised to avoid timed intercourse to reduce the risk of multiple pregnancy. Participants will perform a home urine pregnancy test if menstruation is delayed. At the first positive urine test, the assigned study granules will be withheld pending serum β -hCG confirmation. If pregnancy is confirmed, the study granules will be permanently discontinued and no further letrozole will be administered. The date of the last study drug dose and any potential exposure during early pregnancy will be recorded. Transvaginal ultrasonography will be performed at approximately 5–7 weeks of gestation to confirm an intrauterine gestational sac, after which the participant will enter pregnancy follow-up.

Concomitant Care and Treatment Discontinuation

All participants will receive standardized counseling on a balanced diet, moderate physical activity, weight management, smoking and alcohol avoidance, stress management, and adequate sleep. Concomitant therapies that could affect ovarian function or pregnancy outcomes will be documented. Discontinuation of the assigned treatment for reasons other than pregnancy will not constitute withdrawal from follow-up, and participants will continue to be followed for the primary outcome whenever feasible. Initiation of ART during the 6-month trial period will be documented, with pregnancies resulting from ART recorded separately and excluded from the primary outcome analysis.

Randomization and Allocation Concealment

An independent statistician will generate a centralized computer randomization sequence using R software, with variable block sizes of 4 and 6 and two stratification factors: study center and age (22–29, 30–35, and 36–39 years). Allocation will be implemented via an interactive web response system. After eligibility has been confirmed and baseline data have been entered, the system will assign a unique participant number and corresponding medication number. Investigators at participating centers will not have access to the allocation sequence.

Blinding

Participants, treating clinicians, investigators, outcome assessors, data managers, and statisticians will remain unaware of treatment allocation until the database is locked and the prespecified analysis is completed. The active and placebo granules were matched in appearance, color, odor,

taste, packaging, and administration schedule. The low-herbal-content formulation was selected to facilitate sensory matching while minimizing exposure to herbal ingredients. The adequacy of blinding will be evaluated through a coded-sample similarity assessment conducted by evaluators unaware of sample identity. Sealed emergency codes will be available at each center and may be opened only when knowledge of the treatment allocation is required for the management of a serious adverse event. The reason, date, and personnel involved in any unblinding will be documented.

Outcomes

Primary Outcome

The primary outcome is the cumulative clinical pregnancy rate within 6 months post-randomization, defined as the proportion of participants achieving at least one intrauterine gestational sac confirmed by transvaginal ultrasonography. An event will be attributed to the 6-month window if the first positive serum β -hCG result is obtained within 6 months following randomization, even when ultrasonographic confirmation occurs thereafter.

This endpoint is intended to estimate the effect of the overall 6-month sequential treatment strategy. To delineate the timing of events across the two phases, the cumulative rate by month 3 will be reported separately. For participants who remain nonpregnant at month 3, pregnancies occurring during months 4–6 will also be summarized descriptively as an exploratory phase-specific analysis, without being interpreted as a randomized between-group comparison.

Secondary Outcomes

(1) Changes in AMH, basal FSH, and AFC from baseline to months 3 and 6.

(2) Ovulation rate during the three letrozole cycles, defined as the proportion of monitored menstrual cycles with ovulation confirmed by a serum progesterone concentration >5 ng/mL measured 5–7 days after a positive urinary LH test, or about 7 days before the expected onset of the next menstruation if no LH surge is detected. Transvaginal ultrasonographic evidence of ovulation may also be used when clinically indicated.

(3) Cumulative biochemical pregnancy rate within 6 months after randomization, defined as the proportion of participants with a serum β -hCG concentration >25 IU/L before ultrasonographic confirmation.

(4) Live birth rate, defined as the proportion of participants who deliver at least one infant after 22 completed weeks of gestation, with any sign of life after birth. Births before 37 completed weeks of gestation will be classified as preterm births, with births from 22 to <28 completed weeks of gestation additionally classified as extremely preterm births. Stillbirth will be defined as fetal death at or after 22 completed weeks of gestation with no signs of life after birth, and neonatal death as the death of a live-born infant

within 28 days after birth. These outcomes will be recorded separately.

(5) Miscarriage rate, defined as the proportion of intrauterine clinical pregnancies ending in spontaneous pregnancy loss before 22 completed weeks of gestation.

(6) Changes in TCM kidney-deficiency syndrome score (TCMSS) and quality-of-life score (the 36-Item Short Form Health Survey) from baseline to months 3 and 6. The TCMSS will be assessed using a structured participant-reported questionnaire comprising two primary and six secondary symptom items, with a total score ranging from 0 to 30; higher scores indicate greater symptom severity. Participants will select the response that best reflects their symptoms according to prespecified scoring criteria. Detailed symptom items and scoring criteria are provided in **Supplementary File 3**.

(7) Incidence and severity of adverse events and serious adverse events, clinically relevant laboratory abnormalities, changes in vital signs, and pregnancy-related safety outcomes, including ectopic pregnancy and congenital anomalies.

Assessment Schedule and Follow-Up

At screening, trained research staff will document participants' medical and reproductive histories, perform a physical examination and pregnancy test, conduct safety laboratory assessments, measure serum AMH and basal FSH levels, perform pelvic ultrasonography to assess AFC, and administer the TCM syndrome and quality-of-life instruments. AMH and basal FSH levels will be measured during the early follicular phase (days 2–5 of the menstrual cycle) at the clinical laboratory of each participating center, using locally validated assay platforms and reagents. AFC will be assessed during the same phase via transvaginal ultrasonography, defined as the total number of follicles measuring 2–10 mm in both ovaries. All participating centers will follow a standardized operating procedure, and AFC assessments will be conducted by experienced sonographers who have undergone protocol-specific training. To minimize inter-center variability, all serial assessments for an individual participant will be performed at the same center throughout the study. AMH, basal FSH, and AFC will be reassessed at months 3 and 6 only in participants who remain nonpregnant at the time of assessment, as pregnancy may affect the physiological interpretation of these measures.

During each of the three letrozole cycles, urinary LH results, ovulation status, and pregnancy status will be assessed and documented. Participants will complete an online diary to record medication use, urinary LH and pregnancy-test results, concomitant treatments, and adverse events. Research staff will regularly review diary entries and contact participants when scheduled data are missing. The schedule of enrollment, interventions, and assessments is presented in Fig. 2.

Timepoint (Months) (Days)	TRIAL PERIOD							
	Enrollment		Post-randomization					
	0 -14	0	1M ±7	2M ±7	3M ±7	4M -14	5M -14	6M -14
ENROLLMENT:								
Eligibility screening	x							
Informed consent	x							
Medical history	x							
Pregnancy test	x							
Randomization		x						
INTERVENTIONS:								
XDKTF or placebo		x	→					
Letrozole						x	x	x
ASSESSMENTS:								
Sex hormone levels	x				x			x
Pelvic ultrasound	x				x			x
Safety assessments	x				x			x
Questionnaires	x				x			x
Ovulation monitoring						x	x	x
Serum β -hCG testing ^a								x
Clinical pregnancy status ^b								x
Adverse events			x	x	x	x	x	x

Fig. 2. Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) figure. The arrow indicates continuous administration of the assigned granules. ^a Participants will perform a home urine pregnancy test if menstruation is delayed. Serum β -hCG will be measured after a positive urine test and routinely at the 6-month close-out visit. ^b Clinical pregnancy will be confirmed by transvaginal ultrasonography following serum β -hCG positivity, with pregnancy status recorded at close-out. XDKTF, Xiehe DOR Kidney-Tonifying Formula.

Sample-Size Calculation

The sample size was calculated for the binary primary outcome [21]. Based on preliminary clinical data from our prior application of analogous kidney-tonifying herbal regimens in women experiencing infertility associated with DOR, we assumed 6-month cumulative clinical pregnancy rates of $p_1 = 0.35$ in the intervention group and $p_2 = 0.20$ in the control group. The 15-percentage-point absolute difference was identified as the target treatment effect and deemed clinically significant. With equal allocation, a one-sided α of 0.025 and 80% power ($\beta = 0.20$) for a superiority test, the sample size was calculated as: , where $p = (p_1 + p_2)/2$ and z_q denotes the q th quantile of the standard normal distribution. 138 participants per group are required. The target sample size was increased to 160 participants per group (320 in total), providing an approximately 14% allowance for loss to follow-up or unavailable primary-outcome data.

Statistical Analysis

Statistical analyses will be performed using R software (version to be documented at the time of analysis; R Foundation for Statistical Computing, Vienna, Austria). The primary analysis will follow the intention-to-treat (ITT) principle, with all randomized participants analyzed in their

assigned groups regardless of treatment discontinuation and followed for the primary outcome whenever possible. A per-protocol analysis will serve as a supportive sensitivity analysis. Safety analyses will include all participants who receive at least one dose of the assigned granules. If primary-outcome status remains unavailable despite follow-up attempts, it will be treated as missing and addressed in sensitivity analyses.

Baseline characteristics will be summarized descriptively by treatment group without formal significance testing. The distribution of continuous variables will be assessed using the Shapiro–Wilk test and visual inspection of Q–Q plots. Continuous variables will be presented as means and standard deviations or medians and interquartile ranges, as appropriate, while categorical variables will be reported as numbers and percentages. Model assumptions will additionally be assessed using residual diagnostics where applicable.

The 6-month cumulative clinical pregnancy rate will be compared between groups using a Cochran–Mantel–Haenszel test, stratified by both study center and age stratum (22–29, 30–35, and 36–39 years), consistent with the randomization scheme. The absolute risk difference, relative risk, and corresponding 95% confidence intervals will

be reported. The prespecified superiority test will employ a one-sided alpha level of 0.025. In addition, cumulative clinical pregnancy by month 3 will be summarized by randomized treatment group. Pregnancies during months 4–6 will be summarized among participants who remain non-pregnant at month 3 and will be interpreted as descriptive and exploratory analyses.

Other binary outcomes, including biochemical pregnancy and live birth, will be summarized using effect estimates with 95% confidence intervals and compared using chi-squared tests, Fisher's exact test, or stratified methods, as appropriate. Miscarriage rates will be assessed among participants who achieve a clinical pregnancy, with between-group comparisons interpreted as descriptive and supportive. Ovulation status across the three letrozole cycles will be analyzed as a repeated binary outcome using generalized estimating equations (GEE), with participant specified as the clustering unit. The model will include treatment group, treatment cycle, treatment-by-cycle interaction, study center, and age stratum.

Repeated post-baseline measurements of AMH, basal FSH, TCM syndrome score, and quality-of-life score at months 3 and 6 will be analyzed using mixed-effects models for repeated measures. The models will include treatment group, time point, treatment-by-time-point interaction, study center, and age stratum as fixed effects, with the corresponding baseline value as a covariate. Within-participant correlation will be accounted for using an appropriate covariance structure. Adjusted between-group differences at each time point will be reported with 95% confidence intervals. AFC will be analyzed using a mixed-effects model appropriate to its distribution. For ovarian reserve outcomes (AMH, basal FSH, and AFC), data obtained before pregnancy confirmation will be retained for analysis. Subsequent assessments will not be performed or imputed. The frequency and percentage of missingness due to pregnancy will be reported by treatment group.

Exploratory subgroup analyses will be conducted to evaluate whether the treatment effect varies according to age strata and baseline AMH or FSH categories. For other missing outcomes unrelated to pregnancy, multiple imputation will be considered in sensitivity analyses contingent upon the pattern and extent of the missing data. With the exception of the primary superiority test, all statistical tests will be two-sided with a significance level of $\alpha = 0.05$.

Data Management and Quality Control

Data will be entered into a validated electronic data capture system with role-based access controls, audit trails, and automated range and consistency checks. Clinical research associates will conduct source data verification and periodic monitoring. Before recruitment begins, investigators and relevant research staff at each center will undergo protocol-specific training, including standardization of pelvic ultrasonography and TCM syndrome assessment.

Any data queries will be resolved before database lock. After database lock, a final read-only copy of the database and relevant trial records will be securely archived, with paper copies retained as required by institutional policy. All records will be retained for at least 5 years after study completion.

Safety Monitoring

Adverse events will be collected from enrollment through the 6-month close-out visit and will be assessed for severity, seriousness, and potential association with the study intervention. Pregnancies occurring during the trial will be followed for maternal, fetal, and neonatal safety outcomes, including any documented early-pregnancy exposure to the study treatment. Serious adverse events will be reported and managed according to GCP guidelines and relevant institutional procedures. Emergency unblinding will be authorized solely when necessary for clinical management.

Results

This trial is expected to determine whether the incorporation of XDKTF into a two-stage treatment strategy—3 months of initial granule-only treatment followed by three cycles of letrozole-based ovarian stimulation—improves the cumulative clinical pregnancy rate within 6 months. Secondary outcomes will assess ovulation, ovarian reserve biomarkers, TCM kidney-deficiency symptoms, quality of life, and safety. By emphasizing cumulative clinical pregnancy rather than changes in surrogate biomarkers alone, the study aims to provide clinically significant evidence regarding the efficacy of this integrative treatment strategy.

Discussion

DOR is a major cause of subfertility in women of reproductive age and often leads to a poor response to ovarian stimulation or ART. Conventional management focuses on hormonal stimulation; however, outcomes remain sub-optimal, and few interventions have been shown to restore ovarian reserve. The integrative approach evaluated in this study combines TCM with Western medicine and aims not only to alleviate symptoms but also to improve ovarian function.

XDKTF was developed based on the classical principles of tonifying kidney essence, promoting blood circulation, and regulating the Chong and Ren meridians. Previous small-scale studies and animal experiments have indicated that this formula may enhance follicular development, increase AMH expression, and modulate oxidative stress and inflammatory pathways. Although women with DOR exhibit a reduced follicular pool, residual dormant primordial follicles may persist, and their recruitment and subsequent development are regulated by oocyte-

intrinsic signaling, pregranulosa cells, and the ovarian microenvironment [22]. Several herbs contained in XDKTF, such as *Epimedium brevicornum*, *Rehmannia glutinosa*, and *Angelica sinensis*, have demonstrated antioxidant, anti-apoptotic, and endocrine-modulating effects in preclinical studies [23,24]. Accordingly, the initial 3-month treatment phase is designed to provide sustained exposure across several menstrual cycles, potentially supporting follicular development by improving the local microenvironment and preserving residual follicles. During the subsequent 3 months, the assigned granules will be administered concurrently with up to three cycles of letrozole-based mild ovarian stimulation and timed intercourse, thereby providing multiple opportunities for conception.

The 6-month cumulative clinical pregnancy endpoint thus reflects the impact of the overall sequential, multi-cycle approach while minimizing delays in subsequent fertility treatment, patient dropout, treatment crossover, and initiation of ART. However, robust evidence from large, well-designed randomized trials remains limited, and the current study aims to address this gap.

This protocol has several strengths. Firstly, its rigorous, randomized, double-blind, placebo-controlled design is intended to enhance internal validity and minimize potential biases. Secondly, the integration of TCM syndrome differentiation with biomedical indicators facilitates a comprehensive evaluation of clinical efficacy and associated physiological changes. Furthermore, the multicenter implementation enhances the generalizability of the findings across diverse patient populations and clinical settings. In addition, standardized interventions and outcome measures improve reproducibility and facilitate comparisons with future studies.

The study also has limitations. Conception is affected by multiple factors, including male fertility and lifestyle, which may introduce residual confounding. Adherence to herbal therapy and the duration of follow-up could influence the observed treatment effects. Local measurement of AMH and basal FSH levels may introduce inter-center laboratory variability. In addition, the use of a small amount of XDKTF herbal material in placebo preparation may result in a residual pharmacological effect and attenuate the between-group treatment contrast. Moreover, peripheral biomarkers cannot directly establish mechanisms within the ovary. Finally, the months 4–6 pregnancy analysis is conditional on remaining nonpregnant at month 3 and will therefore be interpreted as descriptive and exploratory rather than as an independent randomized comparison. These limitations will be addressed through standardized procedures and sensitivity analyses.

Conclusions

This multicenter, double-blind, placebo-controlled clinical trial will evaluate the efficacy and safety of inte-

grative Chinese–Western therapy for DOR-associated infertility with a kidney-deficiency pattern among women attempting conception without ART. This study is expected to provide evidence on the role of TCM formulas in improving ovarian reserve markers and fertility outcomes and to inform the development of a standardized integrative treatment model for DOR in reproductive medicine.

Abbreviations

AFC, antral follicle count; AMH, anti-Müllerian hormone; ART, assisted reproductive technology; β -hCG, beta-human chorionic gonadotropin; DOR, diminished ovarian reserve; FSH, follicle-stimulating hormone; GCP, Good Clinical Practice; GEE, generalized estimating equations; LH, luteinizing hormone; SPIRIT, Standard Protocol Items: Recommendations for Interventional Trials; TCM, traditional Chinese medicine; TCMSS, TCM kidney-deficiency syndrome score; XDKTF, Xiehe DOR Kidney-Tonifying Formula.

Availability of Data and Materials

Not applicable.

Author Contributions

YYG and XHH made substantial contributions to the conception and design of the study and drafted the original manuscript. YPW and AJS were responsible for the overall study design and supervision. WX, YD, YZ, XYS, and WQL contributed to methodology, participant recruitment planning, data collection procedures, and quality control. FC and ZYW contributed to the randomization procedures, sample-size calculation, and statistical analysis. All authors critically revised the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The protocol was approved by the Ethics Committee of Peking Union Medical College Hospital (approval number I-25PJ3287; dated 9 December 2025). All participants will provide written informed consent before enrollment. The study will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.24976/Discover.Med.202638213.254>.

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