



# Integrative Management of Acupuncture for Diminished Ovarian Reserve with Insomnia: Study Protocol for a Prospective Randomized Controlled Trial

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**Background:** Insomnia, a prevalent but often overlooked comorbidity in women with diminished ovarian reserve (DOR), remains underaddressed in reproductive care. This study explores the novel integration of acupuncture as a dual-benefit intervention, aiming to simultaneously address insomnia and potentially improve fertility in DOR patients.

**Methods:** This single-blind randomized controlled trial (RCT) will recruit 128 DOR patients with insomnia. They will be randomly allocated to receive either verum acupuncture or sham acupuncture. The acupuncture course lasts for 12 weeks, with three sessions per week, each lasting 30 minutes, and is carried out continuously during menstruation. The primary outcome is the change in sleep quality, assessed by the Pittsburgh Sleep Quality Index (PSQI) scores. Secondary outcomes include the assessment of sleep dysfunction, anxiety and depression status, and ovarian function in DOR patients. Sleep dysfunction will be profiled using the Insomnia Severity Index (ISI) to quantify insomnia severity, whereas daytime somnolence will be assessed using the Epworth Sleepiness Scale (ESS), and daytime fatigue will be evaluated with Flinders Fatigue Scale (FFS). Circadian rhythm will be characterized using both Morningness-Eveningness Questionnaire (MEQ) and Munich Chronotype Questionnaire (MCTQ). Anxiety fluctuations will be tracked with the State-Trait Anxiety Inventory (STAI), and depressive symptomatology will be indexed using the Beck Depression Inventory (BDI). In parallel, ovarian reserve parameters will also be assessed. Safety outcomes will be systematically recorded and reported following the STRICTA-recommended framework.

**Expected Results and Conclusion:** This study develops an acupuncture protocol for DOR patients with insomnia, and aims to generate robust clinical evidence to support the integration of acupuncture into reproductive medicine practice.

**Trial Registration Number:** ITMCTR2025001081.

**Keywords:** acupuncture, diminished ovarian reserve, insomnia, Pittsburgh Sleep Quality Index

## Introduction

Insomnia is a widespread public health concern across the globe, impacting roughly 30% of the population.<sup>1</sup> Recent studies have emphasized the bidirectional pathological link between insomnia and reproductive endocrine disorders.<sup>2</sup> This vicious cycle may be driven by a misalignment of endogenous circadian rhythms, where sleep loss disrupts the rhythmic secretion of key reproductive hormones like FSH and estradiol, thereby potentially accelerating ovarian reserve decline. Conversely, the hypoestrogenic state in DOR can further exacerbate insomnia by impairing the circadian regulation of core body temperature and neuroendocrine pathways.<sup>3</sup> Specifically, a recent cross-sectional study confirmed that insomnia leads to

diminished ovarian reserve (DOR) in women of childbearing age. Notably, the study also revealed that each one-point increase in the Pittsburgh Sleep Quality Index (PSQI) score corresponded to a 17% increased risk of DOR, this association was particularly pronounced in younger individuals and those with low body weight.<sup>4</sup> Epidemiological data indicate that the prevalence of DOR among women of reproductive age ranges from 10% to 35%,<sup>5,6</sup> compared to 33.3% in those with chronic insomnia.<sup>7</sup> However, the current clinical management of DOR patients with insomnia often suffers from a “Island Effect”. This fragmented therapeutic strategy targets ovarian dysfunction and sleep disturbances separately, failing to adequately consider and intervene in the potential psychophysiological interactions and shared pathophysiological mechanisms between the two conditions. A meta-analysis reveals that existing research on the impact of sleep duration and quality on fertility remains remarkably limited.<sup>8</sup> This comorbidity not only severely impairs women’s physical and mental health and quality of life, but also significantly accelerates the pathological progression of DOR.<sup>9</sup> Therefore, developing integrated treatment protocols is urgently needed for DOR patients with insomnia.

The treatment of insomnia primarily involves cognitive therapy and pharmacotherapy.<sup>10,11</sup> However, their efficacy is often limited in patients with diminished ovarian reserve (DOR), owing to the complex and intertwined nature of the comorbid symptomatology. This complexity, characterized by the co-occurrence of endocrine dysfunction, impaired fertility, and sleep disturbances, poses a significant clinical challenge that necessitates a dual-target therapeutic approach.<sup>8,12,13</sup> Acupuncture, as a treasure of Traditional Chinese Medicine (TCM), has a long history in treating insomnia and infertility. Growing evidence suggests that acupuncture can not only safely ameliorate insomnia symptoms<sup>14</sup> by modulating neurotransmitters,<sup>15,16</sup> circadian rhythms,<sup>17</sup> and regulating related hormone levels<sup>18,19</sup> but also benefit DOR by improving ovarian blood flow and regulating the hypothalamic-pituitary-ovarian axis.<sup>20–22</sup> This dual-action mechanism positions acupuncture as a distinctive therapeutic option for DOR patients with insomnia, capable of concurrently addressing both sleep disturbance and ovarian dysfunction.

In conclusion, although acupuncture research on DOR or insomnia as separate entities has advanced, its application for comorbid DOR and insomnia is underexplored. This study aims to delineate the therapeutic efficacy of acupuncture in DOR patients with insomnia. Concurrently, symptomatology network analysis will characterize critical pathological nodes and symptom clusters within this dual-diagnosis population, to provide evidence-based medical evidence for acupuncture treatment of DOR patients with insomnia.

## Methods

### Study Design

This prospective randomized controlled trial (RCT) compares the effects of verum and sham acupuncture on sleep quality and ovarian function in 128 DOR patients with insomnia. The protocol adheres to the Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) checklist (See [Appendix 1](#)). Patients can choose to join or leave the study and must sign an informed consent form before randomization after fully understanding the study design. The study has been reviewed by the ethics committee of Sichuan Jinxin Xinnan Women and Children’s Hospital and was registered on the International Traditional Medicine Clinical Trial Registration Platform (ITMCTR, <http://itmctr.ccebtcm.org.cn/>) on May 30, 2025 (Registration No. ITMCTR202500 1081). [Figure 1](#) shows the study process, and [Table 1](#) details the schedule.

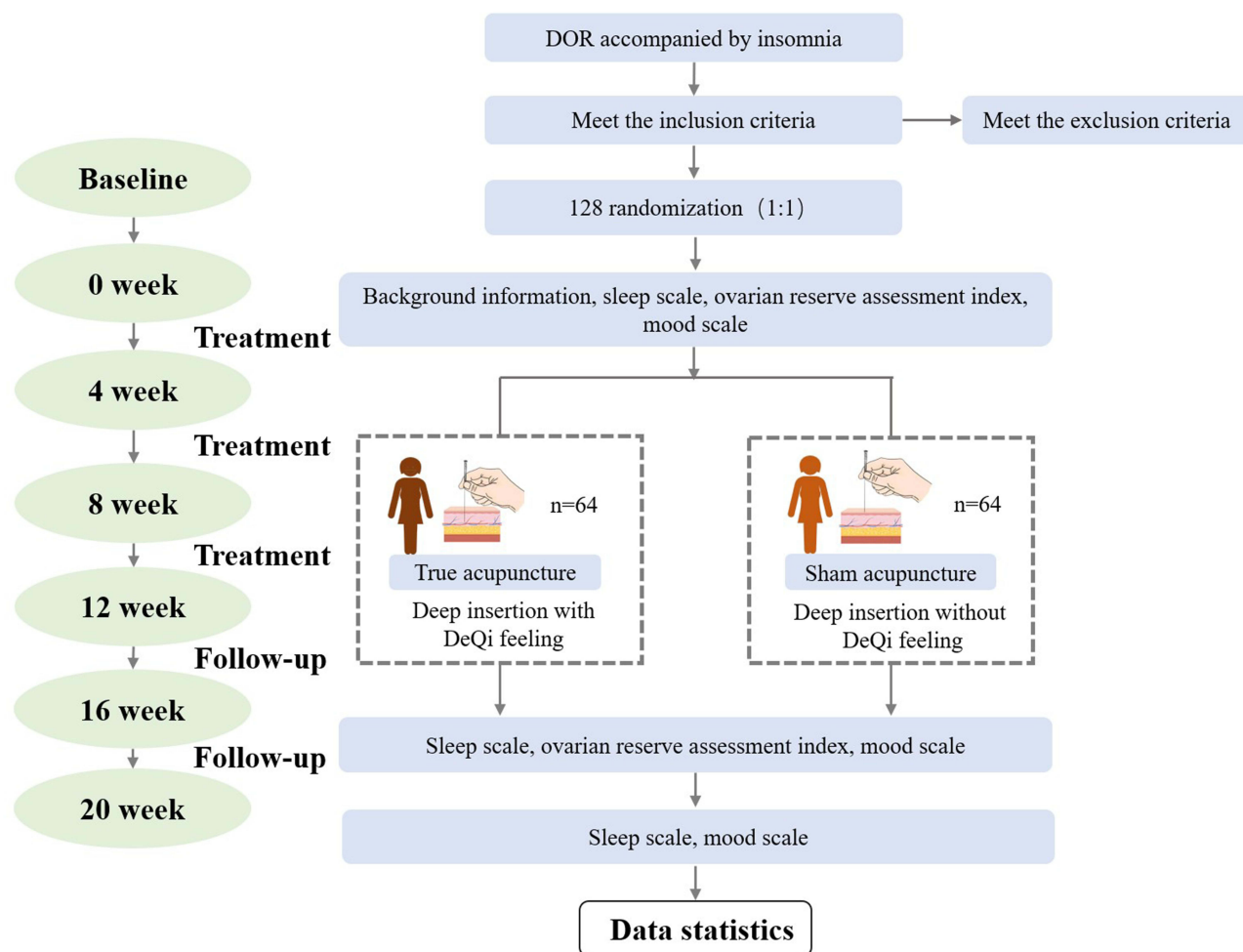
### Study Population

#### Diagnostic Criteria

We will recruit patients diagnosed with DOR and insomnia. DOR diagnosis is based on the guidelines in the 2022 expert consensus document from *the Clinical Diagnosis and Management of Diminished Ovarian Reserve Expert Consensus Group*.<sup>23</sup> Insomnia diagnosis uses the *International Classification of Sleep Disorders-3 (ICSD-3)*.<sup>24</sup> All researchers will screen and select patients per these standards.

#### Diagnostic Criteria for DOR

- ① Antimullerian hormone (AMH)<1.1 ng/mL indicates DOR;
- ② Antral follicle count (AFC)<7 suggests DOR;
- ③ Follicle-Stimulating Hormone (FSH)≥10 IU/L for two consecutive menstrual cycles implies DOR



**Figure 1** The research flowchart.

### Diagnostic Criteria for Insomnia

- ① Difficulty initiating or maintaining sleep, or waking up too early;
- ② Daytime symptoms like fatigue or sleepiness, or impaired social functioning;
- ③ Inability to sleep adequately despite sufficient time and a safe environment.

Note: Symptoms occur  $\geq 3$  times/week. Chronic insomnia lasts  $\geq 3$  months, while short-term insomnia lasts  $< 3$  months.

### Inclusion Criteria

Patients must meet all six criteria are eligible for the study.

- ① Female patients aged between 20 and 45 years.
- ② Patients diagnosed with DOR diagnostic criteria and the chronic or short-term insomnia criteria of ICSD-3, with a PSQI score  $> 7$ ;
- ③ Patients with regular menstrual cycle ( $28 \pm 7$  days);
- ④ No sedative hypnotic use in the past month or acupuncture in the past 3 months;
- ⑤ Not participating in other ongoing clinical studies;
- ⑥ Signed informed consent from the patient or a direct relative.

**Table 1** Schedule of the Trial Process

Specific Entries		Baseline	Treatment			Follow-Up	
		Case Enrollment	4 Week	8 Week	12 Week	4 Week	8 Week
Case enrollment assessment	Confirmed cases included	√					
	Sign the informed consent form	√					
	Patient information	√					
	Routine physical examination of patients	√					
Serum hormone	Six female hormones	√			√		
	AMH	√			√		
Iconography	AFC	√			√		
Clinical assessment	PSQI	√	√	√	√	√	√
	ISI	√	√	√	√	√	√
	ESS	√	√	√	√	√	√
	FFS	√	√	√	√	√	√
	MEQ	√					
	MCTQ	√					
	STAI and BDI	√	√	√	√	√	√
Trial evaluation	Co-Medication						√
	Treatment of other diseases						√
	AEs						√
	Compliance evaluation						√
	Trial completion status						√
	Safety evaluation						√

**Abbreviations:** AMH, antimullerian hormone; AFC, antral follicle count; PSQI, Pittsburgh Sleep Quality Index; ISI, insomnia severity index; ESS, epworth sleepiness scale; FFS, flinders fatigue scale; MEQ, morningsee-eveningness questionnaire; MCTQ, munich chronotype questionnaire; STAI, state-trait anxiety inventory; BDI, beck depression inventory; AEs, adverse events.

### Exclusion Criteria

Patients meeting any of the above criteria will be excluded.

- ① Patients with hypertension, diabetes, coronary heart disease, epilepsy, or severe systemic diseases of the cardiovascular, cerebrovascular, hepatic, renal, oncologic, or hematopoietic systems;
- ② Patients with other sleep disorders, such as sleep apnea, sleep-related movement disorders, central sleep disorders, narcolepsy, or restless legs syndrome;
- ③ Patients with alcohol/drug abuse or dependence;
- ④ Patients with communication/cognitive disorders, or psychiatric disorders (eg, bipolar disorder, anxiety, schizophrenia, or personality change);
- ⑤ Patients with a bleeding tendency, infection susceptibility, severe allergic diseases, or skin conditions (eg, ulcers, scars) that contraindicate acupuncture;
- ⑥ Patients with currently participating in other clinical trials.

## Blinding and Randomization

Patients will be randomly assigned using SAS 9.4. The random sequence will be in sealed, opaque envelopes with two sets of random numbers, opened by an independent research assistant separate from the study team. This assistant will allocate patients in a 1:1 ratio to the verum or sham acupuncture group.

## Sample Size Calculation

This RCT involves verum acupuncture and sham acupuncture groups for DOR patients with insomnia, with the primary outcome being the PSQI score. As no prior cross-sectional studies on DOR with insomnia exist, sample size calculations referenced trials on perimenopausal women with insomnia. Based on a study by Fei-Yi Zhao,<sup>25</sup> the verum acupuncture group showed a significant PSQI reduction (from 11.00±0.34 to 8.23±0.44) versus the sham group (from 10.23±0.29 to 9.45±0.37) after two months, with a Cohen's d of 0.51. Assuming a Cohen's d of 0.65 to this study, two tailed  $\alpha = 0.05$ , and  $1-\beta = 90\%$ , PASS 2021 calculated 51 patients per group. Accounting for a 20% dropout rate, the study will recruit 64 per group, totaling 128 patients.

## Study Protocol

### Verum Acupuncture Group

Acupoint location refers to *the Names and locations of acupoints* in 2021 (GB/T 12346–2021).<sup>26</sup> Two sets of acupoints are alternately used during treatment. The first set will be used as the treatment starting point group, and the patients will be treated in the prone position, including Shenting (GV24), Baihui (DU20), Benshen (GB13), Zhongwan (CV12), Huangshu (KI16), Dai Mai (GB26), Qihai (CV6), Guanyuan (CV4), Dahe (KI12), Zigong (EX-CA1), Zusanli (ST36), Sanyinjiao (SP6), Taichong (LR3), Shenmen (HT7), and Shenmai (BL62). The second set is supine, including Anmian (EX-HN22), Shenshu (BL23), Dachangshu (BL25), Ciliao (BL32), and Taixi (KI3). Details are in [Figure 2](#) and [Appendix 2](#).

Acupuncture is performed by licensed acupuncturists with over five years of experience. The skin at the acupoint is disinfected with 75% ethanol. Sterile, single-use needles (0.25 mm in diameter, lengths of 25 mm, 40 mm, or 75 mm, Hua Tuo, Suzhou, China) are inserted to induce a sense of *de qi*, such as soreness, numbness, heaviness, fullness, and pain. Needles are retained for 30 minutes without manipulation. Treatment is thrice weekly on alternate days for 12 weeks (36 sessions total), starting from the second day of the menstrual cycle and continuing throughout menstruation.<sup>27</sup>

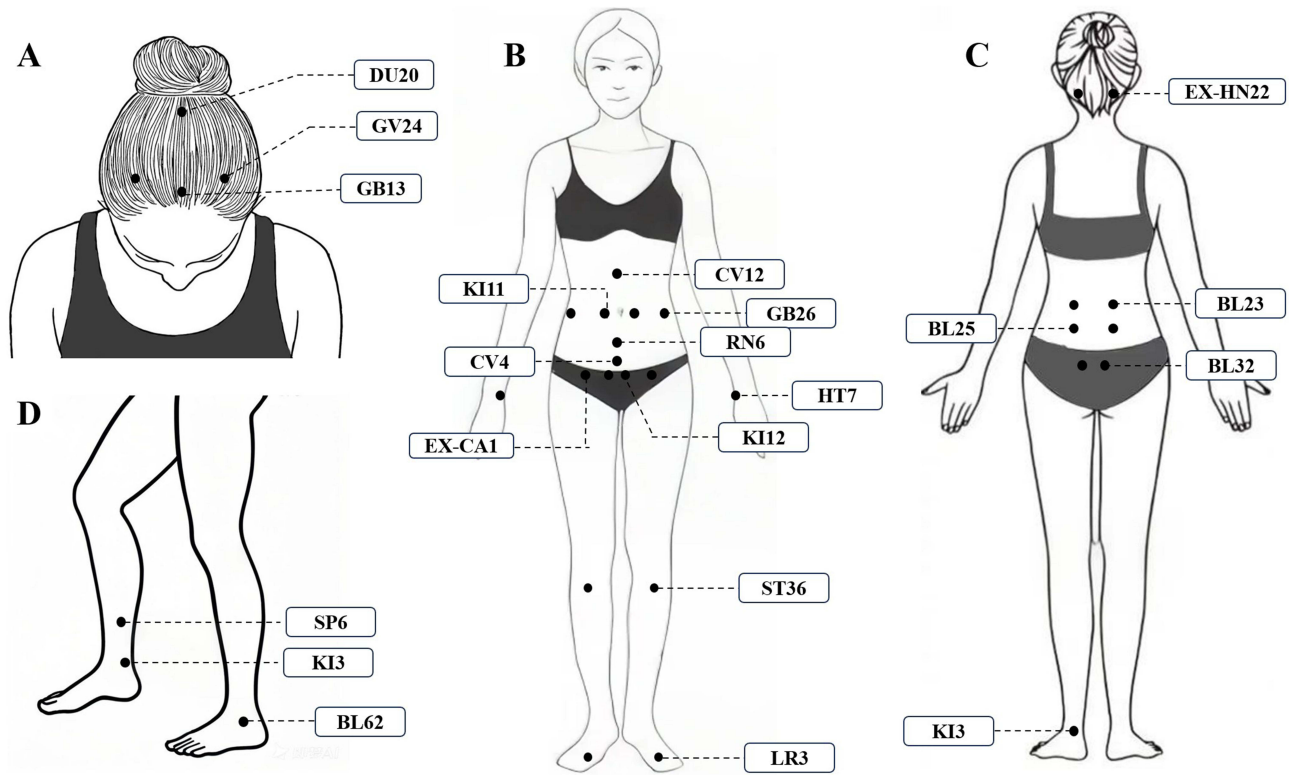
### Sham Acupuncture Group

The sham acupuncture group will follow the SHARE guidelines for sham reporting.<sup>28</sup> Short, shallow needles (0.25 mm in diameter, 25 mm in length, Hua Tuo, Suzhou, China) will be used at non overlapping control points (see [Figure 3](#) and [Appendix 2](#)). Needling is superficial to avoid a sense of *de qi*. The sham procedure, treatment duration, and timing mirrored the verum acupuncture group. The sham acupuncture procedure strictly followed the STRICTA reporting guidelines for sham acupuncture, see [Appendix 3](#) for details.

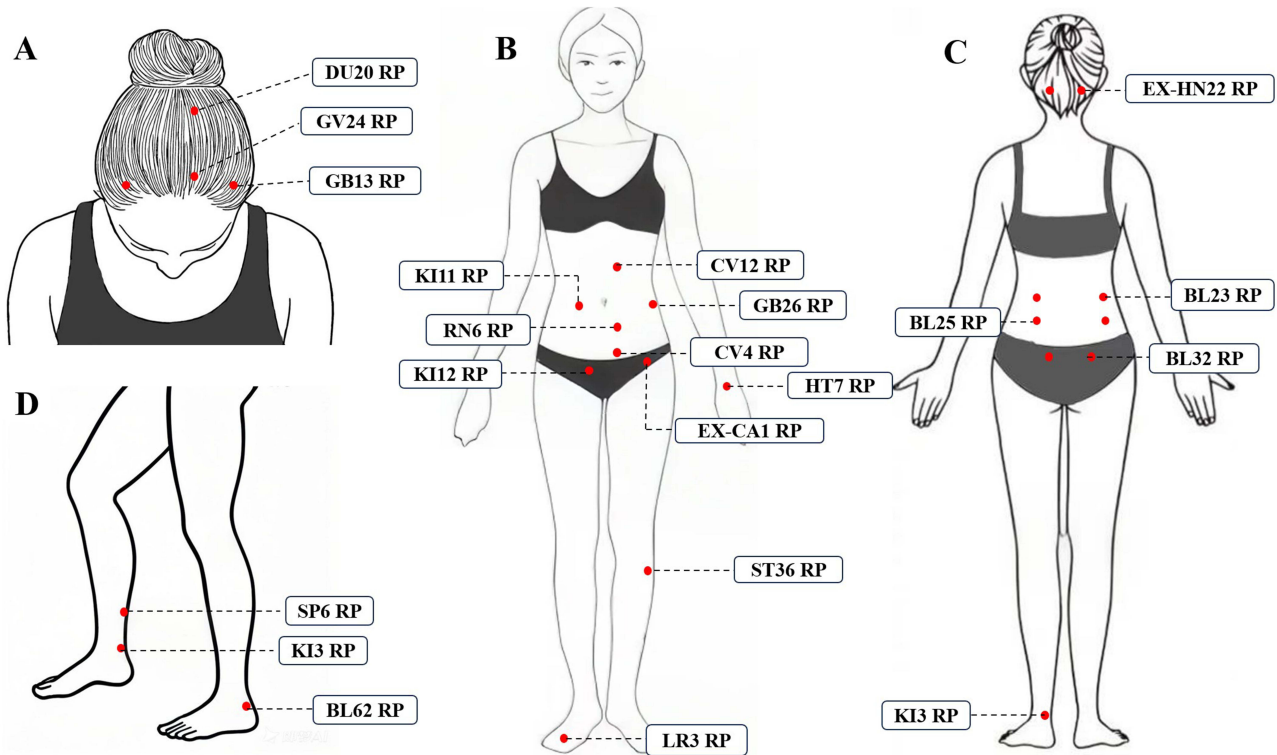
## Outcome Measurement

### Primary Outcomes

The primary outcome will be evaluated through the PSQI, a psychometrically validated instrument assessing sleep quality over the preceding month. Comprising 19 validated items, this multidimensional tool generates seven component scores via algorithmic synthesis: sleep quality, latency, duration, efficiency, disturbances, hypnotic medication usage, and daytime dysfunction, whereas the composite score spans a continuum from 0 to 21. A threshold exceeding 7 points demarcates clinically significant sleep impairment. The higher the score is, the worse the sleep quality is indicated.<sup>29</sup> A 4-point reduction in PSQI scores from baseline operationalize as the minimal important change (MIC), whereas a between-group differential of 2.5 points constitutes the minimal clinically important difference (MCID) threshold.<sup>30</sup> Concurrently, treatment responders are defined a priori as participants demonstrating  $\geq 50\%$  PSQI score reduction relative



**Figure 2** Schematic Representation of Acupoint Localization. (A) Location of head acupoints in the acupuncture group; (B) Location of front acupoints in the acupuncture group; (C) Location of lateral acupoints in the acupuncture group; (D) Location of back acupoints in the acupuncture group.



**Figure 3** Schematic Representation of Sham Acupoint Localization. (A) Location of non-acupoints in the head area of the sham acupuncture group; (B) Location of non-acupoints in the front area of the sham acupuncture group; (C) Location of non-acupoints in the lateral area of the sham acupuncture group; (D) Location of non-acupoints in the back area of the sham acupuncture group. **Abbreviation:** RP, Reference point.

to baseline.<sup>31</sup> The evaluation time points are baseline, therapeutic intervals (4, 8 and 12 weeks), and follow-up period (16 and 20 weeks).

### Secondary Outcomes

Sleep and psychological outcomes will be dynamically assessed at baseline, during treatment (4, 8, and 12 weeks), and at follow-up (16 and 20 weeks). Specifically, the Insomnia Severity Index (ISI) will gauge overall insomnia severity, the Epworth Sleepiness Scale (ESS) daytime sleepiness, and the Flinders Fatigue Scale (FFS) subjective fatigue levels. Concurrently, psychological status will be evaluated using the State-Trait Anxiety Inventory (STAI) for anxiety and the Beck Depression Inventory (BDI) for depressive symptoms. Furthermore, sleep-wake patterns and circadian rhythm characteristics will be captured at baseline and 12 weeks using the Morningness-Eveningness Questionnaire (MEQ) for chronotype preference and the Munich Chronotype Questionnaire (MCTQ) for sleep timing and chronotype based on actual behavior. In parallel with these assessments, AMH, sex hormone levels, and AFC will be conducted at baseline and on 2–3 days of the menstrual period at 12 weeks of treatment to evaluate the ovarian reserve function of DOR patients with insomnia.

### Safety Outcomes

At each visit, researchers identify adverse events (AEs) through questioning or patient reports. Common acupuncture related AEs include intolerable needle pain, local hematoma, or infection. The case report form documents the name, dates, severity, relation to the intervention, impact on treatment, and outcome of AEs.

### Statistical Analysis

Baseline demographic data will be reported descriptively using the intent-to-treat (ITT) population. All efficacy analyses will be conducted using the ITT dataset and the per-protocol (PP) set, which is defined as all randomized patients who received at least 29 (80% of the total number of treatments) interventions. The primary outcome, operationalized as PSQI scores, will be analyzed through a mixed-model repeated measures (MMRM) framework incorporating to estimate efficacy, with time as a within-group factor and intervention as a between-group factor. To evaluate the robustness of the primary findings, sensitivity analyses will be performed using multiple imputation and last observation carried forward methods to handle missing data. This analytical paradigm is generated least squares mean (LSM) changes from baseline to 20 weeks, between-group LSM differentials, and associated 95% confidence intervals with two-tailed probability values. The percentage of effective responders to the PSQI during therapeutic intervals (4, 8 and 12 weeks), and follow-up period (16 and 20 weeks) will be calculated and summarized by groups. The percentage of PSQI effective responders is compared between groups using chi-square tests. Secondary outcomes will be analyzed in the same way. Cohen's *d* effect sizes are calculated by dividing the absolute value of the least squares mean difference in scores between groups from baseline to 20 weeks by the combined standard deviation of the MMRM estimates. AEs are categorized by severity and assessed for their relationship to study treatment. Fisher's exact test is used to compare the incidence of AEs between groups. Statistical analysis is performed using SPSS version V26.0, and a two-sided  $P < 0.05$  is considered statistically significant.

To delineate the potential mediation effect of insomnia amelioration on acupuncture-mediated modulation of DOR, structural equation modeling will be employed to systematically characterize causal effects. The model included patients with baseline and post-intervention data, using baseline PSQI scores as the independent variable, post-intervention AMH as the dependent variable, and PSQI scores at 4, 8 and 12 weeks as mediators (M1, M2, M3). Adjustments PSQI scores and AMH at these time points. Mediating effects are calculated via 10,000 bootstrap samples, producing bias-corrected confidence intervals.

We will build a network visualization model for the PSQI, ISI, MEQ, MCTQ, and ESS scales to explore the complex relationships among insomnia symptoms in DOR patients. All data will be analyzed using R software. The qgraph package will be used to estimate and visualize sleep symptom networks. For continuous data, a gaussian graphical model (GGM) will be used to construct a partial correlation network; for mixed data types, mixed graphical models (MGM) will be applied. The bootnet package will assess network accuracy and stability. Centrality metrics will identify core scale

items, and node predictability will be calculated with the *mgm* package. The *NetworkComparisonTest* package will generate and compare network graphs for the acupuncture and sham groups across time points, analyzing network structure and dynamics to identify key node changes and acupuncture targets.

## Discussion

To the best of our knowledge, this is the first prospective study to comprehensively investigate the efficacy and safety of acupuncture for DOR patients with insomnia. By leveraging mediation analysis and scales network analysis, this research will thoroughly evaluate acupuncture's effects, identify core symptoms and intervention targets, and explore the interrelationship between DOR and insomnia. These findings are expected to provide novel insights and therapeutic targets for this complex comorbidity, which often presents as a vicious cycle. Last but not least, the study aims to alleviate symptoms and improve the life quality of patients.

Sleep, as an indispensable physiological activity in human life,<sup>32,33</sup> exerts extensive and profound effects on reproductive health.<sup>34–36</sup> Therefore, while focusing on the improvement of ovarian function in DOR patients by acupuncture, this study places particular emphasis on the impacts of acupuncture on their sleep quality and physical-mental health. Multiple sleep and emotion-related scales will be employed to comprehensively and accurately evaluate sleep quality, insomnia severity, daytime somnolence, daytime fatigue, circadian rhythm disorder, anxiety, and depression status in DOR patients. Additionally, this study employs mediation analysis to go beyond the limitations of previous research that only observed correlations, aiming to test the internal causal pathways through which acupuncture improves ovarian function and sleep quality. Specifically, it intends to clarify whether sleep improvement acts as a mediator in acupuncture-promoted ovarian function recovery.<sup>37</sup> Meanwhile, scale network analysis will be used to identify core nodes within the symptom clusters of DOR patients with insomnia and observe changes in these core nodes before and after acupuncture.<sup>38</sup> This approach helps avoid the shortcomings of relying solely on total score assessments of outcomes and provides a basis for accurately locating core intervention targets for acupuncture in the future.

## Limitations

This study has several limitations. Firstly, the use of standardized scales as assessment tools introduces the potential for measurement bias due to subjective patient factors such as self-perception, recall, emotional fluctuations, or environmental influences. Secondly, the single-center design within China limits the generalizability of the findings. Furthermore, the study did not track pregnancy outcomes in DOR patients, potentially limiting its relevance for reproductive outcome-focused clinical decision-making.

## Declaration of Generative AI Use

During the preparation of this work, we used *Deepseek* in order to improve readability and language fluency of initial drafts of specific sections and check grammar and spelling in the final draft.

## Abbreviations

DOR, diminished ovarian reserve; PSQI, Pittsburgh Sleep Quality Index; TCM, Traditional Chinese Medicine; RCT, randomized controlled trial; STRICTA, standards for reporting interventions in clinical trials of acupuncture; ITMCTR, international traditional medicine clinical trial registration platform; AMH, antimullerian hormone; AFC, antral follicle count; FSH, follicle-stimulating hormone; MIC, minimal important change; MCID, minimal clinically important difference; ISI, insomnia severity index; ESS, epworth sleepiness scale; FFS, flinders fatigue scale; STAI, state-trait anxiety inventory; BDI, beck depression inventory; MEQ, morningness-eveningness questionnaire; MCTQ, munich chronotype questionnaire; AEs, adverse events; ITT, intent-to-treat; PP, per-protocol; MMRM, mixed model repeated measures; LSM, least squares mean; GGM, gaussian graphical model; MGM, mixed graphical models.

## Ethics and Dissemination

This trial is conducted in accordance with the Declaration of Helsinki. The protocol has been approved by the Medical Ethics Committee of Sichuan Jinxin Xinnan Women and Children's Hospital (No. (2024) Ethics Committee for

Reproductive Ethics (039)). The trial was registered on the International Traditional Medicine Clinical Trial Registration Platform (ITMCTR, <http://itmctr.ccebtcm.org.cn/>) on May 30, 2025 (Registration No. ITMCTR2025001081). Informed consent will be obtained from all participants. The results will be published in a peer-reviewed academic journal.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare that there are no potential conflicts of interest or financial relationships in this study.

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