

Investigating the Therapeutic Effects of Bushen Huoxue Formula Combined with Aerobic Exercise on Reproductive Function in Obese Polycystic Ovary Syndrome: An Experimental Study Based on the Integration of Sports and Medicine

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Objective: To investigate the therapeutic effects of Bushen Huoxue Formula combined with aerobic exercise (COMB) on reproductive function in obese polycystic ovary syndrome (PCOS) mice.

Methods: Seventy-six 3-week-old female C57BL/6JGpt mice were randomly divided into a control group (CON, $n=14$) and a model group (MOD, $n=62$). The PCOS model was established using a high-fat diet (HFD) combined with dehydroepiandrosterone (DHEA). Successfully modeled mice were randomly allocated into the MOD group ($n=12$), metformin group (MET, $n=12$), Bushen Huoxue Formula group (BHF, $n=12$), aerobic exercise group (AE, $n=12$), and BHF combined with AE group (COMB, $n=12$). After 8 weeks of intervention, 6 female mice were randomly selected from each group. Body weight, body fat percentage (BFP), and fasting plasma glucose (FPG) were measured. Fasting insulin (FINS) and serum sex hormone levels (follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), and estradiol (E2)) were determined using enzyme-linked immunosorbent assay (ELISA). The remaining female mice in each group were co-housed with 10-week-old male mice. Pregnancy rate, abortion rate, and embryonic resorption rate were recorded.

Results: After 8 weeks of intervention, MET, BHF, AE, and COMB groups showed significant improvements in ovarian morphology, body weight, BFP, insulin resistance (HOMA-IR), and serum sex hormone levels ($P<0.05$). Compared with the BHF and AE groups, the COMB group demonstrated superior effects in reducing BFP, T levels, and HOMA-IR ($P<0.05$). All intervention groups showed higher pregnancy rates compared to the MOD group. Notably, the BHF, AE, and COMB groups exhibited lower abortion and embryo loss rates than the MET group. COMB group showed the most favorable pregnancy outcomes, including higher pregnancy rate, lower abortion and embryo absorption rates, and increased total number of embryos.

Conclusion: The combined intervention of the COMB group demonstrated superior therapeutic effects on reproductive function in obese PCOS mice compared to the individual interventions of the BHF group or AE group, with a better safety profile. The integrated sports-medicine approach shows promising clinical potential.

Keywords: bushes Huoxue formula, aerobic exercise, polycystic ovary syndrome, obesity, reproductive function

Introduction

PCOS is a prevalent gynecological endocrine disorder characterized by its chronic nature, with increasing incidence rates in recent years.¹ Studies indicate that approximately 30–70% of PCOS patients present with overweight or obesity, predominantly exhibiting central obesity.² The correlation between obesity and female reproductive dysfunction has been documented in ancient Chinese medical literature. The Danxi's Experiential Therapy (Danxi Xinfu) from the Yuan Dynasty states: "For



obese women with excessive nourishment and indulgence in alcohol and food, menstrual irregularities and infertility may occur due to fat accumulation obstructing the uterus...”,³ explicitly indicating that dietary excess-induced obesity can lead to menstrual disorders and infertility. Fu Qingzhu’s Gynecology (Fu Qingzhu Nüke) from the Qing Dynasty records: “Dampness predominance often leads to obesity, obesity often accompanies qi deficiency, and qi deficiency results in phlegm retention. While appearing robust externally, internal deficiency is present. When the spleen fails to transform, dampness accumulates in the uterus, gradually transforming it into a reservoir of fluid. Moreover, in obese women, excessive internal fat obstructs the uterus, preventing fertilization”.⁴ These classical theories provide the foundation for treating obese PCOS through traditional Chinese medicine (TCM) approaches focusing on phlegm dampness and qi deficiency.

In recent years, the concept of “sports-medicine integration” has garnered increasing attention, with its core principles rooted in traditional Chinese medical theories such as “smooth circulation of qi and blood” and “balance between activity and rest”. This integrative approach has been explicitly proposed in the “Healthy China 2030” Planning Outline.⁵ Sports-medicine integration emphasizes the combination of physical exercise and medical care, utilizing medical expertise and technology to guide exercise interventions for health promotion. Currently, researchers have explored the application of this integrated model in various fields, including pulmonary function improvement in pneumoconiosis patients,⁶ postural correction in children and adolescents,⁷ and cardiac function enhancement in chronic heart failure patients,⁸ demonstrating significant therapeutic outcomes.

The optimization of therapeutic strategies for obese PCOS remains a crucial focus in clinical research, with cost-effectiveness and treatment efficacy being primary considerations. Clinical studies have demonstrated that BHF (comprising Shoutai Wan and Guizhi Fuling Wan) combined with lifestyle interventions significantly enhances therapeutic outcomes compared to monotherapy approaches, revealing a notable synergistic effect. This integrated intervention model fully embodies the modern medical concept of “sports-medicine integration”, providing novel insights into the comprehensive management of obese PCOS.

Building upon these findings, this study innovatively investigates the therapeutic effects and underlying mechanisms of BHF combined with aerobic exercise on reproductive function in obese PCOS from the perspective of sports-medicine integration. The research outcomes will provide valuable experimental evidence for clinical decision-making in PCOS treatment while offering scientific support for the application of traditional Chinese medicine in reproductive endocrine disorders.

Materials and Methods

Experimental Drugs

The BHF, composed of Shoutai Wan and Guizhi Fuling Wan, contains the following components: Guizhi (Cassia bark tree Twig) [Guangxi, Batch No. 24090150] 10 g, Fuling (Indian Buead Tuokahoe) [Yunnan, Batch No. 24030379] 15 g, Baishao (White Paeoniae Trichocarpaceae) [Anhui, Batch No. 24080180] 15 g, Taoren (Peach Seed) [Henan, Batch No. 24110015] 15 g, Danpi (Tree Peony Root-bark) [Anhui, Batch No. 24090174] 15 g, Sangjisheng (Chinese Taxillus Herb) [Yunnan, Batch No. 24070073] 15 g, Xuduan (Asper-like easel Root) [Sichuan, Batch No. 24100090] 20 g, and Tusizi (Chinese Dodder Seed) [Ningxia, Batch No. 24100040] 15 g. The herbal pieces were processed into instant granules by Sichuan New Green Pharmaceutical Technology Development Co., Ltd. All Chinese herbal decoction pieces (CHDP) used in this study were procured from certified suppliers of the company, with botanical origin authentication and quality control strictly adhering to the relevant monographs of the Pharmacopoeia of the People’s Republic of China (2020 Edition).⁹ Metformin tablets were obtained from Bristol-Myers Squibb (Shanghai) Co., Ltd. (Batch No. H20023370).

Experimental Animals

Seventy-six specific pathogen-free (SPF) female C57BL/6JGpt mice (3 weeks old) were purchased from Chengdu Yaokang Biotechnology Co., Ltd. (License No. SCXK(Chuan)2020-034). The animals were housed in a quiet, well-ventilated environment with controlled temperature (22±2°C) and humidity (45–60%). A 12-hour light/dark cycle was maintained, with free access to food and water. This study has been reviewed and approved by the Animal Ethics Committee of Chengdu University of Traditional Chinese Medicine. Laboratory animal procedures complied with

China's Guidelines on Ethical Treatment of Laboratory Animals (MoST) and the National Standard Guidelines for Welfare and Ethical Review of Laboratory Animals (GB/T 35892–2018).

Experimental Reagents and Experimental Equipment

The main reagents used in this experiment, along with their manufacturers and catalog numbers, are listed in Table 1. Similarly, the experimental equipment and their manufacturers and models are listed in Table 2.

Animal Grouping and Modeling

Following a 3-day acclimatization period, 76 female C57BL/6JGpt mice were randomly allocated into two groups: 62 mice as the MOD group and 14 mice as the CON group. The MOD group was modeled using a HFD (60% fat, 20% carbohydrate, 20% protein) combined with daily subcutaneous injections of 0.6 mg/kg DHEA dissolved in 0.1 mL sesame oil. The CON group received a standard diet (12% fat, 65% carbohydrate, 23% protein) and equivalent-volume injections of sesame oil. Both diets were procured from Beijing Huafukang Bioscience Co., Ltd. (China), with interventions maintained for 21 days. From day 10 of modeling, vaginal smears were collected daily for 10 consecutive days to monitor estrous cycles. When 50% of mice in the MOD group exhibited estrous cycle disorders, a statistically significant difference in body weight between the two groups was confirmed. Subsequently, two mice were randomly selected from each of the MOD and CON groups. Successful model establishment was validated through assessment of BFP and histological examination of ovarian tissues.^{10,11}

After successful modeling, the mice were randomly divided into five groups (n=12 each): MOD group, MET group, BHF group, AE group, and COMB group. The CON group retained 12 mice as controls, resulting in a total of 72 mice included in the experiment.

Table 1 Experimental Reagents

Main Reagents	Manufacturer/Brand	Catalog Number
HE Staining Kit	Leagene	DH0020
ELISA Kit (E2)	Elabscience	E-OSEL-M0008
ELISA Kit (T)	Elabscience	E-OSEL-M0003
ELISA Kit (FSH)	Elabscience	E-EL-M0511c
ELISA Kit (LH)	Elabscience	E-EL-M3053
ELISA Kit (INS)	Elabscience	E-EL-M1382c

Table 2 Experimental Equipment

Name	Manufacturer	Model
Treadmill	Jiangsu Siansi Biotechnology Co., Ltd.	SA101BR
Analytical Balance	Shimadzu	AUY120
Benchtop High-Speed Micro Centrifuge	Scilogex (USA)	D3024
Benchtop High-Speed Refrigerated Centrifuge	Scilogex (USA)	D3024R
Vortex Mixer	Scilogex (USA)	MX-S
Digital Thermostatic Water Bath Shaker	Changshu Langyue Instrument Manufacturing Co., Ltd.	THZ-82A
High-Speed Low-Temperature Tissue Grinder	Wuhan Servicebio Technology Co., Ltd.	KZ-III-F
Spectrophotometer	BioTek (USA)	µQuant
Embedding Machine	Wuhan Junjie Electronics Co., Ltd.	JB-P5
Microtome	Thermo Fisher Scientific	HM325
Pathology Slide Scanner	3DHISTECH Kft	Pannoramic SCAN II

Intervention Methods for Each Group of Mice

The BHF and COMB groups received oral gavage of 0.5 g/kg/d (equivalent dose calculated based on human-mice body surface area ratio of 0.0026) at 10:00 AM daily. The drug was prepared as a ready-to-use granule solution containing 100 g/L raw herbs (concentration 27.3 mg/kg). The MET group was administered 6.5 g/kg/d (equivalent to the adult dose of 500 mg bid), while the remaining groups received 2 mL saline, all for 8 weeks. Referring to the research by Matheus Santos de Sousa Fernandes et al,¹² the AE and COMB groups underwent 8 weeks of treadmill training (5 times/week, 60 min/session). Initial incremental load testing (starting at 6 m/min, speed increased by 3 m/min every 3 minutes until exhaustion) determined the maximal running capacity. For the first 4 weeks, training intensity was set at 60% of maximal capacity (10 m/min), and after re-measurement, it was increased to 12 m/min for the subsequent 4 weeks. No mice died during the experiment, and 12 mice were retained in each group at completion.

Body Weight and Body Fat Percentage

Body weight was monitored every 2–3 days during modeling and intervention using a precision electronic balance (accuracy: 0.001 g). At the experimental endpoint, abdominal and perivisceral adipose tissues were dissected and weighed. Body fat percentage (BFP) was calculated as follows:¹³

$$\text{BFP (\%)} = (\text{adipose tissue weight/body weight}) \times 100$$

Estrous Cycle Monitoring

From day 47 of drug administration and exercise intervention, vaginal smears were collected daily at 10:00 AM. After hematoxylin-eosin (HE) staining, changes in vaginal epithelial cells were observed under an optical microscope to assess estrous cycle stages.

Ovarian Morphological Observation

Bilateral ovarian tissues were collected and fixed in a 4% paraformaldehyde solution. After conventional paraffin embedding, 3- μm -thick ovarian tissue sections were prepared. Following hematoxylin-eosin (HE) staining, whole-slide scanning was performed using a digital slide scanner. Histomorphological evaluation was conducted under light microscopy at 200 \times magnification to assess follicular development and pathological changes.

Serum Biochemical Analysis

Upon completion of the 8-week drug and exercise intervention, 6 female mice were randomly selected from each group for sample collection. The mice were fasted for 12 hours prior to tissue sampling. FBG was measured via tail vein puncture. Subsequently, the mice were anesthetized using isoflurane inhalation, and blood was collected from the retro-orbital plexus for serological analysis. Serum levels of FSH, LH, T, E2, and FINS were quantitatively determined using ELISA kits. HOMA-IR was calculated using the following formula:¹⁴

$$\text{HOMA - IR} = [\text{Glu (mmol/L)} \times \text{FINS (mIU/L)}] / 22.5$$

Cohabitation Scheme

Following completion of the drug and exercise interventions and subsequent tissue sampling, the remaining female mice in each group received an intraperitoneal injection of 10 IU pregnant mare serum gonadotropin (PMSG) at 12:00 noon the next day. Forty-eight hours later, 10 IU human chorionic gonadotropin (HCG) was administered intraperitoneally to induce superovulation. On the evening of HCG injection (20:00), the females were co-housed with 10-week-old adult male mice at a 2:1 female-to-male ratio. Vaginal plugs were checked before 08:00 the following morning. Mice exhibiting vaginal plugs were designated as embryonic day 0.5 (E0.5) and housed individually. Thereafter, all mice were maintained on standard diets without further intervention.

Reproductive Outcome Assessment

On gestational day 8.5 (E8.5), mice were euthanized by cervical dislocation under anesthesia. Uterine morphology was examined after laparotomy, and the number of implantation sites and resorbed embryos were recorded. Reproductive indices were calculated as follows:^{15,16}

$$\text{Pregnancy rate (\%)} = (\text{Number of pregnant mice} / \text{Total number of mated mice}) \times 100\%$$

$$\text{Abortion rate (\%)} = (\text{Number of mice with abortion} / \text{Total number of pregnant mice}) \times 100\%$$

$$\text{Embryo resorption rate (\%)} = [\text{Number of resorbed embryos} / (\text{Number of resorbed embryos} + \text{Number of normal embryos})] \times 100\%$$

Statistical Analysis

Statistical analyses were performed using SPSS 25.0 and GraphPad Prism 9.3.1471. Continuous data were expressed as mean \pm standard deviation (\pm s). Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene’s test. For data satisfying normality and homogeneity of variance, one-way analysis of variance (ANOVA) was applied, followed by the least significant difference (LSD) test for post hoc pairwise comparisons. Non-normally distributed data were analyzed using the Kruskal–Wallis *H*-test. Categorical data were compared using the chi-square test, and Fisher’s exact test was employed when the expected frequency was <5 or the total sample size was <40 . A significance level of $\alpha = 0.05$ was used, with $P < 0.05$ considered statistically significant.

Results

Changes in Estrous Cycle Patterns Across Intervention Groups

We analyzed the vaginal smears of experimental mice (Figure 1a–d). Results showed that: the CON group exhibited regular estrous cycles, while the MOD group displayed significant cycle irregularities with a markedly reduced frequency of estrus phases. Compared to the MOD group, both the BHF group and AE group showed improved cycle regularity and a significant increase in estrus frequency. Notably, the MET group and COMB group demonstrated near-normal estrous cycle patterns, with cycle characteristics closely resembling those of the CON group (Figure 1e–j).

Ovarian Histological Observations

Ovarian tissue sections in the CON group showed thick and regularly arranged granulosa cell layers, large mature follicles with abundant follicular fluid, numerous normal follicles at various stages, multiple well-developed corpora lutea, and fewer atresic follicles (Figure 2a). The MOD group exhibited significantly thinned and disorganized granulosa cell layers with darkened staining, markedly reduced primordial follicles, increased atresic follicles, local inflammatory cell infiltration in the stroma, and absence of corpora lutea (Figure 2b). The BHF, AE, MET, and COMB groups demonstrated varying degrees of reduction in atresic follicles, increased numbers of corpora lutea, and decreased inflammatory cell infiltration.(Figure 2c–f).

Changes in Body Weight and Body Fat Percentage Across Intervention Groups

As shown in Figure 4, compared to the CON group, the MOD group exhibited a significantly higher weight gain trend after 1 week of modeling ($P < 0.05$, Figure 4a). Compared to the MOD group, the MET, BHF, AE, and COMB groups displayed reduced weight gain trends from week 3 to week 8 of intervention ($P < 0.05$, Figure 4b). After the intervention period, visceral adipose tissue samples were collected from experimental mice (Figure 3) for subsequent BFP (Body Fat Percentage) calculation. In terms of BFP, the MOD, MET, BHF, and AE groups showed increased BFP compared to the CON group ($P < 0.05$, Figure 4c), while the COMB group exhibited a significant reduction in BFP compared to the MOD group ($P < 0.05$, Figure 4c).

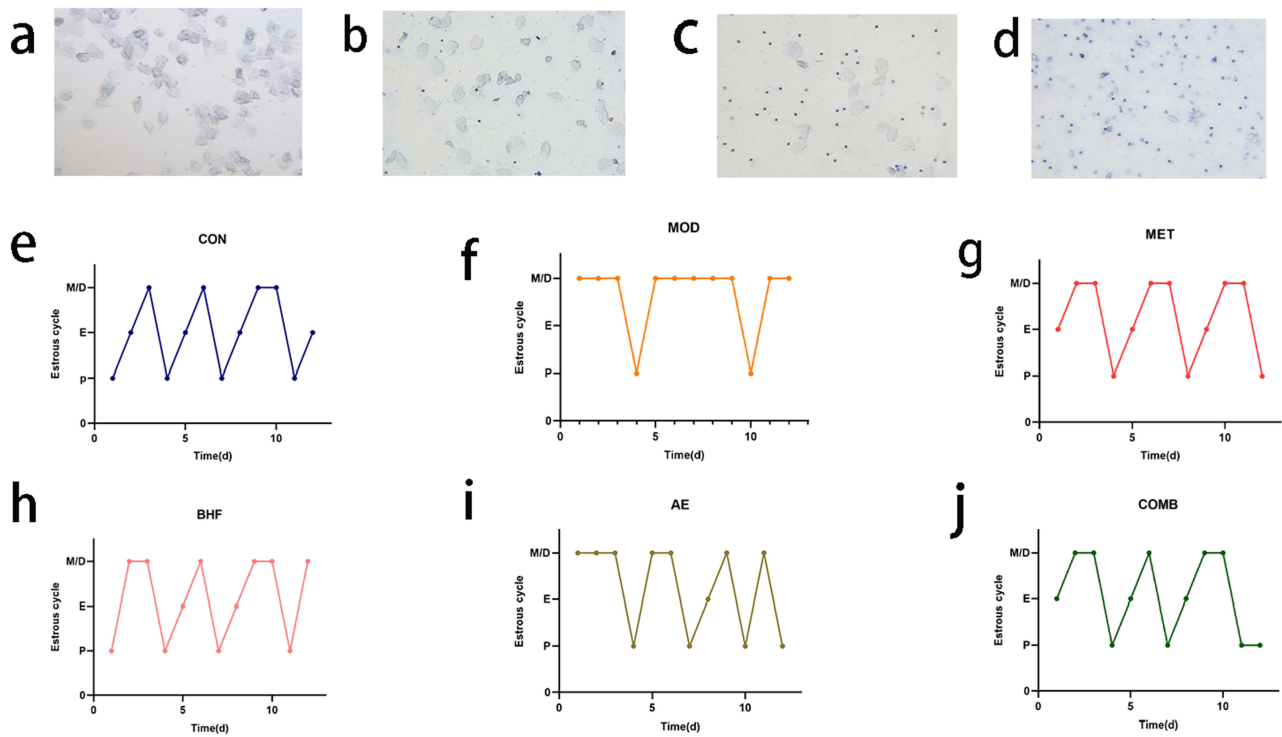


Figure 1 Mouse Vaginal Smear (HE staining, 20x) and Estrous Cycle Changes in Intervention Groups. (a–d): Proestrus, Estrus, Metestrus, Diestrus of vaginal smear; (e–j): Estrous cycle changes in CON, MOD, MET, BHF, AE, COMB groups, respectively.

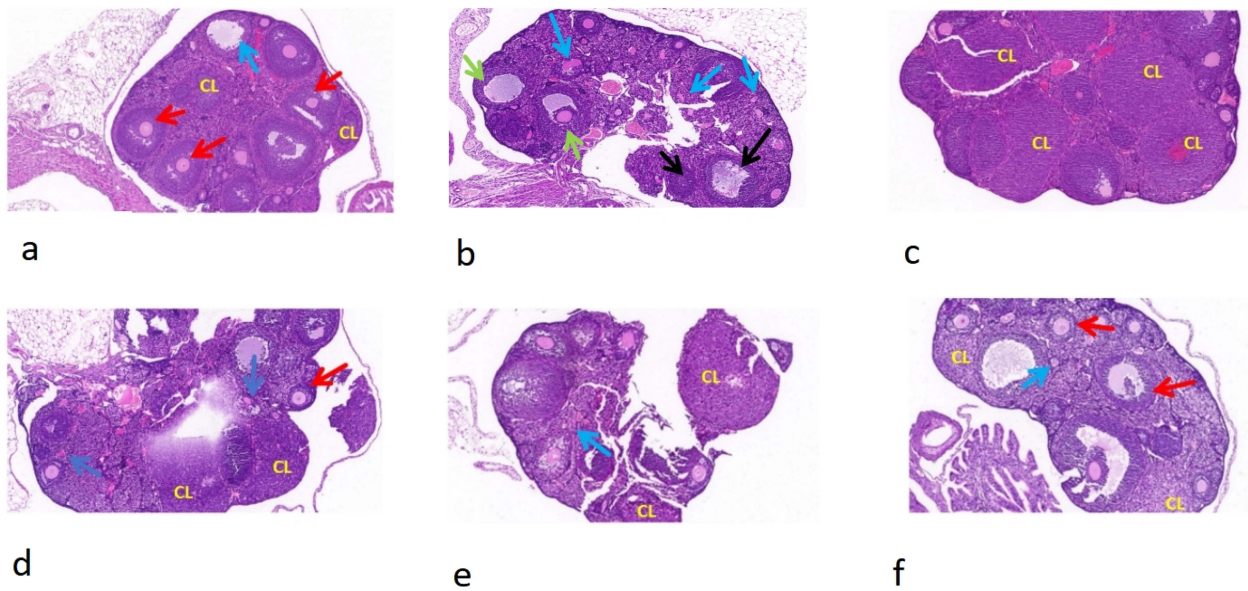


Figure 2 Ovarian histopathology sections across experimental groups (HE staining, 200x magnification). (a) CON, (b) MOD, (c) MET, (d) = BHF, (e) AE, (f) COMB. Red arrows: Normal follicles at various stages; Blue arrows: Atretic follicles; Green arrows: Pyknotic follicular cells; Black arrows: Local inflammatory cell infiltration. **Abbreviation:** CL, Corpus luteum.

Changes in Sex Hormone Levels Across Intervention Groups

Compared to the CON group, the MOD, MET, and BHF groups showed increased LH levels, while the MOD, BHF, and AE groups exhibited elevated T levels. Additionally, the MOD and MET groups demonstrated reduced FSH levels, with all differences being statistically significant ($P < 0.05$, Figure 5a–c). In comparison to the MOD group, the MET, BHF, AE, and

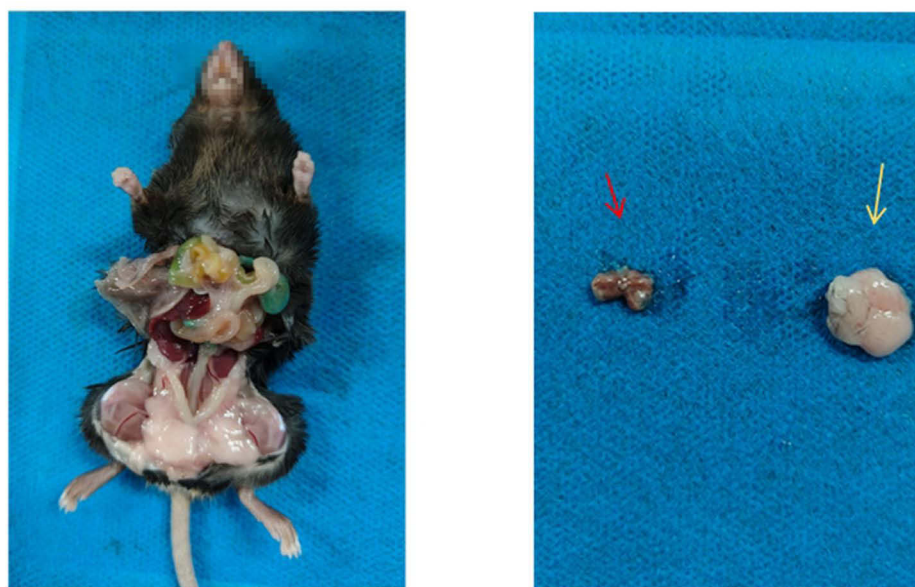


Figure 3 Visceral fat in mice (red arrow → brown fat, yellow arrow → visceral fat).

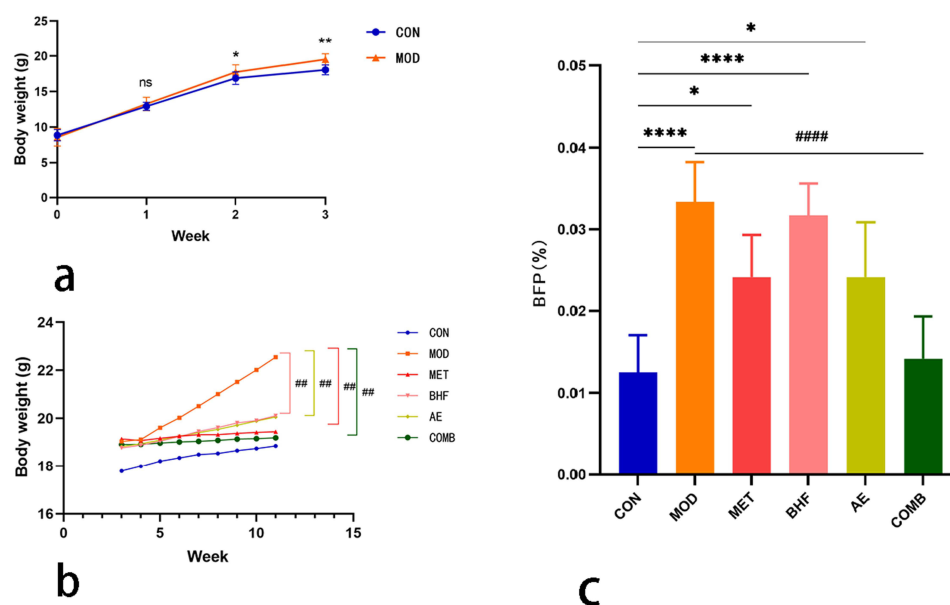


Figure 4 (a) shows the trend of body weight gain in each group during the modeling period, (b) shows the trend of body weight gain in each group during the intervention period, and (c) shows the body fat percentage of each group (Compared with the CON group: * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$; Compared with the MOD group: ### $P < 0.01$, #### $P < 0.0001$).

COMB groups displayed decreased LH levels, while the MET and COMB groups showed reduced T levels. Furthermore, the MET, BHF, AE, and COMB groups exhibited increased FSH levels, with all differences being statistically significant ($P < 0.05$, Figure 5a–c). No significant differences in E2 levels were observed among the groups ($P > 0.05$, Figure 5d).

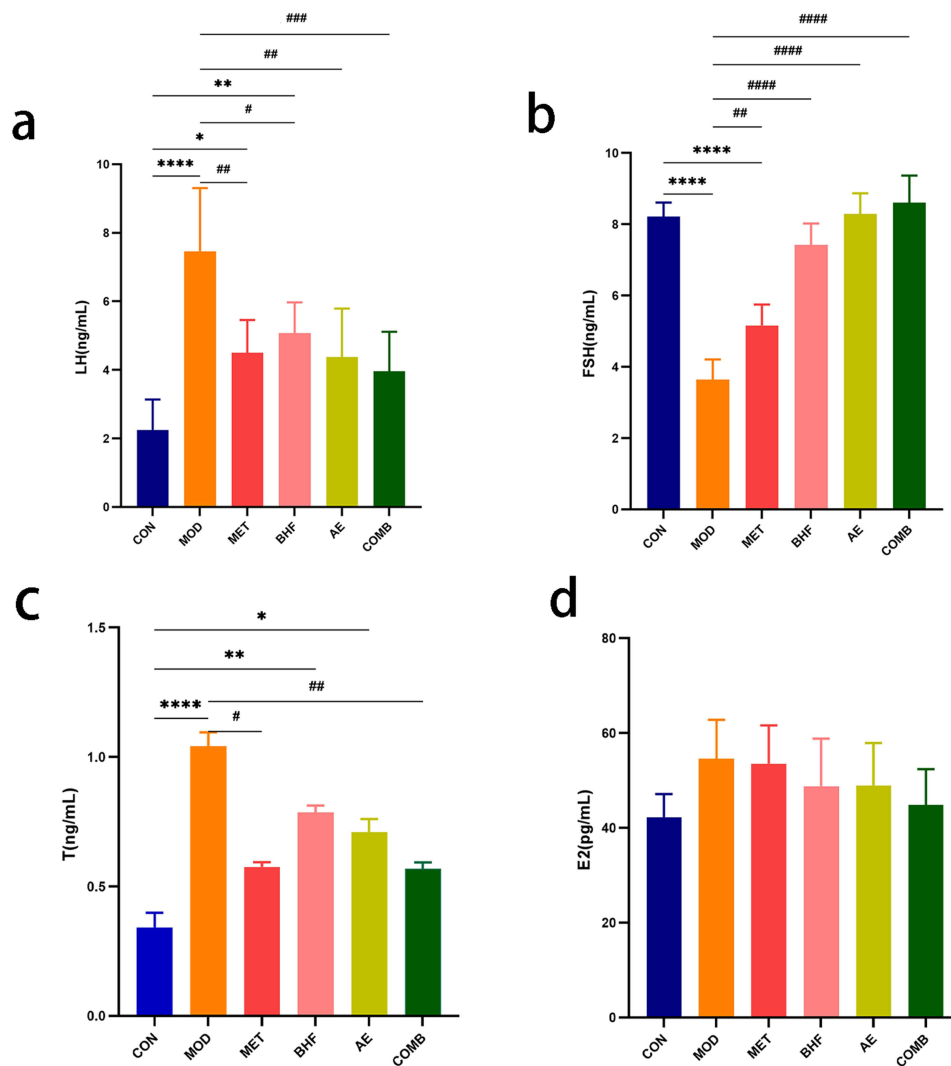


Figure 5 Serum concentrations of reproductive hormones. (a) Shows the LH concentrations in each group, (b) shows the FSH concentrations in each group, (c) shows the T concentrations in each group, and (d) shows the E2 concentrations in each group (Compared with the CON group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$; Compared with the MOD group: # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$).

Changes in Insulin Resistance Across Intervention Groups

Compared to the CON group, the MOD, BHF, and AE groups exhibited increased FPG levels ($P < 0.05$, Figure 6a). In contrast, the MET, BHF, and COMB groups showed reduced FPG levels compared to the MOD group ($P < 0.05$, Figure 6a).

The MOD group demonstrated significantly elevated FINS levels compared to the CON group ($P < 0.05$, Figure 6b), while the MET, BHF, AE, and COMB groups displayed FINS levels similar to the CON group ($P > 0.05$, Figure 6b).

HOMA-IR results revealed that the MOD, BHF, and AE groups had increased HOMA-IR levels compared to the CON group ($P < 0.05$, Figure 6c). Conversely, the MET and COMB groups showed significantly reduced HOMA-IR levels compared to the MOD group ($P < 0.05$, Figure 6c).

Effects on Pregnancy Rate, Abortion Rate, and Embryo Resorption Rate Across Intervention Groups

Figure 7 respectively presents the normal and abnormal embryos of the mice we have dissected. As shown in Table 3, the pregnancy rates for the CON, MOD, MET, BHF, AE, and COMB groups were 66.67%, 50%, 83.33%, 66.67%, 50%, and

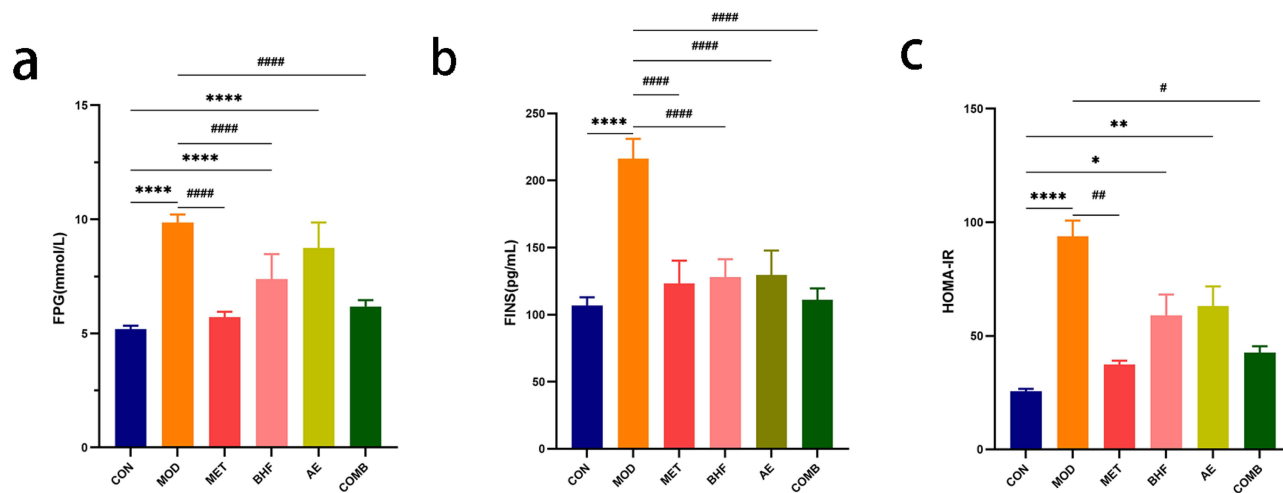


Figure 6 Fasting metabolic parameters. (a) Shows the FPG concentrations in each group, (b) shows the FINS concentrations in each group, (c) shows the HOMA-IR in each group (Compared with the CON group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$; Compared with the MOD group: # $P < 0.05$, ## $P < 0.01$, ### $P < 0.0001$).



Figure 7 The red arrow indicates normal mouse embryos, while the yellow arrow indicates abnormal embryonic tissue.

Table 3 Pregnancy Rate, Abortion Rate, and Embryo Resorption Rate in Mice

Group	Number of Pregnancies (n)	Number of Abortions (n)	Total Number of Embryos (n)	Total Number of Resorbed Embryos (n)	Pregnancy Rate (%)	Abortion Rate (%)	Embryo Resorption Rate (%)
CON	4	0	28	0	66.67	0	0 [#]
MOD	3	2	27	10	50.00	33.33	37.04*
MET	5	3	75	12	83.33	50	16.00
BHF	4	0	25	0	66.67	0	0 [#]
AE	3	0	22	0	50.00	0	0 [#]
COMB	3	0	34	0	50.00	0	0 [#]

Notes: Compared with the CON group: * $P < 0.05$; Compared with the MOD group: [#] $P < 0.05$.

50%, respectively. Compared to the CON group, the MOD and MET groups exhibited abortion rates of 33.3% and 50%, and embryo resorption rates of 37.04% and 16.00%, respectively. The MOD group showed a significantly increased embryo resorption rate compared to the CON group ($P < 0.05$).

In contrast, the CON, BHF, AE, and COMB groups demonstrated reduced embryo resorption rates compared to the MOD group ($P < 0.05$). No abortion cases were observed in the CON, BHF, AE, and COMB groups. Although the MET group had the highest number of pregnant mice and total embryos, it also exhibited the highest abortion and embryo resorption rates, which may be attributed to the small sample size.

Discussion

PCOS is a polygenic disorder influenced by both epigenetic and environmental factors.¹⁷ Studies have shown that PCOS is closely associated with insulin resistance, metabolic disorders, and cardiovascular risks, with environmental factors such as diet and lifestyle playing a significant role in disease progression.¹⁸ Obesity, which is highly prevalent in PCOS patients, not only acts as an independent metabolic risk factor but also exacerbates the pathological progression of PCOS.¹⁹ Although diagnostic criteria for obese PCOS are relatively well-established, optimizing individualized treatment strategies remains a major clinical challenge. Traditional Chinese medicine (TCM), as an important complement to modern medicine, has extensively explored the etiology and pathogenesis of obese PCOS based on the “syndrome differentiation and treatment” theory. Various TCM schools, building on traditional theories, generally agree that the core pathogenesis involves dysfunction of the liver, spleen, and kidney, characterized by a deficiency in origin and excess in manifestation. Based on clinical observations and research, our team has identified the core pathogenesis of obese PCOS as “kidney deficiency as the root and phlegm-stasis as the manifestation”. This finding provides a theoretical basis for the clinical application of BHF and has driven further in-depth research.

Our preliminary experimental studies have demonstrated that BHF exerts multi-target therapeutic effects on PCOS model rats. This formula significantly improves ovarian histomorphology and endometrial pathology, regulates serum sex hormone levels, and reduces inflammatory factors such as TNF- α .^{20,21} Additionally, studies have confirmed that BHF downregulates endometrial TGF- β and NF- κ B protein expression while promoting E-cadherin expression, thereby inhibiting the epithelial-mesenchymal transition (EMT) process.^{20,21} Furthermore, treated rats showed significantly improved pregnancy rates and embryo counts, indicating enhanced reproductive function.^{20,21} Clinical efficacy observations have also found that Bushen Huoxue therapy effectively increases pregnancy and ovulation rates in patients.²²

Research has shown that aerobic exercise improves adipose tissue lipolysis, oxidative metabolism, mitochondrial function, and oxidative stress.^{12,23} Regular aerobic exercise enhances insulin sensitivity in PCOS patients,^{24,25} reduces BMI and AMH levels, and positively impacts cardiovascular health and oxidative stress.^{26,27} It also alleviates PCOS symptoms by reducing systemic inflammatory factors and mitigating inflammatory responses.^{28,29} These findings align with our current results, where both BHF and AE alone significantly improved ovarian morphology, sex hormone levels, and insulin resistance in obese PCOS mice while increasing pregnancy rates and reducing embryo resorption rates. However, we found that the COMB group exhibited effects comparable to the MET group in regulating estrous cycles and restoring ovarian function while outperforming MET in controlling body weight and reducing BFP ($P < 0.01$).

Notably, the COMB group showed the most favorable trends in increasing total embryo count and improving reproductive outcomes, along with a favorable safety profile.

An unexpected finding was that while the pregnancy rate in the MET group did not significantly differ from the CON group, the MET group exhibited higher abortion and embryo loss rates compared to the BHF, AE, and COMB groups. This observation differs from previous literature. Prior studies have suggested that metformin is safe during pre-pregnancy and pregnancy in PCOS patients, improving pregnancy and live birth rates.^{30,31} Tosti et al³² also supported the safety and efficacy of metformin during pre-pregnancy, pregnancy, and postpartum periods. However, in IVF-related studies, while metformin improves ovarian hyperstimulation syndrome (OHSS) and pregnancy rates, there is no conclusive evidence that it increases live birth rates. In some cases, such as short-course GnRH antagonist protocols, metformin may even reduce live birth rates.³³ A large-scale study involving 50,000 infants with congenital malformations found that maternal metformin use during early pregnancy (first trimester) was associated with an increased risk of pulmonary valve atresia in offspring.³⁴ Therefore, we believe that the observed outcomes in the MET group may be influenced by the small sample size, but they also highlight the need for larger sample sizes in future metformin-related studies and suggest cautious evaluation of glucose-lowering strategies for PCOS patients with recent or ongoing pregnancy plans.

Conclusion

In summary, the COMB group significantly improved ovarian pathology, promoted normal follicular development, regulated sex hormone levels, ameliorated metabolic disorders, reduced BFP, and enhanced reproductive function (increased pregnancy rates and reduced abortion and embryo resorption rates) in obese PCOS mice. This integrated approach provides a safe, effective, and easily implementable treatment strategy for obese PCOS patients, with the potential to optimize clinical management. However, the underlying mechanisms remain incompletely understood, and future studies should explore molecular mechanisms using transcriptomics, metabolomics, and other advanced technologies.

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Disclosure

The authors declare no conflicts of interest in this work.

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