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ORIGINAL RESEARCH

Sleep Quality and Premature Ovarian Insufficiency: Genetic Evidence and Clinical Benefits of NTZF Combined with HRT

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Purpose: This study aimed to investigate the causal relationship between sleep and Premature ovarian insufficiency (POI) using genetic methods and to evaluate the efficacy and safety of the Chinese herbal medicine Ningxin-Tongyu-Zishen Formula (NTZF) combined with hormone replacement therapy (HRT) on reproductive hormone levels and sleep quality in patients with POI through a clinical controlled trial.

Patients and Methods: A multi-stage research design was implemented. Initially, a cross-sectional analysis was conducted on 200 POI patients. Two -sample Mendelian randomization analysis (MR) was then performed using publicly available data from the UK Biobank and FinnGen to infer causal relationships. Finally, a clinical observational study involving 119 patients compared three regimens over a 6-month treatment period: NTZF combined with HRT, NTZF alone, and HRT alone. The primary outcomes were reproductive hormone levels and the Pittsburgh Sleep Quality Index (PSQI).

Results: Cross-sectional analysis showed that decreased sleep quality was an independent risk factor for altered reproductive hormone levels in POI patients. MR analysis further provided genetic evidence that insomnia may be a causal risk factor for POI. The clinical study indicated that NTZF combined with HRT was significantly more effective than either monotherapy in improving reproductive hormone levels, enhancing sleep quality, and overall clinical efficacy, with a favorable safety profile.

Conclusion: This study confirmed a strong association between sleep quality and POI from both epidemiological and genetic perspectives. NTZF combined with HRT, as an integrated traditional Chinese and Western medicine therapy, can synergistically improve reproductive endocrine and sleep disturbances in POI patients, providing a new and effective strategy for clinical management.

Keywords: premature ovarian insufficiency, sleep quality, reproductive hormone levels, ningxin-tongyu-zishen formula, clinical observational study

Introduction

Premature ovarian insufficiency (POI) is defined as the loss of ovarian function before the age of 40, affecting approximately 3.7% of women of reproductive age.[1] The clinical impact of POI extends beyond menstrual disturbances and loss of fertility. Long-term estrogen deficiency resulting from POI increases the risk of future complications, including osteoporosis, cardiometabolic diseases, and cognitive decline.[2] Furthermore, a POI diagnosis imposes a substantial psychosocial burden on young women, often manifesting as anxiety, depression, identity crises, and challenges in intimate relationships, which can significantly compromise quality of life and social functioning.[3] POI is therefore recognized as a multisystem disorder involving reproductive endocrinology, metabolism, cardiovascular health, and mental well-being. This complexity underscores the importance of investigating modifiable risk factors and developing comprehensive management strategies.

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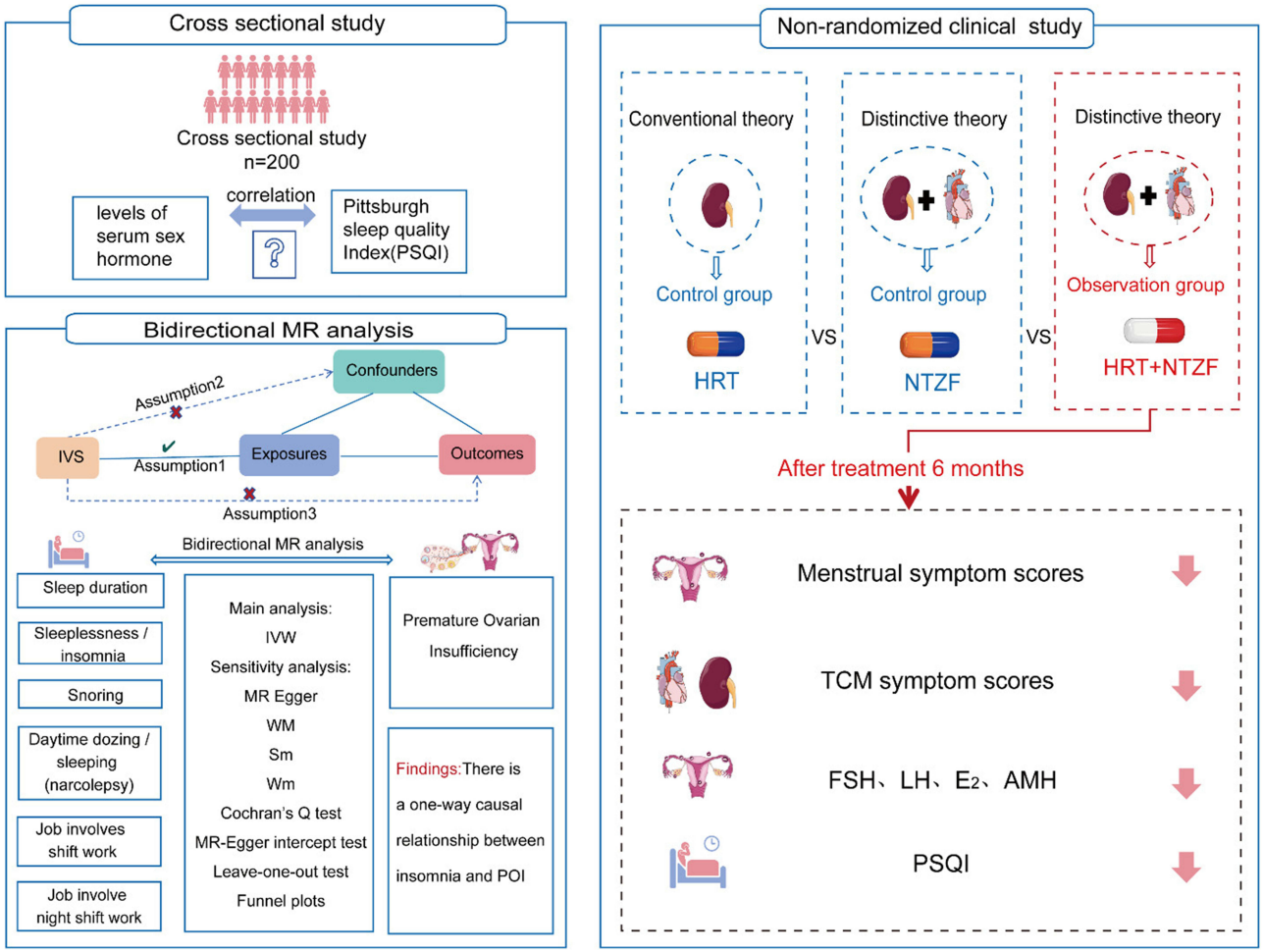
Graphical Abstract



Pathological sleep patterns are closely associated with impaired female reproductive health.[4] Recent systematic reviews identify sleep disorders as a key modifiable environmental risk factor for female fertility, with a significant association observed between sleep disturbances and diminished ovarian reserve.[5] Mechanistically, sleep disorders may accelerate the decline of ovarian function through multiple pathways, including disruption of the neuroendocrine axis, interference with circadian clock genes, and induction of oxidative stress and inflammation. Specifically, insomnia can disrupt the homeostasis of the hypothalamic-pituitary-ovarian (HPO) axis, altering the pulsatile secretion of gonadotropin-releasing hormone (GnRH) and leading to dysregulation of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion. This process directly interferes with follicular development and steroid hormone synthesis.[6] The expression of core circadian clock genes such as CLOCK and BMAL1 interacts with estrogen signaling pathways, and sleep deprivation may disrupt ovarian biological rhythms, affecting follicular quality and atresia.[7] Chronic sleep loss can also induce systemic low-grade inflammation and oxidative stress, accelerating apoptosis of ovarian granulosa cells and impairing ovarian reserve.[8–10] Despite progress in mechanistic understanding, robust evidence from prospective or genetic studies establishing a causal link between sleep quality and POI onset is lacking. Addressing this gap is the primary objective of the present study.

Hormone replacement therapy (HRT) is the primary treatment for managing POI and alleviating hypoestrogenic symptoms. However, HRT demonstrates limited long-term efficacy in restoring intrinsic ovarian function and produces inconsistent effects on comorbid conditions such as sleep disturbances. Traditional Chinese Medicine (TCM) may offer additional benefits by regulating the overall functional state of the body. The Ningxin-Tongyu-Zishen Formula (NTZF), utilized in this study, is based on the TCM theory of Heart-Kidney Interaction, which describes the inter-regulation between the central nervous and reproductive endocrine systems.[11] Preliminary experimental studies indicate that NTZF can delay the senescence of ovarian granulosa cells and improve ovarian function in a POI mouse model, suggesting its potential therapeutic effects through modulating neuroendocrine signaling and the local ovarian microenvironment.[12] Although both HRT and TCM are used clinically for POI management, high-quality research on their combined use, particularly for synergistically improving reproductive endocrine parameters and addressing sleep disturbances, remains limited.

To address this critical evidence gap, our study employed a multi-phase design. To overcome the confounding biases and reverse causality inherent in traditional observational studies, we incorporated Mendelian randomization (MR). This approach utilizes genetic variants as instrumental variables, leveraging the early and random assignment of genotypes, to provide more reliable evidence for inferring causal exposure-outcome relationships in the insomnia-POI hypothesis.[13]

Based on this background, this present study advances two core hypotheses. First, decreased sleep quality is hypothesized to be an independent risk factor for POI, with a potential genetic-level causal relationship between insomnia and POI. Second, the TCM formula NTZF, developed based on the Heart-Kidney Interaction theory, is expected to exhibit synergistic effects with standard HRT in improving reproductive endocrine profiles and sleep quality in POI patients.

To evaluate these hypotheses, a three-phase study was designed. The first phase explores the association between sleep and POI via cross-sectional analysis. The second phase infers causality using MR. The final phase assesses the efficacy and safety of NTZF combined with HRT compared to either therapy alone through a clinical observational study.

Materials and Methods

Study Population and Design

The research design of this project is shown in Figure 1. First of all, from June 8, 2022, to December 31,2024,200 patients with POI were included in the gynecology clinic of the Affiliated Hospital of Zhejiang University of Traditional Chinese Medicine. According to the sample size formula of the cross-sectional study,[14] the number of patients included met the research needs. The demographic data and clinical indicators of the patients were collected to evaluate the risk factors affecting the incidence of POI. In order to confirm the causal relationship between sleep quality and POI, we used the UK Biobank (http://www.nealelab.is/uk-biobank/) and the FinnGen database to obtain genome-wide association studies (GWAS) data and performed two-sample two-way MR analysis.[15] The analysis methods include random inverse variance weighted (IVW), weighted median (WM), MR-Egger regression, simple mode, and weighted mode method.[16,17] Cochran's Q test was used to evaluate heterogeneity. The sample size estimation for this clinical trial was based on previous studies of a similar nature,[18] with the change in FSH level serving as the primary outcome measure. Assuming a significance level (α) of 0.05 and a statistical power (1-β) of 0.90, the calculated minimum sample size required for the three groups was 105 participants, accounting for an anticipated dropout rate of approximately 15%. This study ultimately enrolled and completed data analysis for 119 participants, thereby meeting the sample size requirement. Finally, 119 patients were included and divided into NTZF combined with Hormone Replacement Therapy (HRT) treatment group, NTZF control group and HRT control group according to the patient's wishes. The course of treatment was 6 months, aiming to evaluate the efficacy and safety of NTZF in the treatment of sex hormone levels and sleep quality in patients with POI. This study has been approved by the Ethics Committee of the Third Affiliated Hospital of Zhejiang Chinese Medical University (approval No.: ZSLL-KY-2022-037-01). Before the inclusion of the study, the researchers explained the purpose, plan and possible risks of the study to the patients in detail, and included the participants in the study after signing the written informed consent.

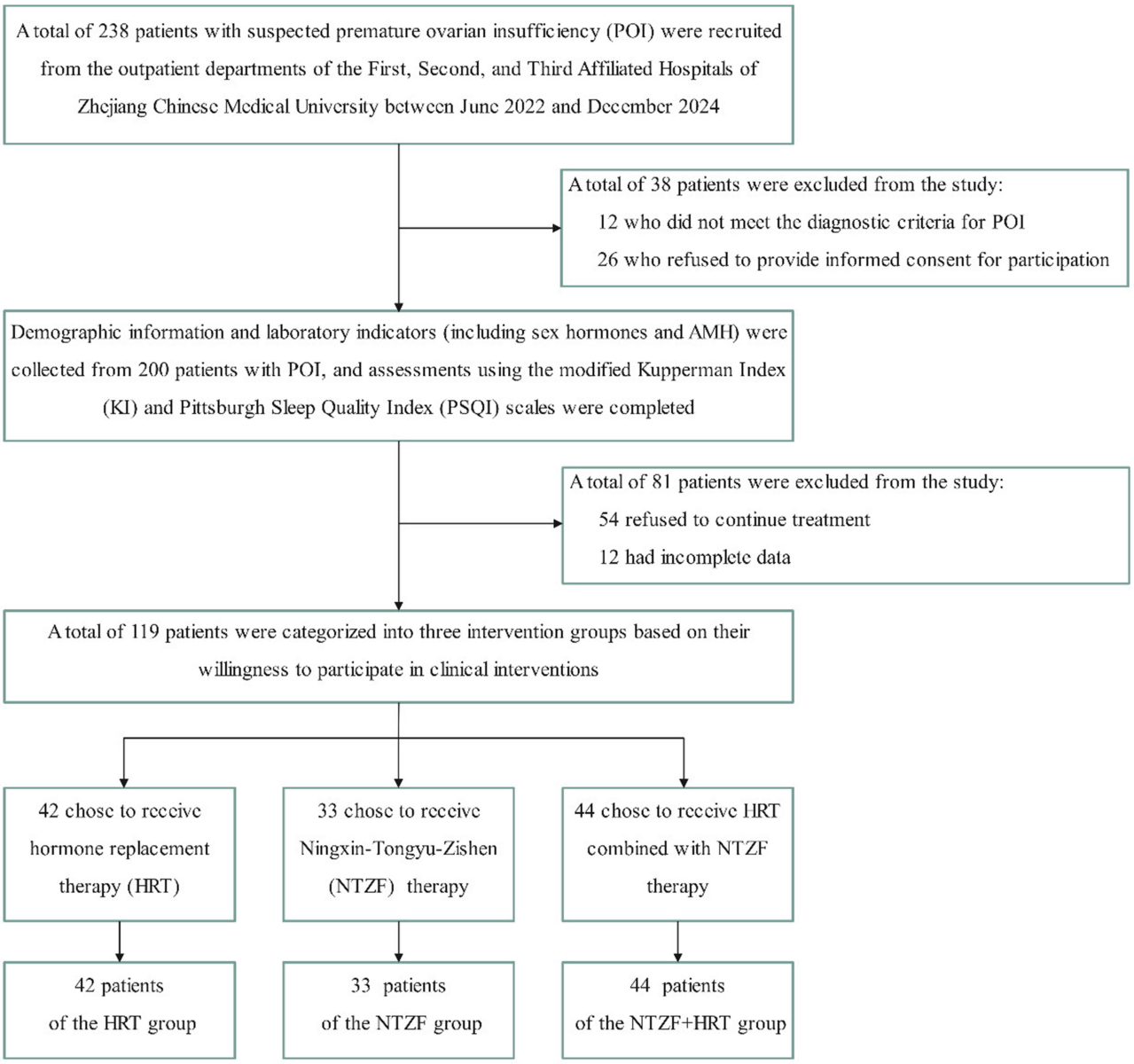


Figure 1 Study flow diagram of participants.

We registered on the International Traditional Medicine Clinical Trial Registry (ITMCTR) (No. ITMCTR2025000521). The GWAS data used in the MR analysis were derived from a publicly available database, and its ethical review was completed in the original study. This study strictly followed *the Helsinki Declaration* and followed STROBE to report the results of this study (Supplementary Tables S1 and S2).

Standards

The diagnostic criteria of POI refer to the Chinese expert consensus on clinical diagnosis and treatment: requiring age < 40 years, oligomenorrhea or amenorrhea for ≥ 4 months, and two consecutive Follicle-stimulating hormone (FSH) levels > 25 IU/L (measured ≥ 4 weeks apart).[19] Inclusion criteria: 18 ≤ age < 40 years old female patients, meet the diagnostic criteria of POI, voluntarily participate in and sign informed consent. Exclusion criteria: primary amenorrhea; patients with various diseases affecting ovarian function, such as chromosomal abnormalities, thyroid or adrenal diseases, chronic wasting diseases, malignant tumors, nutritional and metabolic diseases, severe malnutrition, etc. Patients with abnormal

liver and kidney function; unable to control and/or diagnose medical conditions, may interfere with or affect research and treatment, such as serious liver, heart, kidney, brain and other diseases; those who are unable to cooperate with patients with severe mental illness; those who had participated in other clinical trials within 3 months; allergic constitution or allergy to the tested drug; incomplete data; those who did not sign the informed consent.

The curative effect was determined by referring to the menstrual disease in the *TCM syndrome diagnosis and curative effect standard* and *Chinese medicine new drug clinical research guiding principle (Trial)*.[20,21] The calculation formula is: total effective rate (%) = (number of cases with obvious effect + number of effective cases)/total number of cases × 100%, as shown in supplementary Table S3.

The screening criteria of instrumental variables in the MR study: single nucleotide polymorphisms (SNPs) ($P < 5\times10^{-6}$), linkage disequilibrium (LD) $r^2 = 0.001$, region width = 10,000 kb, excluding weak instrumental variables (F statistic < 10).[22]

Treatment Plan

NTZF control group: The prescription is composed of Radix Rehmanniae Preparata, Tortoise Shell, Codonopsis, Yam, Semen Ziziphi Spinosae, Bupleuri, Cuscutae Semen, Angelica, Paeoniae Alba, and Cortex Moutan (All medicinal materials are sourced from the Third Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China). The detailed information is shown in Supplementary Table S4. One dose a day, water frying to 300mL (150mL/bag, a total of 2 bags), morning and evening each take a bag. It was taken on the 5th day of menstruation and stopped during the menstrual period. HRT control group: Patients received sequential HRT consisting of estradiol valerate tablets (Progynova®, Bayer AG, Berlin, Germany) at a dosage of 1 mg orally once daily, initiated on day 5 of the menstrual cycle for 21 days, with the addition of dydrogesterone tablets (Duphaston®, Abbott Biologicals B.V., Veerweg 12, 8121 AA Olst, Netherlands) at 20 mg orally once daily from day 12 to day 21 (10 days total). Each treatment cycle lasted 21 days. NTZF + HRT treatment group: taking NTZF while adding Progynova and Duphaston, taking the same method as above.

Data Collection and Management

In the cross-sectional study, demographic data and clinical indicators [including onset time, diet, previous menstrual period, previous cycle, abortion history, reproductive history, gynecological-related diseases, modified Kupperman index (KI) score, Pittsburgh Sleep Quality Index (PSQI) and reproductive hormone levels] were collected. The data were double-checked and electronically entered. In MR, the genetic data of sleep phenotype and POI were collected, and the data were integrated and standardized. In the clinical study, the clinical outcome indicators [including reproductive hormone levels (FSH), Luteinizing hormone (LH), estradiol (E_2), Anti-Müllerian hormone (AMH), the total effective rate of treatment (supplementary Table S5)], sleep quality (PSQI score), and safety indicators (gynecologic ultrasound, breast ultrasound, complete blood count (CBC), biochemical testing) were observed. The data were collected before treatment, 3 months after treatment, and 6 months after treatment. The data were collected through the paper case report form. After the treatment, the information was electronically entered and checked by two people to ensure accuracy.

Statistical Analysis

All data are checked for missing data before analysis. For variables with a missing ratio of more than 20%, they will be removed from the analysis to ensure data quality. In the cross-sectional study, SPSS 29.0 software was used. First, univariate analysis was used to screen potential influencing factors. Variables with $P < 0.05$ in univariate analysis were considered as potential risk factors, and binary logistic regression was included to further calculate the odds ratio (OR) and 95% confidence interval (CI) of independent risk factors. In the MR study, R 4.3.2 software was used for IVW, weighted median, MR-Egger regression, simple mode, and weighted mode to analyze the two-way causal effect of sleep and POI, and Cochran's Q test was used to evaluate heterogeneity. In the clinical study, the baseline data were analyzed by single factor analysis according to the treatment group, and the group balance of the three groups of drugs in the population was evaluated. For inter-group comparisons, independent data that followed a normal distribution with homogeneity of variance were analyzed using a two-sample *t*-test or F-test; otherwise, the Mann–Whitney *U*-test or Kruskal–Wallis *H*-test was applied. Categorical data were analyzed using the chi-square test. To control for the inflation

of type I error due to multiple comparisons, the Bonferroni correction was applied for comparisons such as the total effective rate, with the significance level adjusted to $\alpha = 0.05 / 3 = 0.0167$. Demographic characteristics and research results are described. Continuous variables conforming to normal or approximately normal distribution are described by $(\bar{x} \pm s)$. Those conforming to skewed distribution are represented by medians with quartiles, and classified data are represented by rates (%). In all analyses, the significance threshold is $P < 0.05$.

Results

Analysis of Risk Factors Related to Reproductive Hormones

A total of 200 patients with POI were included in this study. According to previous studies,[23,24] patients with POI were grouped according to the reproductive hormones FSH (≤ 40 IU/L and > 40 IU/L), AMH (≤ 0.2 ng/mL and > 0.2 ng/mL) and E_2 (≤ 73.2 pmol/L and > 73.2 pmol/L), and the demographic data and clinical characteristics of patients were compared (Tables 1–3). The results of the univariate analysis showed that there were significant differences in onset time, diet, modified KI score and PSQI score when grouped by FSH and AMH ($P < 0.05$), and there were significant differences in PSQI score when grouped by E_2 ($P < 0.05$), suggesting that PSQI is a potential risk factor for FSH, AMH and E_2 (Tables 1–3). The potential risk factors in the univariate analysis were included in the binary logistic regression. It was found that PSQI was an independent risk factor for FSH, E_2 and AMH in patients with POI. With the increase of

Table 1 Baseline Demographic and Clinical Characteristics of Study Participants Stratified by FSH Levels

Characteristic	FSH		*P* value
	≤ 40 IU/L (n=99)	> 40 IU/L (n=101)	
Age at onset (years)	38.00 (36.00, 39.00)	38.00 (36.00, 39.00)	0.559
BMI (kg/m^2)	21.23 (20.20, 22.66)	21.48 (19.69, 23.23)	0.969
Duration of disease			0.005
≤ 5 years	92 (92.9)	80 (79.1)	
> 5 years	7 (79.2)	21 (20.8)	
Dietary status			0.034
Normal	78 (78.8)	66 (65.3)	
Overeating or undereating	21 (21.2)	35 (34.7)	
Previous menstrual duration			0.855
≤ 7 days	89 (89.9)	90 (89.1)	
> 7 days	10 (10.1)	11 (10.9)	
Previous menstrual cycle length			0.802
≤ 30 days	81 (81.8)	84 (83.2)	
> 30 days	18 (18.2)	17 (16.8)	
History of miscarriage			0.403
No	45 (45.5)	40 (39.6)	
Yes	54 (54.5)	61 (60.4)	
History of childbirth			0.714
No	12 (12.1)	14 (13.9)	
Yes	87 (87.9)	87 (86.1)	
Gynecological diseases			0.365
No	64 (64.6)	59 (58.4)	
Yes	35 (35.4)	42 (41.6)	
KI score	8.78 ± 3.31	10.18 ± 2.98	0.002
PSQI score	10.46 ± 5.19	14.3 ± 4.61	< 0.001

Note: Continuous variables are presented as mean ± SD for normally distributed data or as median (25th, 75th percentile) for non-normally distributed data. Categorical variables are presented as n (%).

Abbreviations: FSH, follicle-stimulating hormone; BMI, Body Mass Index; KI, Kupperman Index; PSQI, Pittsburgh Sleep Quality Index.

Table 2 Baseline Demographic and Clinical Characteristics of Study Participants Stratified by AMH Levels

Characteristic	AMH		*P* value
	≤ 0.2 ng/mL (n=128)	> 0.2 ng/mL (n=72)	
Age at onset (years)	38.00 (36.00, 39.00)	38.00 (36.00, 39.00)	0.690
BMI (kg/m^2)	21.48 (20.03, 25.41)	21.11 (20.09, 22.39)	0.496
Duration of disease			0.003
≤ 5 years	103 (80.5)	69 (95.8)	
> 5 years	25 (19.5)	3 (4.2)	
Dietary status			0.043
Normal	86 (67.2)	58 (80.6)	
Overeating or undereating	42 (32.8)	14 (19.4)	
Previous menstrual duration			0.453
≤ 7 days	113 (88.3)	66 (91.7)	
> 7 days	15 (11.7)	6 (8.3)	
Previous menstrual cycle length			0.877
≤ 30 days	106 (82.8)	59 (81.9)	
> 30 days	22 (17.2)	13 (18.1)	
History of miscarriage			0.905
No	54 (42.2)	31 (43.1)	
Yes	74 (57.8)	41 (56.9)	
History of childbirth			0.661
No	16 (12.5)	10 (13.9)	
Yes	112 (87.5)	62 (86.1)	
Gynecological diseases			0.603
No	77 (60.2)	46 (63.9)	
Yes	51 (39.8)	26 (36.1)	
KI score	10 ± 2.98	8.57 ± 3.43	0.002
PSQI score	13.8 ± 4.79	9.92 ± 5.15	<0.001

Note: Continuous variables are presented as mean ± SD for normally distributed data or as median (25th, 75th percentile) for non-normally distributed data. Categorical variables are presented as n (%).
Abbreviations: AMH, anti-Müllerian hormone; BMI, Body Mass Index; KI, Kupperman Index; PSQI, Pittsburgh Sleep Quality Index.

Table 3 Baseline Demographic and Clinical Characteristics of Study Participants Stratified by E_2 Levels

Characteristic	E_2		*P* value
	≤ 73.2 pmol/L (n=128)	> 73.2 pmol/L (n=72)	
Age at onset (years)	38.00 (37.00, 39.00)	38.00 (36.00, 39.00)	0.293
BMI (kg/m^2)	21.88 (19.95, 23.23)	21.09 (20.06, 22.43)	0.360
Duration of disease			0.454
≤ 5 years	73 (83.9)	99 (87.6)	
> 5 years	14 (16.1)	14 (12.4)	
Dietary status			0.248
Normal	59 (67.8)	85 (75.2)	
Overeating or undereating	28 (32.2)	28 (24.8)	
Previous menstrual duration			0.950
≤ 7 days	78 (89.7)	101 (89.4)	
> 7 days	9 (10.3)	12 (10.6)	

(*Continued*)

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Table 3 (Continued).

Characteristic	E_2		*P* value
	≤ 73.2 pmol/L (n=128)	> 73.2 pmol/L (n=72)	
Previous menstrual cycle length			0.933
≤ 30 days	72 (82.8)	93 (82.3)	
> 30 days	15 (17.2)	20 (17.7)	
History of miscarriage			0.085
No	31 (35.6)	54 (47.8)	
Yes	56 (64.4)	59 (52.2)	
History of childbirth			0.895
No	11 (12.6)	15 (13.3)	
Yes	76 (87.4)	98 (86.7)	
Gynecological diseases			0.661
No	55 (63.2)	68 (60.2)	
Yes	32 (36.8)	45 (39.8)	
KI score	9.82 ± 3.12	9.23 ± 3.28	0.198
PSQI score	13.56 ± 4.93	11.50 ± 5.34	0.012

Note: Continuous variables are presented as mean ± SD for normally distributed data or as median (25th, 75th percentile) for non-normally distributed data. Categorical variables are presented as n (%).
Abbreviations: E_2, estradiol; BMI, Body Mass Index; KI, Kupperman Index; PSQI, Pittsburgh Sleep Quality Index.

PSQI score by one unit, the probability of FSH elevation increased to 1.13 times (OR = 1.13,95% CI: 1.06–1.20, $P \leq 0.001$), and the probability of E_2 elevation decreased to 0.93 times (OR = 0.93,95% CI: 0.88–0.98, $P \leq 0.001$). The probability of AMH elevation was reduced to 0.89 times (OR = 0.89,95% CI: 0.84–0.95, $P \leq 0.001$) (Table 4). Therefore, we included sleep quality in the follow-up analysis.

Table 4 Binary Logistic Regression Analysis of Factors Associated with FSH, AMH, and E_2 Levels

Indicator	Variables	Beta	Standard Error	Wald Chi-Square Value	OR (95% CI)	*P* value
FSH (IU/L)	Duration of disease					0.031
	≤ 5 years	-	-	-	-	
	> 5 years	1.069	0.496	4.650	2.91 (1.10–7.70)	
	Dietary status					0.008
	Normal	-	-	-	-	
	Overeating or undereating	1.001	0.378	7.025	2.72 (1.30–5.71)	
	KI score	0.119	0.055	4.617	1.13 (1.01–1.26)	0.032
	PSQI score	0.117	0.033	12.734	1.12 (1.05–1.20)	<0.001
AMH (ng/mL)	Duration of disease					0.019
	≤ 5 years	-	-	-	-	
	> 5 years	−1.535	0.656	5.464	0.22 (0.06–0.78)	
	Dietary status					0.009
	Normal	-	-	-	-	
	Overeating or undereating	−1.059	0.405	6.845	0.35 (0.16–0.77)	
	KI score	−0.118	0.058	4.209	0.89 (0.79–0.99)	0.040
	PSQI score	−0.111	0.033	11.201	0.90 (0.84–0.96)	<0.001
E_2 (pmol/L)	PSQI score	−0.078	0.029	7.395	0.93 (0.88–0.98)	0.007

Abbreviations: FSH, follicle-stimulating hormone; AMH, anti-Müllerian hormone; E_2, estradiol; KI, Kupperman Index; PSQI, Pittsburgh Sleep Quality Index; OR, odds ratio; CI, confidence interval.

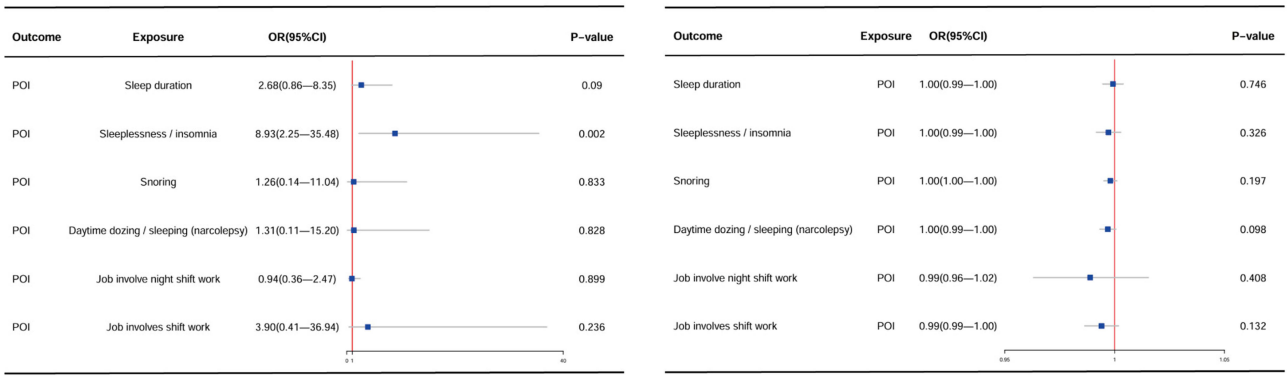

Figure 2 IVW results of MR between exposure and outcome.

The Causal Relationship Between Sleep Phenotype and POI in Genetic Variation

In the MR Analysis, the basic information of the genome-wide association studies of all the variables included in the study is presented in (Supplementary Table S3). Positive MR Analysis of sleep phenotypes and POI. After removing SNPs with LD and moderate palindromic sequences, 112 SNPS were ultimately retained, with an F value >10. IVW analysis indicated that insomnia might increase the risk of POI occurrence (IVW: OR = 8.93, 95% CI: 2.25–35.48, P = 0.002) (Figure 2). The OR of the other four methods was all > 1, which was consistent with the OR direction of IVW (Supplementary Table S6). The intercept of the MR-Egger regression analysis was 0.013 (P = 0.584), indicating that there was no horizontal pletropy in this study. Cochran's Q heterogeneity test did not detect heterogeneity in either the IVW analysis method or the MR-Egger regression method (IVW: Q = 95.699, P = 0.375; MR-Egger: Q = 95.383, P = 0.356) (Supplementary Table S4). The remaining five sleep phenotypes were not related to the risk of POI onset (Supplementary Table S4 and Supplementary Figure S1 and 2). In addition, POI was also not related to the risk of POI onset for the six sleep phenotypes (Supplementary Table S4 and Supplementary Figure S3 and 4). In the sensitivity analysis, the results of MR-presso and the retention one method showed that the MR Results of this study were robust (Supplementary Table S4 and Supplementary Figure S1–4).

The Clinical Outcome of NTZF Combined with HRT in the Treatment of POI

Primary Outcome Indicator

There was no significant difference in baseline among the three groups before treatment (P > 0.05) (Table 5). For intergroup comparison, at 3 months of treatment, there was no significant difference in E_2 among the three groups (P > 0.05), while

Table 5 Baseline Demographic and Clinical Characteristics of Study Participants by Treatment Group

Characteristic	HRT Group (n=42)	NTZF Group (n=33)	NTZF+HRT Group (n=44)	*P* value
Age at onset (years)	38.00 (36.00, 39.00)	38.00 (35.50, 39.00)	38.00 (36.00, 39.00)	0.992
BMI (kg/m^2)	21.16 (20.07, 22.85)	22.03 (20.67, 24.01)	21.25 (19.56, 22.25)	0.069
Duration of disease				0.857
≤ 5 years	37 (88.1)	28 (84.8)	37 (84.1)	
> 5 years	5 (11.9)	5 (15.2)	7 (15.9)	
Dietary status				0.768
Normal	31 (73.8)	22 (66.7)	30 (68.2)	
Overeating or undereating	11 (26.2)	11 (33.3)	14 (31.8)	
Previous menstrual duration				0.183
≤ 7 days	36 (85.7)	26 (78.8)	41 (93.2)	
> 7 days	6 (14.3)	7 (21.2)	3 (6.8)	

(*Continued*)

Table 5 (Continued).

Characteristic	HRT Group (n=42)	NTZF Group (n=33)	NTZF+HRT Group (n=44)	*P* value
Previous menstrual cycle length				0.331
≤30 days	31 (73.8)	27 (81.8)	38 (86.4)	
>30 days	11 (26.2)	6 (18.2)	6 (13.6)	
History of miscarriage				0.183
No	14 (33.3)	18 (54.5)	19 (43.2)	
Yes	28 (66.7)	15 (45.5)	25 (56.8)	
History of childbirth				0.462
No	5 (11.9)	4 (12.1)	9 (20.5)	
Yes	37 (88.1)	29 (87.9)	35 (79.5)	
Gynecological diseases				0.134
No	26 (61.9)	13 (39.4)	25 (56.8)	
Yes	16 (38.1)	20 (60.6)	19 (43.2)	

Note: Continuous variables are presented as mean ± SD for normally distributed data or as median (25th, 75th percentile) for non-normally distributed data. Categorical variables are presented as n (%).
Abbreviations: HRT, Hormone replacement therapy; NTZF, Ningxin-Tongyu-Zishen Formula; BMI, Body Mass Index.

there were differences in the other outcome indicators ($P < 0.05$) (Table 6); After 6 months of treatment, there were differences in all outcome indicators among the three groups ($P < 0.05$) (Table 6), pairwise comparison results among the three groups (Table 6), and intra-group comparison results (Supplementary Table S7). After 6 months of treatment, the total effective rates of the three groups were 93.2% in the NTZF+HRT group, 39.4% in the NTZF group, and 69.0% in the HRT control group respectively. The therapeutic effect was significantly higher than that after 3 months of treatment. There was a statistical difference in the total effective rate between the three groups ($P < 0.05$), and there was a statistical difference between the three groups ($P < 0.017$). HRT + NTZF group and NTZF group, the effective rate difference between the two groups and 95CI (%) 53.79 (31.52,76.06), NTZF + HRT group compared with HRT group, the effective rate difference between the two groups and 95CI (%) 24.13 (4.81,43.45), HRT compared with NTZF group, the effective rate difference between the two groups and 95CI (%) 29.96 (3.43,56.49). The results showed that the efficacy of NTZF combined with HRT was better than the other two treatment methods (Table 7).

Secondary Outcome Indicators

There was no significant difference in the modified KI score between the three groups at baseline ($P > 0.05$). After 3 months and 6 months of treatment, there were differences between the three groups and within the group ($P < 0.05$) (Table 8 and Supplementary Table S7). During the 6-month treatment period, some patients had mild breast-distending pain, which was related to the use of progesterone, which was normal. No obvious symptoms of gastrointestinal dysfunction were found, and no adverse events such as drug allergy were found. The CBC and biochemical testing of the three groups were within the normal range. Except for 5 patients with anemia before treatment, there was no special report of breast ultrasound and gynecologic ultrasound.

Discussion

This study adopted a multi-faceted methodological integration strategy, employing cross-sectional analysis, MR, and a clinical observational study to investigate the relationship between sleep and POI. This research pathway, combining genetic epidemiology with clinical intervention, constitutes an innovative methodological exploration in POI research. Our findings indicate that decreased sleep quality is an independent risk factor for POI, and that a potential unidirectional causal relationship exists between insomnia and POI at the level of genetic variation. Furthermore, the combination of the NTZF with HRT improved reproductive hormone levels in POI patients and enhanced sleep quality, with good safety and tolerability. This integrated approach offers a novel strategy for the combined use of traditional Chinese and Western medicine treatment of POI.

Table 6 Primary Outcomes After Treatment

Timepoint	Indicator	HRT Group	NTZF Group	NTZF+HRT Group	*P* value	HRT Group vs NTZF Group		NTZF Group vs NTZF+HRT Group		HRT Group vs NTZF+HRT Group	
		OR (95% CI)	OR (95% CI)	OR (95% CI)		OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
0 months	FSH (IU/L)	50.20 (27.45, 70.13)	58.40 (30.17, 75.11)	50.92 (28.16, 67.90)	0.800	−1.79 (−14.00, 6.68)	1.000	2.31 (−5.96, 14.87)	1.000	0.10 (−8.07, 9.99)	1.000
	AMH (ng/mL)	0.11 (0.03, 0.19)	0.13 (0.04, 0.28)	0.13 (0.05, 0.32)	0.328	−0.02 (−0.08, 0.02)	1.000	−0.01 (−0.08, 0.04)	1.000	−0.04 (−0.10, 0.01)	0.418
	E_2 (p mol/L)	96.67 (51.73, 218.17)	91.53 (53.93, 190.98)	64.20 (45.35, 142.07)	0.434	0.02 (−31.70, 32.47)	1.000	12.46 (−10.94, 47.71)	0.891	12.11 (−9.20, 42.53)	0.745
	LH (IU/L)	35.12 ± 12.04	33.95 ± 11.87	33.95 ± 11.87	0.668	−1.43 (−8.52, 5.65)	1.000	2.60 (−4.42, 9.61)	1.000	1.17 (−5.41, 7.74)	1.000
	PSQI	16.00 (11.75, 18.25)	14.00 (8.00, 16.50)	15.00 (8.00, 17.00)	0.108	2.00 (0.00, 4.00)	0.111	−1.00 (−3.00, 1.00)	1.000	1.00 (−1.00, 3.00)	0.613
3 months	FSH (IU/L)	23.01 (20.94, 62.06)	50.16 (35.01, 73.71)	36.23 (22.27, 40.76)	0.007	−17.17 (−29.56, −4.91)	0.019	18.74 (7.83, 31.95)	0.012	−0.96 (−10.55, 13.30)	1.000
	AMH (ng/mL)	0.31 (0.27, 0.33)	0.16 (0.12, 0.27)	0.43 (0.35, 0.57)	<0.001	0.13 (0.07, 0.17)	0.026	−0.26 (−0.31, −0.20)	<0.001	−0.13 (−0.19, −0.09)	<0.001
	E_2 (p mol/L)	139.80 (113.80, 238.98)	109.70 (63.65, 204.74)	143.31 (99.15, 197.49)	0.206	27.91 (−3.04, 62.26)	0.321	−24.94 (−52.26, 7.23)	0.377	0.90 (−13.08, 33.15)	1.000
	LH (IU/L)	26.77 ± 12.38	24.51 ± 12.99	24.51 ± 12.99	0.003	−7.94 (−15.38, −0.51)	0.032	10.21 (2.85, 17.57)	0.003	2.26 (−4.63, 9.16)	1.000
	PSQI	12.00 (8.25, 16.00)	11.00 (5.00, 15.00)	11.00 (6.00, 11.00)	0.002	1.00 (−1.00, 3.00)	1.000	2.00 (0.00, 5.00)	0.045	3.00 (1.00, 5.00)	0.002
6 months	FSH (IU/L)	30.89 (8.26, 41.43)	38.09 (23.64, 60.55)	10.14 (5.34, 21.53)	<0.001	−13.81 (−24.22, −3.49)	0.040	23.82 (16.75, 35.09)	<0.001	12.92 (3.77, 22.06)	0.008
	AMH (ng/mL)	0.49 ± 0.14	0.58 ± 0.15	0.58 ± 0.15	<0.001	0.11 (0.02, 0.19)	0.008	−0.19 (−0.27, −0.11)	<0.001	−0.08 (−0.16, −0.01)	0.032
	E_2 (p mol/L)	191.92 (147.79,274.59)	128.55 (83.44, 213.35)	188.28 (180.75, 227.35)	0.001	54.51 (20.74, 83.27)	0.006	−63.24 (−95.14, −32.76)	0.002	−4.44 (−31.60, 11.19)	1.000
	LH (IU/L)	16.88 (12.34, 26.46)	26.32 (21.22, 37.96)	5.78 (3.52, 11.25)	<0.001	−9.53 (−15.34, −3.74)	0.047	19.69 (16.41, 22.87)	<0.001	10.16 (6.65, 13.22)	0.001
	PSQI	8.50 (4.00, 10.25)	9.00 (4.50, 12.50)	6.00 (4.00, 7.00)	0.005	−1.00 (−3.00, 1.00)	1.000	3.00 (1.00, 5.00)	0.011	2.00 (1.00, 4.00)	0.023

Note: Continuous variables are presented as mean ± SD for normally distributed data or as median (25th, 75th percentile) for non-normally distributed data.

Abbreviations: HRT, Hormone replacement therapy; NTZF, Ningxin-Tongyu-Zishen Formula; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; AMH, anti-Müllerian hormone; PSQI, Pittsburgh Sleep Quality Index.

Table 7 Analysis of Therapeutic Efficacy at 3 and 6 months Among Different Treatment Groups

Treatment Duration	Group	Ineffective [n (%)]	Effective [n (%)]	Significantly Effective [n (%)]	χ^2 value	*P* value
3 months	HRT group	20 (47.6)$^{\#}$	17 (40.5)	5 (11.9)	13.885	0.008
	NTZF group	28 (84.8)	3 (9.1)	2 (6.1)		
	NTZF+HRT group	21 (47.7)$^{\#\#}$	18 (40.9)	5 (11.4)		
6 months	HRT group	13 (31.0)$^{\#}$	16 (38.1)	13 (31.0)	31.802	<0.001
	NTZF group	20 (60.6)	11 (33.3)	2 (6.1)		
	NTZF+HRT group	3 (6.8)$^{\#\#*}$	17 (38.6)	24 (54.5)		

Note: Data were analyzed using the Chi-square test. At 3 months: $^{\#}P = 0.003$, HRT group vs NTZF group; $^{\#\#}P = 0.003$, NTZF group vs NTZF +HRT group. At 6 months: $^{\#}P = 0.009$, HRT group vs NTZF group; $^{\#\#}P < 0.001$, NTZF group vs NTZF+HRT group; $^{*}P = 0.009$, HRT group vs NTZF+HRT group.
Abbreviations: HRT, Hormone replacement therapy; NTZF, Ningxin-Tongyu-Zishen Formula.

Table 8 Secondary Outcomes After Treatment

Timepoint	Indicator	HRT Group	NTZF Group	NTZF+HRT Group	*P* value	HRT Group vs NTZF Group		NTZF Group vs NTZF+HRT Group		HRT Group vs NTZF+HRT Group	
		OR (95% CI)	OR (95% CI)	OR (95% CI)		OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
0 months	KI	10.71 ± 2.75	9.97 ± 2.81	9.97 ± 2.81	0.304	−1.43 (−8.52, 5.65)	1.000	2.60 (−4.42, 9.61)	0.467	1.17 (−5.41, 7.74)	0.708
3 months	KI	6.00 (4.88, 7.63)	9.00 (6.75, 11.50)	4.00 (3.50, 4.88)	<0.001	−3.00 (−4.50, −2.00)	<0.001	5.50 (4.00, 6.50)	<0.001	2.00 (1.50, 3.00)	0.002
6 months	KI	3.50 (2.88, 4.63)	6.00 (4.50, 7.00)	1.50 (0.50, 1.50)	<0.001	−2.50 (−3.00, −1.50)	0.006	5.00 (4.00, 5.50)	<0.001	2.50 (2.00, 3.00)	<0.001

Note: Continuous variables are presented as mean ± SD for normally distributed data or as median (25th, 75th percentile) for non-normally distributed data.
Abbreviations: HRT, Hormone replacement therapy; NTZF, Ningxin-Tongyu-Zishen Formula; KI, Kupperman Index.

The present findings align closely with recent research trends. For instance, a systematic review by Caetano et al identified sleep disorders as an independent environmental factor affecting female fertility.[5] This study reinforces that conclusion through genetic evidence and specifically focus on POI as a distinct population, providing a new perspective for understanding its pathogenesis.

The results indicate that a higher PSQI score, reflecting poor sleep quality, is an independent risk factor for POI. This association may be attributable to insomnia-induced dysfunction of the HPO axis. Previous studies have shown that insomnia disrupt the function of both the hypothalamic-pituitary-adrenal (HPA) axis and the HPO axis, leading to dysregulation in the secretory rhythms of GnRH, LH, and FSH, which in turn impairs follicular development and ovulation.[25,26] In addition, sleep disturbances and circadian rhythm disruptions can affect the expression of clock genes such as BMAL1, CLOCK, which are critical for regulating the ovarian cycle.[27] Our findings further validate the clinical relevance of the circadian clock-ovary hypothesis. Furthermore, clinical and experimental evidence suggests that insomnia can enhance systemic inflammatory response, increasing levels of inflammatory factors like IL-6 and TNF-α.[28] Individuals with insomnia may experience chronic low-grade inflammation, which could damage ovarian granulosa cells and contribute to the development of POI. The interaction of these mechanisms may accelerate the depletion of ovarian reserve, potentially explaining the observed association between insomnia and increased POI risk. Further research combining basic experiments approaches is required to clarify the specific molecular pathways involved.

Against this theoretical and clinical backdrop, the combined application of NTZF and HRT for treating POI and improving sleep quality demonstrates unique value. While both therapies are individually employed in clinical practice, evidence for their long-term combined use in POI treatment is very limited, making this study a significant exploration in this field. The synergistic effect observed between NTZF and HRT can be elucidated through modern pharmacological mechanisms. Core herbal components including Ziziphi Spinosae Semen and Bupleuri Radix, which serve essential functions in tranquilizing the heart and relieving stagnation, contain active constituents such as jujuboside and saikosaponins. These compounds demonstrate modulatory effects on the central GABAergic system, providing a pharmacological foundation for sleep quality improvement that aligns with Western medical principles of sleep hygiene.[29]

Concurrently, kidney-tonifying and essence-replenishing herbs like Rehmanniae Radix Praeparata and Testudinis Carapax et Plastrum have been scientifically validated to exhibit antioxidant properties and inhibit granulosa cell apoptosis.[30] Consequently, NTZF likely achieves multi-target therapeutic outcomes through dual mechanisms of central nervous system regulation and ovarian microenvironment improvement, thereby complementing HRT at the neuro-endocrine level to jointly facilitate the functional recovery of the HPO axis.

Placing our findings within the landscape of current POI management strategies highlights their importance. Standard HRT for POI primarily focuses on hormone replacement, symptom improvement, and prevention of long-term complications, but has capacity to restore intrinsic ovarian function and shows inconsistent efficacy for comorbidities like sleep disturbances. Alternative treatments, including stem cell and exosome therapies, either lack sufficient evidence or remain in the experimental stage.[31,32] Furthermore, recent research underscores the influence of endocrine and metabolic factors, such as thyroid function, insulin resistance, on ovarian morphology and function.[33,34] The NTZF and HRT combination regimen presented in this study offers further benefits in improving AMH, a key marker of ovarian reserve, and enhancing sleep quality. This indicates that for complex conditions like POI require a multi-dimensional management strategy that addresses neuroendocrine, metabolic, and ovarian microenvironmental aspects is needed. The integrated Chinese and Western medicine approach compensates for the shortcomings of single-modality therapies through multi-target regulation, offering a more comprehensive management approach for POI.

The findings of this study possess clear clinical translational value and broad prospects. They strongly suggest that sleep quality should be considered a key modifiable factor in the diagnosis and treatment of POI. Routine assessment of sleep in POI patients using tools like the PSQI can facilitate the identification of individuals most likely to benefit most from sleep interventions, supporting earlier and more personalized management. Future research could focus on establishing specific PSQI cutoff values in broader populations to aid in risk stratification for early intervention. These findings could potentially change clinical practice, prompting the consideration of sleep quality as a key element in POI management, allowing for earlier intervention in patients suffering from both sleep disorders and ovarian insufficiency. Moreover, this study paves the way for further exploration of how integrated Chinese and Western medicine therapies can be applied to female hormonal and reproductive health issues, expanding the range of POI management strategies.

Methodologically, integrating MR from genetic epidemiology, the holistic approach of TCM, and standard Western medical treatment provides convergent evidence for the insomnia-POI hypothesis from different methodological perspectives. This interdisciplinary approach demonstrates significant potential for addressing complex gynecological endocrine disorders. It also establishes a preliminary framework for building an integrated research paradigm in female reproductive health that combines biomedicine, genetics, and traditional medicine.

Certainly, translating the findings of this study into widely adopted clinical guidelines requires ongoing effort. As a pioneering exploration, this study lays a solid foundation for subsequent research. Future studies should prioritize the following directions: conducting large-scale, multicenter, randomized, double-blind, placebo-controlled trials with follow-up periods of 12 months or longer to confirm the long-term efficacy, safety, and impact on reproductive outcomes of the NTZF + HRT combination regimen; delving deeper into its mechanisms of action, exploring the biological basis of the synergistic effects from multiple angles such as the ovarian microenvironment, HPO axis regulation, and central sleep-wake pathways; and applying the research framework of "etiological exploration - clinical validation - mechanism research" established in this study to the evaluation of integrated Chinese and Western medicine treatments for other complex gynecological endocrine disorders, aiming to construct a more comprehensive female reproductive health management strategy.

Limitations

Several limitations should be acknowledged in this study. The primary limitation is the non-randomized clinical design. Although patient self-selection of treatment groups reflects real-world clinical decision-making, it inevitably introduces the risk of selection bias. Despite baseline characteristics were balanced between groups and statistical methods were used for control, unmeasured confounding factors may still be present. For example, patients more receptive to TCM might possess certain unmeasured characteristics, such as a preference for natural therapies or specific lifestyle factors, which could themselves influence outcomes. Future randomized controlled studies should consider advanced statistical

techniques, such as propensity score matching, to more precisely control for potential confounders. The 6-month observation period is sufficient for assessing short-to-medium term efficacy but does not address the long-term effects and safety of NTZF combined with HRT. Furthermore, the single source study sample may limit the generalizability of the findings across different ethnic and geographical populations. Future research should prioritize multicenter, randomized, double-blind, placebo-controlled designs, increase sample size, and extend follow-up to 12 months or longer to validate the conclusions and assess long-term outcomes such as pregnancy rates. Additionally, although the MR analysis provides strong evidence for a causal relationship between insomnia and POI, and horizontal pleiotropy was assessed using methods including MR-Egger regression, certain limitations remain. Genetic instrumental variables might influence POI through biological pathways independent of insomnia, or unmeasured confounding factors might exist, potentially affecting the precision of the causal inference. Moreover, the genetic data used were primarily derived from European populations, and the applicability of the conclusions to populations with different genetic backgrounds and environments requires further validation.

Conclusion

An elevated PSQI score, indicating poor sleep quality, is an independent risk factor for POI. Genetic evidence supports a potential causal link between insomnia and POI risk, suggesting that insomnia may accelerate ovarian function decline by affecting patients' reproductive hormone levels. Therefore, insomnia may accelerate ovarian function decline by affecting the level of reproductive hormones in patients. The long-term treatment of NTZF combined with HRT can not only significantly improve the reproductive hormone levels of POI patients, but also significantly improve the sleep quality, which is safe and well tolerated. This study provides a new strategy for the treatment of POI with integrated traditional Chinese and Western medicine.

Abbreviations

POI, Premature ovarian insufficiency; NTZF, Ningxin-Tongyu-Zishen Formula; MR, Mendelian randomization; HRT, Hormone replacement therapy; PSQI, Pittsburgh Sleep Quality Index; KI, Kupperman index; TCM, traditional Chinese medicine; FSH, Follicle-stimulating hormone; LH, Luteinizing hormone; E_2, estradiol; AMH, Anti-Müllerian hormone; IVW, inverse variance weighted, WM, weighted median; SNPs, single nucleotide polymorphisms; LD, linkage disequilibrium; GWAS, genome-wide association studies; OR, odds ratio; 95% CI, 95% confidence interval; CBC, complete blood count; HPO, hypothalamic-pituitary-ovarian axis; GnRH, gonadotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal axis.

Data Sharing Statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Ethics Approval and Informed Consent

This study was approved by the Ethics Committee of the Third Affiliated Hospital of Zhejiang University of Traditional Chinese Medicine (approval number: ZSLL-KY-2022-037-01), All patients were included in the study after signing informed consent. This study was registered in the International Traditional Medicine Clinical Trial Registry (ITMCTR), registration number (ITMCTR2025000521). GWAS data are derived from public databases, and their ethical review has been completed in the original study.

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The capsules, ovary, heart and kidney icons of graphical abstract are acquired from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Common Attribution 4.0 Generic License (https://creativecommons.org/licenses/by/4.0/).

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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