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Internal and external factors affecting polycystic ovary syndrome

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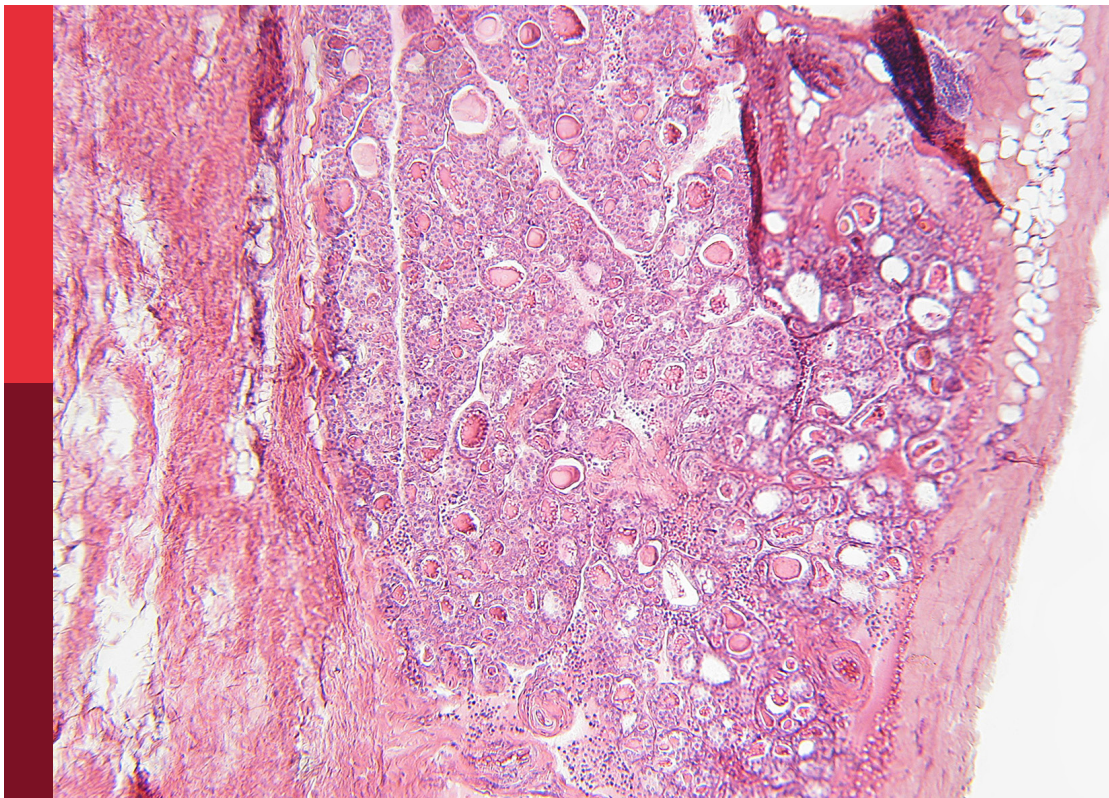

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Internal and external factors affecting polycystic ovary syndrome

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Editorial: Internal and external factors affecting polycystic ovary syndrome

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polycystic ovary syndrome, PCOS polycystic ovarian syndrome, genetics, lifestyle, fertility

Editorial on the Research Topic
Internal and external factors affecting polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) pathophysiology is modified by a multitude of internal and external factors that go far beyond the traditional stimulators and inhibitors of endocrine response and function. This Research Topic call was to specifically include state-of-the art methodologies and review articles, with the result that 21 manuscripts were published that could be broadly divided into mendelian randomisation studies (RMS), articles regarding lifestyle and PCOS pathophysiology, and fertility including *in vitro* fertilization studies, the overview of which is shown in Figure 1.

Wang et al. reported a RMS showing that blood lipid metabolites and potential metabolic pathways may have a genetic association with PCOS and that there may be a causal relationship between hexadecanedioate and dihomo-linolenate and risk of PCOS. These compounds could potentially serve as metabolic biomarkers for screening PCOS and selecting drug targets; however, metabolomic studies on a robust population will be needed to answer these questions (Wang et al.) A second RMS looked at the association of PCOS and thyroid disease to address the debate as to whether PCOS is associated with hyperthyroidism, hypothyroidism, neither or both. The study reported that the occurrence of PCOS was associated with increased risk of hyperthyroidism (Zhao et al.) This suggests that, at the very least, a TSH should be measured in the investigation of PCOS; however, to confirm this, a large prospective study would likely be needed and could be combined with a long-term study looking at cardiovascular disease prevalence in PCOS. Several studies have shown a potential link between PCOS and immune system dysregulation, though the results are conflicting. In the RMS reported by Aru et al., a causal association between immune cells and susceptibility to PCOS was found suggesting a

true association, but it is unclear whether the immune system is modulating PCOS or whether the converse occurs through inflammation and insulin resistance (IR) combined with obesity. It is of interest, though still unclear, whether immune modulation in PCOS is a viable treatment option particularly in the setting of infertility; the creation of new selective drugs may address this. Interestingly, a study looking at the genes for necroptosis, critical for reproductive and endocrine disorders, reported that enrichment analysis for differentially diagnostic genes for necroptosis were augmented in immune-related signaling pathways such as B cells, T cells, and natural killer cells (Wang et al.) In addition, immune microenvironment analysis revealed that differentially diagnostic genes for necroptosis were significantly correlated with 13 markedly different immune cells, data that would support the findings reported in the RMS focused upon the immune system and PCOS, as noted above (Aru et al.).

Lifestyle issues were addressed in several articles. A systematic review and meta-analysis on dietary consumption in PCOS concluded that there was limited evidence regarding the association between dairy consumption and PCOS; however, in accord with several dietary-based studies in PCOS, a low-dairy/low-starch diet may improve some anthropometric and metabolic features, likely through calorie restriction rather than any inherent food characteristics (Rastad et al.) Another study looked at the association of mild depressive states in PCOS and an unhealthy lifestyle, concluding that generally depressive symptoms in PCOS are mild and related to physical issues such as acne and hirsutism, and to poor lifestyle choices such as frequent consumption of high-fat diet, regularly staying up late and a lack of exercise, (Li et al.) all features noted to contribute to low quality of life (QOL) issues in PCOS in previous studies. This then raises the question of how this can be addressed in PCOS; a systematic review focused upon the effect of behavioral interventions on anthropometric, clinical and biochemical parameters reported that, as anticipated, behavioral intervention strategies contribute to weight loss, reduction in BMI and in waist circumference, and improvement in depression (Xie et al.) However, surprisingly, no significant improvement was observed in biochemical indices or QOL (Xie et al.), the latter being discordant with the improvement in depression reported. This data may predict that PCOS patients will not adhere to lifestyle and exercise, the cornerstone of the treatment strategy, if QOL is not improved by the intervention. The question then arises whether there is a physiological contribution to the psychological stress indices that have been reported in the literature. A study investigating both physiological and mental stress indices showed no difference in salivary cortisol levels, suggesting the physiological markers of stress are not elevated in PCOS even though the mental stress score was higher in PCOS, (Marschalek et al.) findings that are in accord with previous studies: prolactin was suggested as a measure of physical stress, though avoiding an acute stress-induced prolactin response leading to a misleading positive result may be challenging. Differing results within these reported studies for the PCOS subjects, when compared to the literature, may be a result of not defining the PCOS phenotype within the study group. This was highlighted by a report showing that differing phenotypes of PCOS were metabolically dissimilar: those with hirsutism and anovulation, and hirsutism with PCOS ovary features, had the most adverse metabolic profile including IR, dyslipidemia, impaired glucose tolerance and non-alcoholic fatty liver disease (Wen et al.).

How such research may be undertaken to identify novel research areas and knowledge gaps was reported in a bibliometric analysis for metabolic dysfunction in PCOS using software to analyze the status of the field, illustrating the key words that included lipid profile, androgen receptors, phosphorylation, luteinizing hormones, proteomics, metabolomics and gut microbiota, (Xu et al.) providing a valuable tool for researchers in the field.

In an intervention study of metformin versus spironolactone plus metformin, a not uncommon combination in clinical practice, the combination treatment improved BMI and serum androgen levels though, unexpectedly, hirsutism was not different, an outcome which differs to other published studies (Zeng et al.). After six months, metabolic indices were improved with lower IR and fasting blood glucose; this was, however, a relatively short duration study and it would be important to determine what happens at 12 months and beyond to see if this improvement is maintained.

A review of the autonomic nervous system (ANS) in PCOS presented evidence that there is increased ANS activation in PCOS that could be due to the induction of inflammation, for example, though whether this was a contributory cause or an effect of the underlying pathophysiology of PCOS is unclear. In addition, it is not clear whether the ANS activation contributes to the decreased QOL, emotional stress issues or depression indices or, conversely, whether these conditions cause its activation (Yu et al.)

Nesfatin-1 is a 82 amino acid peptide that is involved in glucose homeostasis, anxiety and depression and cardiovascular function and therefore would be suspected to differ in PCOS. Nesfatin-1 levels are discrepant in the PCOS literature and this question was addressed in a systematic review and meta-analysis that showed that blood nesfatin-1 levels did not differ in PCOS compared to control subjects (Wang et al.), suggesting this factor has no role in PCOS, though phenotype heterogeneity was not taken into account.

Unsurprisingly, fertility aspects accounted for nearly 50% of all accepted articles. Wilms tumor-1 is critical for endometrial decidualization and its function may be dysregulated in PCOS due to epigenetic factors allowing androgen receptors to recruit cofactors that affect the process (James et al.); this may, in part, explain the increased miscarriage rate in PCOS that is then further exacerbated by IR and β-cell dysfunction. In another report, levels of the endocrine disruptor polychlorinated biphenyl (PCB) were shown to correlate with luteal phase hormones and unexplained infertility but not in PCOS patients suggesting that PCBs are not involved and do not contribute to ovulatory dysfunction in PCOS, though they may have other independent effects on fertility.

PCOS patients, following a suboptimal response to IVF, can achieve a reasonable live birth response per fresh embryo transfer.

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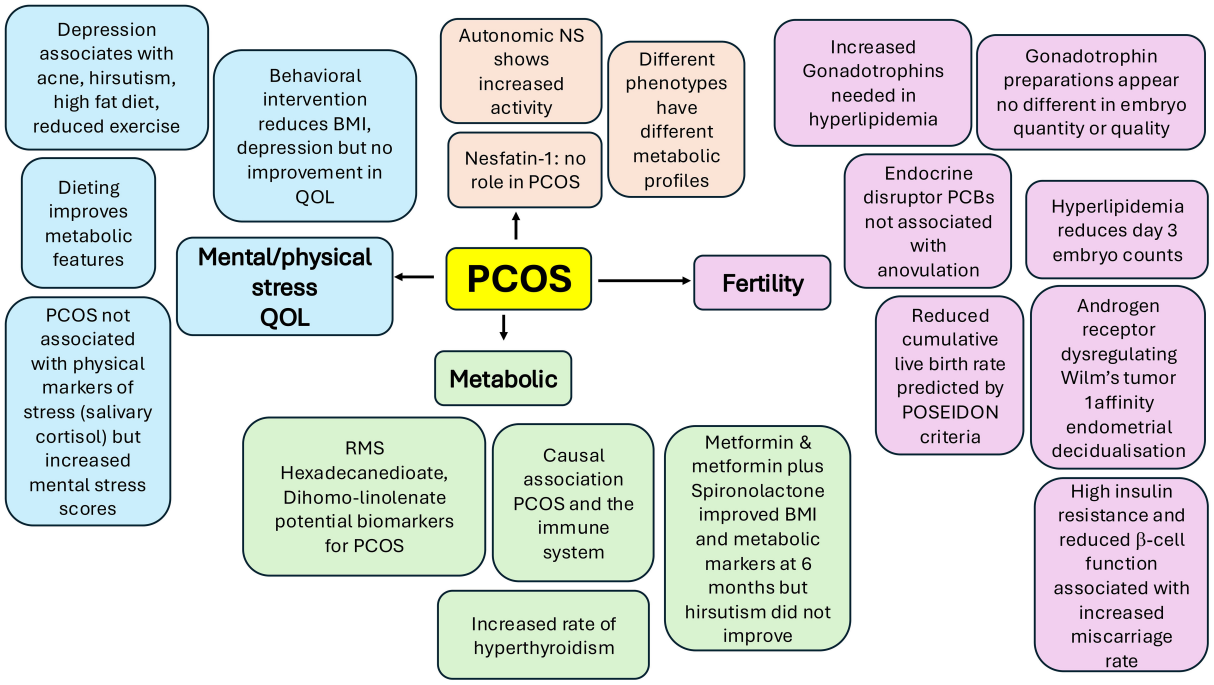

FIGURE 1
A schematic to illustrate the broad range of articles included in this Research Topic . The articles can be broadly divided into Mendelian randomisation studies (RMS), articles regarding lifestyle and PCOS pathophysiology, and fertility including in vitro fertilization studies.

However, the cumulative live birth rate per aspiration cycle differed in women with PCOS as defined by the Patient-Oriented Strategy Encompassing Individualized Oocyte Number (POSEIDON) criteria, indicating that prediction of future clinical outcome can be made for certain patient groups that are at risk following a poor outcome – though there was no difference for subjects with good reproductive outcomes (Jiang et al.). A further study reported that both high IR and HOMA-B, as a measure of β-cell function, were associated with an increased miscarriage rate, IR being the most important of the two (Huang et al.). This is clearly of importance in PCOS due to the higher IR that patients have, and there is a need to understand the mechanism of this increased miscarriage rate to determine if weight loss, for instance, is sufficient or if further pharmaceutical intervention for weight loss or insulin resistance is needed. A study looking at four different gonadotrophin preparations in a GnRH agonist protocol showed no difference in the preparations for PCOS for ovarian hyperstimulation syndrome or embryo quantity and quality (Hu et al.), which is reassuring for clinical practice; however, a study also reported in this Research Topic showed that the dose of the gonadotrophin in IVF may have to be increased in the presence of hyperlipidemia, and that hyperlipidemia per se may also reduce embryo quality, endometrial receptivity and clinical outcomes in PCOS patients (Yang et al.); notwithstanding, it may be difficult to address hyperlipidemia and potential treatment teratogenic effects prior to an IVF cycle. The causality of these detrimental effects of dyslipidemia were investigated in another article within this Research Topic, showing the resultant inflammation from increasing serum triglyceride and low-density lipoprotein cholesterol levels and decreasing serum high-density lipoprotein cholesterol levels, resulted in a reduction of day-3 embryo counts (Jiang et al.).

Finally, in a review article, a novel treatment for infertility was proposed using stem cells and exosomes, both of which exhibit cytokine effects that may defend against the metabolic consequences found as part of the PCOS condition (Hadidi et al.) Exosome therapy in rats has shown beneficial effect on enhancing fertility though mechanistic control of the exosome process has still not been fully elucidated. Exosomes derived from mesenchymal stem cells have been shown to suppress chronic inflammation by decreasing the generation of inflammatory mediators such as tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ), elevating interleukin-10 (IL-10) levels and anti-inflammatory cytokines and reducing ovarian granulosa cell apoptosis. In addition, these exosomes were shown to regulate androgen synthesis in an *in vitro* model and restore fertility in a mouse model suggesting their promise as a therapy for PCOS fertility in the future.

The diverse nature of the individual manuscripts in this Research Topic highlights the breadth of research directed towards understanding and treating the PCOS disorder. Many of the articles in this Research Topic were, in fact, complementary to others in the series providing further evidence regarding the underlying pathophysiology of PCOS and its future treatment.

Author contributions

AB: Visualization, Writing – review & editing, Writing – original draft. TS: Writing – original draft, Writing – review & editing. HR: Writing – review & editing, Writing – original draft. SA: Writing – original draft, Conceptualization, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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A case-control study about markers of stress in normal-/overweight women with polycystic ovary syndrome and in controls

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Background: Polycystic ovary syndrome (PCOS) is linked to an elevated risk of psychological disorders, decreased quality of life and emotional distress. Serum cortisol as a potential stress marker has been found to be increased in women with PCOS. The aim of this study was to evaluate both saliva stress markers and subjective psychological distress in women with PCOS.

Methods: In a prospective case-control study, 31 PCOS women and 31 healthy controls were included. Salivary cortisol, and metanephrines were collected in the morning and in the evening. Emotional distress and quality of life were assessed by means of the Perceived Stress Scale (PSS-10) and the Short Form-36 (SF-36). Multivariable generalized linear models were applied to test the influence of various parameters on numerical outcome parameters.

Results: After correction for age and body mass index (BMI), there were no statistically significant differences of salivary biomarkers between PCOS women and healthy controls ($p>0.05$). PCOS patients revealed significantly higher increased PSS total scores and lower quality of life in all SF-36 modules apart from pain ($p< 0.05$). The PSS total score was positively correlated to prolactin in PCOS women ($r= 0.450$; $p= 0.011$). In overweight/obese PCOS patients, a higher BMI, a higher Ferriman Gallwey score and higher age significantly predicted the PSS total score ($p< 0.05$).

Conclusion: Stress measured by salivary biomarkers did not differ between PCOS women and healthy controls, whereas stress scores evaluated by questionnaires were significantly greater in women with PCOS. A higher BMI, hirsutism and a higher age seem to be the main modulators of subjective stress in PCOS. Prolactin might serve as a biomarker for chronic stress in PCOS women.

KEYWORDS

polycystic ovary syndrome, stress, saliva, biomarkers, cortisol, emotion, quality of life

Introduction

Polycystic ovary syndrome (PCOS) is the most widespread female endocrinopathy, affecting 5-10% of women in the reproductive age. It is primarily characterized by ovulatory dysfunction and androgen excess. However, women with PCOS also fall prey to psychological distress, due to body dissatisfaction and impaired health-related quality of life. This psychological distress may manifest itself as depression and anxiety (1, 2) imposing a substantial health care burden. A higher prevalence of anxiety and depressive disorders in women with PCOS has been confirmed (3). The extents of these diseases could be connected to higher stress levels, which is also widely encountered in women with PCOS (4, 5). The stress response is modulated by the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system by synthesizing and releasing adrenal glucocorticoids and catecholamines. A recent meta-analysis of 41 studies reports cortisol to be elevated in PCOS subjects (6). However, the overall effect is accounted for a few large studies with the majority of included studies not reporting significant differences. Further, there are variations in the methodological characteristics, in particular of cortisol sampling, with most studies evaluating cortisol in the serum and only a few attempting to assess cortisol in saliva. Notably, the assessment of salivary biomarkers has received recognition because of the relaxed and non-invasive self-collection, as well as the easy handling of the saliva samples (7–9). Salivary cortisol represents an established tool for evaluating acute and chronic stress responses, as increased secretions of salivary stress biomarkers under different distressing conditions have been demonstrated (10–12). However, free metanephrines reflect the sympathetic/adrenomedullary system of the stress response.

The international evidence-based guidelines for the assessment and management of PCOS emphasize the importance of considering quality of life and stress in PCOS research, and the application of the correct methods in clinical care in order to recognize and highlight the priorities of patients (13). Appropriate evaluation, identification of high-risk individuals and management of stress at an early stage may prevent the onset of secondary disorders like depression and anxiety, and thereby facilitate positive, long-term mental health outcome and have a favorable impact on the health and financial burden on the health-care system (14, 15). To our knowledge, no studies have yet examined the association of perceived stress and cortisol secretion in PCOS women.

Moreover, sympathetic activity is increased in subjects with obesity (16) and there seems to be a complex and bidirectional relationship between sympathetic activity and insulin resistance (17). Given that insulin resistance is common in PCOS with a prevalence of 75-95% according to clamp studies (18), that the PCOS prevalence increases with obesity (19) and that the risk for insulin resistance and type 2 diabetes is higher in overweight and obese PCOS women (20), one could hypothesize that sympathetic activity and perceived stress might differ between lean/normal weight and overweight/obese PCOS women. It has already been reported that concerns about weight were associated with a decreased quality of life in patients with PCOS (21).

Thus, in light of the prevalence of distress among PCOS patients, our aim was to evaluate both self-perceived severity of symptoms measured by the Perceived Stress Score (22) and Short Form-36 (23), and the salivary biomarkers cortisol and free metanephrines. As a secondary study aim, we also focused on differences between lean/normal weight and overweight/obese PCOs patients.

Materials and methods

Study population and setting/study design

This was a prospective case-control study at the Clinical Department of Gynecologic Endocrinology and Reproductive Medicine of the Medical University of Vienna, Austria. The study was approved by Institutional Review Board of the Medical University of Vienna (IRB number 1804/2016), and each subject provided written informed consent to participate. Thirty-one non-infertile patients diagnosed with PCOS and 31 healthy female controls were recruited. Patients with PCOS were subsequently divided into an overweight/obesity (BMI > 25) group and a lean/normal-weight (BMI ≤ 25) group. Control subjects were women with a regular menses and without any signs of clinical or biochemical hyperandrogenism, who did not use any hormonal contraception. Diagnosis of PCOS was made according to the revised Rotterdam criteria (24), having two of the following criteria: 1) biochemical and/or clinical hyperandrogenism; 2) polycystic ovaries seen on transvaginal ultrasound; 3) an- or oligoovulation (24). Furthermore, participants were eligible if they met each of the following criteria: age 18-40 years; able to read, understand and sign the informed consent; not having non-classic adrenal hyperplasia; not having PCOS-specific treatment within the last three months before study enrollment.

Study participants were recruited from the outpatient clinic of the Department of Gynecologic Endocrinology and Reproductive Medicine of the Medical University of Vienna. All patients were referred by a gynecologist, seen by a specialist at our clinic and underwent medical routine examination including laboratory measurements. After initial diagnosis of PCOS, the patients were recruited and, if they were willing to participate, they were invited for an extra study visit.

The recruitment of participants was initiated in January 2017. During the extra study-visit, participants were given two questionnaires, which were completed by themselves on-site. Serum samples of participants with PCOS were collected beforehand as part of clinical routine. All participants were instructed to collect saliva samples. Furthermore, clinical parameters were evaluated and documented in the case report form.

Measures

Blood samples were analyzed for total and free testosterone, the free androgen index (FAI) calculated as 100 x (total testosterone/sex hormone binding globulin), androstenedione,

dehydroepiandrosterone sulfate (DHEAS), the ratio of luteinizing hormone (LH)/follicle-stimulating hormone (FSH), antimullerian hormone (AMH), sex hormone binding globuline (SHBG), prolactin and cortisol-binding globulin (CBG). Insulin resistance was defined by a HOMA-IR >2.5, calculated as fasting insulin (mU/l) * fasting glucose (mg/dl)/405 (25).

Saliva samples of salivary cortisol and free metanephrines were collected with the Salivette sampling device (Sarstedt, Germany) in the morning, within 30 minutes after awakening, and in the evening, by the participants themselves. Participants returned samples within a week. Samples were then centrifuged and stored at -80°C until assayed.

Perceived stress was assessed with the German version of the 10-item Perceived Stress Scale (PSS-10) which calculates the point at which events are considered incontrollable, unpredictable and/or overloading (22). The German version of the PSS-10 has also been validated (26, 27). Answers to all questions were rated on a five-point Likert scale (0= never, 4= very often). The scale correlates with different psychological measures specifically depression, anxiety as well as decreased satisfaction with self, job and life in general. A score of "perceived helplessness subscale" and a score of "perceived self-efficacy subscale" is obtained. The total Perceived Stress Scale (PSS-10) score is the sum of all "perceived helplessness subscale" items and reversed "perceived self-efficacy subscale" items. Quality of life was assessed with the German version of the Short Form-36 (SF-36), an established and validated 36-item instrument including a total of eight subscales (Physical Function, Physical Role Function, Emotional Role Function, Energy/Fatigue, Emotional wellbeing, Social Function, Bodily Pain, General Health (28). According to the guidelines, scores were converted to a 0-100 scale. Higher values of the transformed scale indicate better health status. Lower values indicate lower health status, show lower functional limitation, distress and further social and role disability.

In addition, we assessed clinical information of PCOS. Weight and height were calculated by weight/height squared (kg/m2) in all patients to BMI. The clinical assessment of hirsutism was determined using the Ferriman-Gallwey scoring system (29). Acne vulgaris was evaluated by use of the global acne grading system (30).

Blood samples were obtained during the early follicular phase visit (cycle days 2-5). All biochemical analyses were carried out at the Department of Laboratory Medicine, Medical University of Vienna using CE IVD labeled assays according to ISO 15189 standards. As reported previously (31), prolactin, DHEAS and cortisol measurements were performed with the corresponding Cobas electrochemiluminescence immunoassays (ECLIA) on Cobas e 801 immunologyanalyzers (Roche Diagnostics, Mannheim, Germany). Metanephrine and Normetanephrine were measured by ELISAs (LDN, Nornhorn, Germany) and CBG by RIA (DiaSource, Louvain-la-Neuve, Belgium).

Polycystic ovarian morphology was defined by a number of follicles per ovary >12. For vaginal ultrasound, an "Aloka Prosound 6" ultrasound machine and an "UST-9124 Intra Cavity transducer" (frequency range 3.0 - 7.5 MHz; Wiener Neudorf, Austria) were used (31).

Statistical analysis and sample size calculation

The sample size was calculated to detect a 0.2 difference in saliva cortisol levels between PCOS women and controls with an expected SD of 0.25 at a power of 80% and an alpha of 0.05. This required 26 participants in each group. Due to the medium effort that was expected of the participants, we anticipated a dropout rate of 20%, five participants respectively per group. To account for dropouts, we therefore aimed to recruit 31 participants per group. Accordingly, the total sample size was estimated with 62.

Statistical analysis

Data are presented as median and interquartile range for numerical parameters and as number and frequency for categorical parameters. The SF-36 and PSS-10 questionnaires were scored and analyzed according to the published guidelines. To test differences between groups, numerical parameters were compared using the unpaired t-test (in case of a normal distribution) or the Welch-test. Pearson correlation was used to examine relationships between numerical parameters. Statistical analyses were performed in SPSS 24.0 (IBM, Vienna, Austria). For comparison of groups with correction for age and BMI, univariable binary logistic regression models were used, where age and BMI were also entered as covariates. Multivariable generalized linear models were applied to test the influence of various parameters on numerical outcome parameters. For these analyses, ß-values (B) with their standard deviations (standard errors) as well as 96% confidence intervals (95% CI) and the Wald's tests are provided. A p-value < 0.05 was considered statistically significant.

Results

Table 1 provides details about basic patient and clinical characteristics in PCOS patients and healthy controls. A higher BMI was found for women with PCOS. Moreover, PCOS patients revealed a higher median Ferriman Gallwey score, higher levels of LH, testosterone, DHEA-S, prolactin, AMH, an increased FAI, and higher rates of polycystic ovarian morphology on ultrasound ($p< 0.05$).

When PCOS women were compared to controls after correction for age and BMI (Table 2), there were no differences between the two groups concerning laboratory stress markers. In contrast, women with PCOS revealed significantly increased PSS total scores as well as significantly lower quality of life in all SF-36 modules apart from pain.

Correlation analyses were performed between the PSS total score and SF-36 general health as well as possible salivary and serum stress markers, namely morning saliva cortisol, DHEAS and prolactin in PCOS patients (Figures 1A–D) and controls (Figures 1E–H) separately. The only significant correlation was found for the PSS total score and prolactin in PCOS women ($r= 0.450$; $p= 0.011$; Figure 1C). Notably, there was no significant correlation between

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TABLE 1 Basic patient and clinical characteristics of PCOS women and healthy controls.

	PCOS patients	Controls	p
Age (years)	25 (22,30)	28 (24,31)	0.071
BMI (kg/m^2)	24.9 (21.5;35.5)	21.5 (19.5;23.9)	0.001
Insulin resistance (HOMA-IR >2.5)	20 (64.5)	–	–
Ferriman Gallwey Score	10 (5,15)	0 (0;0)	<0.001
GAGS	4 (4;11)	0 (0;6)	0.198
LH (mlU/mL)	12.6 (8.5;14.6)	5.2 (3.4;7.1)	<0.001
FSH (mlU/mL)	5.6 (4.9;7.5)	5.9 (4.7;8.1)	0.548
LH: FSH ratio	2.18 (1.51;3.09)	0.93 (0.55;1.4)	<0.001
Testosterone (ng/mL)	0.51 (0.39;0.72)	0.25 (0.16;0.31)	<0.001
SHBG (nmol/L)	35.3 (29.1;73.7)	82.3 (68;114.6)	0.001
Free androgen index	1.15 (0.72;2.53)	0.29 (0.20;0.38)	<0.001
DHEA-S (µg/ml)	3.17 (2.53;4.33)	2.19 (1.81;2.80)	0.001
Prolactin (ng/mL)	13.1 (8.9;17.8)	9.5 (8.3; 13.1)	0.017
AMH (ng/mL)	7.83 (5.94;11.00)	3.01 (2.03-4.07)	<0.001
Presence of polycystic ovarian morphology on ultrasound	25 (80.6)	4 (12.9)	<0.001

Data are provided as median (interquartile range) for numerical parameters and as number (frequency) for categorical parameters
–, not applicable.
BMI, body mass index; HOMA-IR, HOMA index of insulin resistance; GAGS, global acne grading system; LH, luteinizing hormone; FSH, follicle stimulating hormone; DHEA-S, dehydroepiandrosterone-sulfate; SHBG, sexual hormone binding globulin; AMH, anti-Mullerian hormone.

TABLE 2 Salivary and serum stress markers as well as main results of the SF-36 and PSS questionnaires in PCOS women and controls.

	PCOS patients	Controls	p*
Morning saliva cortisol (µg/dL)	0.326 (0.201;0.490)	0.416 (0.217;0.529)	0.798
Evening saliva cortisol (µg/dL)	0.054 (0.054;0.054)	0.054 (0.054;0.054)	0.168
Morning saliva metanephrines (pg/mL)	13.7 (10.0;28.2)	11.2 (8.2;27.6)	0.937
Evening saliva metanephrines (pg/mL)	12.5 (7.1;17.5)	14.2 (8.8;19.7)	0.338
Morning saliva normetanephrines (pg/mL)	175.0 (58.6;370.5)	187.0 (86.2;340.8)	0.182
Evening saliva normetanephrines (pg/mL)	118.0 (80.9;336.0)	136.0 (93.9;256.5)	0.466
Serum CBG (µg/mL)	51.0 (47.1;56.8)	54.0 (51.6;59.4)	0.214
PSS: PHS score	18 (15;22)	14 (11;16)	<0.001
PSS: PSE score	11 (10;13)	15 (13;16)	<0.001
PSS: total score	7 (4;11)	0 (6;4)	<0.001
SF-36: Physical functioning	90 (80-100)	100 (95-100)	0.002
SF-36: Role functioning/physical	100 (75-100)	100 (100-100)	0.025
SF-36: Role functioning/emotional	66.6 (33.3-100)	100 (67-100)	0.007
SF-36: Energy/fatigue	40 (35-50)	55 (50-65)	<0.001
SF-36: Emotional wellbeing	64 (48-72)	76 (60-80)	<0.001
SF-36: Social functioning	75 (62.5-87.5)	87.5 (50-100)	0.021
SF-36: Pain	77.5 (67.5-100)	90 (68-100)	0.698
SF-36: General health	60 (50-75)	80 (70-90)	0.002

Data are provided as median (interquartile range).
*p was adjusted for age and BMI.
PSS, Perceived Stress Scale; PHS, perceived helplessness subscale; PSE, perceived self-efficacy subscale; SF-36, Short Form-36.

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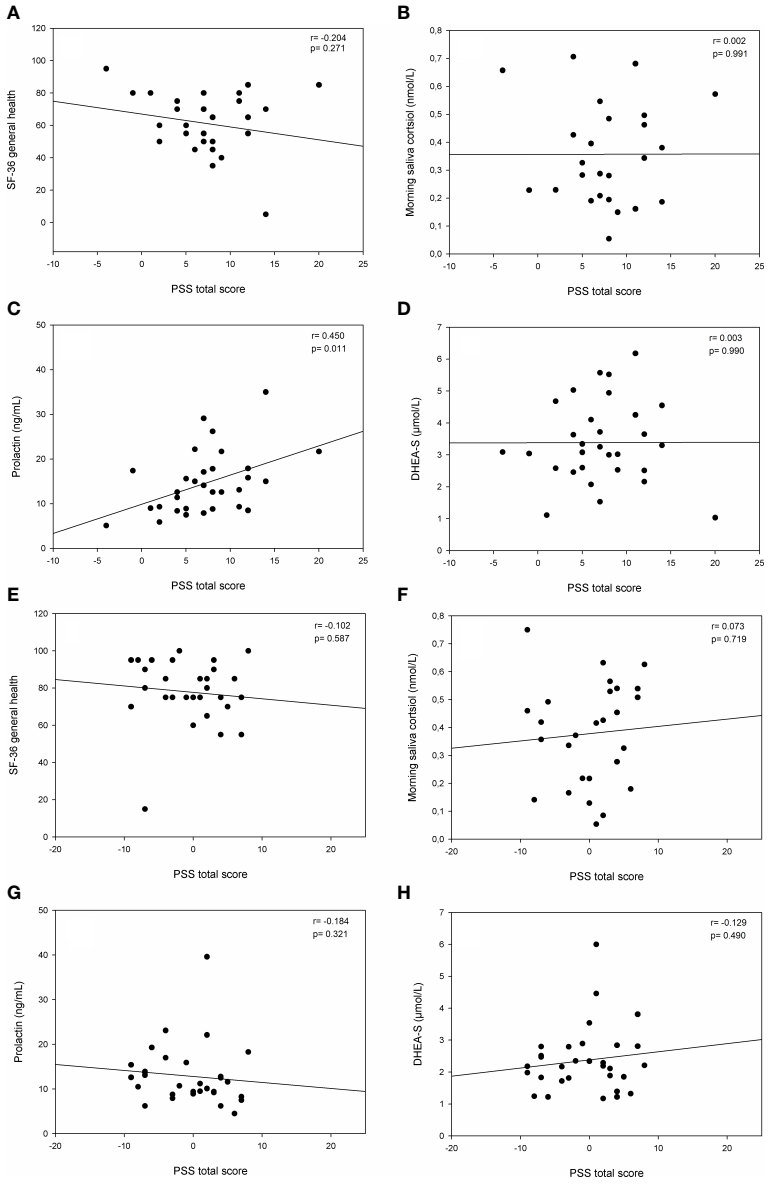

FIGURE 1
Correlation analyses between the Perceived Stress Scale's total score and general health/stress-specific parameters. **(A)** PSS total score versus SF-36 general health in PCOS patients; **(B)** PSS total score versus morning saliva cortisol (nmol/L) in PCOS patients; **(C)** PSS total score versus Prolactin (ng/mL) in PCOS patients; **(D)** PSS total score versus DHEA-S (µmol/L) in PCOS patients; **(E)** PSS total score versus SF-36 general health in controls; **(F)** PSS total score versus morning saliva cortisol (nmol/L) in controls; **(G)** PSS total score versus Prolactin (ng/mL) in controls; **(H)** PSS total score versus DHEA-S (µmol/L) in controls.

SF-36 general health and the PSS total score, neither in PCOS patients (Figure 1A) nor in controls (Figure 1E).

In a next step, we evaluated whether PCOS-typical parameters were associated with two main outcome parameters, namely the PSS total score and the overall quality of life in SF-36, by the use of generalized linear models. Age, BMI, the Ferriman Gallwey Score (for PCOS patients), total testosterone and AMH were included into these models. When focusing on the PSS total score (Table 3), only a higher BMI was linked to increased stress in controls (n= 31), whereas none of the parameters was found to be of significant influence in the total PCOS population (n= 31). Similar results were found in lean/normal weight PCOS women (n= 15) despite a non-significant trend for testosterone and higher perceived stress. However, in overweight/obese PCOS patients a higher BMI, a higher Ferriman Gallwey score and higher age were significantly associated with stress ($p< 0.05$).

Concerning overall quality of life in the SF-36 questionnaire (Table 4), a similar pattern was found. This time, an increased BMI was the major modulator associated with a decreased quality of life and this was the case in the whole PCOS group as well as in overweight/obese PCOS patients ($p< 0.05$).

Discussion

The PCOS patients in our study population revealed typical general and PCOS-specific characteristics with a median age of 25

TABLE 3 Generalized linear models for the Perceived Stress Scale's total score.

	Controls (n= 31)			PCOS: all patients (n= 31)			PCOS: lean and normal weight (n= 15)			PCOS: overweight and obese (n= 16)		
	ß	SD (ß)	p	ß	SD (ß)	p	ß	SD (ß)	p	ß	SD (ß)	p
Constant	-0.035	5.845	0.995	-3.112	5.316	0.558	0.596	10.685	0.956	-25.119	4.686	<0.001
Age (years)	0.301	0.192	0.118	0.176	0.170	0.300	0.256	0.189	0.175	0.351	0.150	0.019
BMI (kg/m2)	-0.533	0.167	0.001	0.145	0.107	0.174	0.084	0.405	0.835	0.532	0.087	<0.001
Ferriman Gallwey Score	–	–	–	0.076	0.106	0.473	-0.068	0.104	0.509	0.334	0.098	<0.001
Testosterone (ng/mL)	8.696	6.877	0.206	-4.982	3.206	0.120	8.407	4.392	0.056	0.354	2.412	0.883
AMH (ng/mL)	0.271	0.604	0.653	0.424	0.223	0.057	0.406	0.223	0.069	-0.206	0.251	0.410

–, not applicable.

TABLE 4 Generalized linear models for total health in SF-36.

	Controls (n= 31)			PCOS: all patients (n= 31)			PCOS: lean and normal weight (n= 15)			PCOS: overweight and obese (n= 16)		
	ß	SD (ß)	p	ß	SD (ß)	p	ß	SD (ß)	p	ß	SD (ß)	p
Constant	598.665	100.519	<0.001	512.710	100.092	<0.001	498.977	198.176	0.012	741.939	137.744	<0.001
Age (years)	-3.860	3.177	0.224	-1.254	3.085	0.684	-3.293	3.507	0.348	2.332	4.421	0.598
BMI (kg/m2)	0.027	1.859	0.988	-5.754	1.919	0.003	-4.629	7.509	0.538	-13.897	2.554	<0.001
Ferriman Gallwey Score	–	–	–	-1.796	1.914	0.348	-2.255	1.923	0.241	-1.655	2.891	0.567
Testosterone (ng/mL)	-230.095	121.519	0.058	53.526	57.806	0.354	-2.369	81.461	0.977	99.349	70.893	0.161
AMH (ng/mL)	-14.834	10.203	0.146	-2.167	4.087	0.596	2.952	4.136	0.475	-7.494	7.367	0.309

–, not applicable.

years, a rate of overweight/obesity of about 52%, and increased testosterone, DHEA-S and LH levels (Table 1). Notably, controls were significantly older and had a lower BMI. Thus, all results of the laboratory stress markers and the questionnaires had to be corrected for age and BMI when compared between PCOS women and controls (Table 2). We did not find differences in laboratory stress biomarkers between PCOS women and controls. Despite the fact that salivary stress markers have been implemented to assess distress in several studies (7, 12, 32, 33), with salivary cortisol being the most established biological marker in stress research, only few authors have attempted to evaluate salivary stress markers in PCOS women so far (34–36). Tock et al. similarly found no hyperactivity of the HPA axis in PCOS women compared to controls. However, non-obese PCOS women had higher salivary cortisol levels when compared to obese PCOS women in the mentioned study (36). On the other hand, an overactivity of the HPA-axis triggered by stressful stimuli was seen as a characteristic of hirsute adolescents, detected by increased salivary glucocorticoid measurements (35).

Noteworthy, studies about cortisol production in PCOS have presented heterogeneous results. While some studies found elevated serum cortisol levels (37–39), others found normal levels (40–42). A recent meta-analysis performed by Benjamin et al. summarized that women with PCOS had higher cortisol levels than controls. However, significant heterogeneity existed across the various studies included. Moreover, the overall effect was accounted for a few large studies, whereas the majority of included studies did not report significant differences (6).

As stated previously, in addition to the objective biochemical assessment of stress, we equally performed subjective evaluation of mental and general health and self-perceived severity of symptoms. Women with PCOS had significantly higher total stress scores, higher perceived helplessness scores and lower perceived self-efficacy scores in the PSS compared to healthy controls. This is in line with other studies, such as Khafagy et al., who found a significant difference in PSS scores among adolescents with and without PCOS (43). Furthermore, significant differences of health related quality of life in terms of physical health and emotional health using the SF-36 were found on the majority of subscales in our study, showing lower scores among PCOS women and therefore indicating a compromised quality of life. Similarly, a recent case-control-study observed significant differences in various domains of the short form health survey-36 between PCOS and healthy control cases (44). Comparable results have been previously published and our findings confirm the overall convergence that women with PCOS are at increased risk of emotional distress and diminished quality of life (5, 45–49). A recent systematic review and meta-analysis conducted by Yin et al. concluded that women with PCOS experience a lower quality of life and more frequently suffered from depression and anxiety (49), which supports the well-known and well-established effect of PCOS on general health and quality of life (48).

Despite the fact that sample sizes were small for generalized linear models, a higher BMI and hirsutism seemed to influence perceived stress mainly in obese PCOS women (Table 3). However, with a focus on the comparably small ß-values in the lean/normal weight PCOS group, BMI and the Ferriman Gallwey Score did not seem to be of major influence on the PSS total score and overall quality of life. Although being overweight is known to be a contributor to an impaired quality of life and depression in the general public, there are conflicting results concerning the influence of BMI on mental distress in PCOS women. Previous studies reported perceived stress scores to be independent of BMI (43) and that an obesity category was not connected to emotional quality of life (5). Other studies demonstrated that an increased BMI did alter health related quality of life (44). The study by Karsten et al. looked at whether obese women suffered specifically if PCOS was detected, with a particular mental health impact. However, despite the fact that these issues are related to the PCOS condition, the authors concluded that the impaired mental quality of life, anxiety, depression and physical quality of life seemed to be more connected to the obesity rather than to the PCOS condition (50). Moreover, females with PCOS report lower body image satisfaction compared to the females without PCOS (51, 52). This may be due to being overweight, or to the androgen related disorders such as hirsutism affecting women's feelings of attractiveness (53). Our findings demonstrate a possible influence of an increased BMI and hirsutism on mental health in particular of obese PCOS women, which could be due to the impact on femininity and body image.

Interestingly, reproductive and metabolic PCOS characteristics are associated with specific PCOS susceptibility loci. As reported, increasing BMI appeared to be causal for PCOS development, whereas having PCOS did not affect BMI (54). While these data support a genetic background, PCOS development also seems to be induced or at least intensified by overweight/obesity. Thus, one could assume different pathophysiologic pathways in overweight/obese PCOS women, more based on a (genetically) higher BMI, and in lean/normal weight PCOS patients, more likely due to a direct genetic risk for PCOS. Likewise, the source of stress could differ between these populations. Notably, a shared genetic basis of PCOS with psychiatric diseases has been refuted (55). However, our data suggest that the higher BMI itself and hirsutism were of major influence on stress in overweight/obese women, whereas in lean/normal weight PCOS patients testosterone as a marker of overall disease severity showed a trend (p= 0.056; Table 3).

In addition to these findings, prolactin was the only serologic stress marker, which was correlated to the PSS total score. In detail, there was a positive correlation. Prolactin has been mentioned as a serum parameter possibly elevated in patients with chronic stress like burn out (56). This would also explain the significantly higher prolactin levels in PCOS patients (p= 0.017; Table 1). From a pathophysiologic point of view, chronic stress induces an intense cortisol production. For the production of cortisol, the POMC-neurons must produce ACTH. By doing this, they also secrete GABA and glutamate. GABA acts a stimulator of prolactin and can therefore lead to an increased prolactin secretion (57). We are aware of the fact that cortisol levels did not differ between PCOS patients and controls, a fact, which we find hard to comment on. In addition to prolactin, PCOS women revealed higher DHEA-S levels, which have also been claimed to be linked to chronic stress (56), but do not seem to be specific for a stress response rather than for the hyperandrogenemic state.

To our best knowledge, this study is the first trial evaluating both salivary stress biomarkers including salivary cortisol measurement and self-perceived severity of symptoms of stress and quality of life in adult PCOS women in one population. Further strengths of our study include the use of validated general and condition- specific questionnaires and an excellent participant retention. Although adequately powered, we are aware of the fact that the study sample size was nevertheless small, and that future studies with a larger sample size are required. A further limitation is that healthy controls had a slightly, but significantly, lower BMI than the PCOS participants at baseline, despite a proactive matching strategy. We realize that the generalizability of our trial results is limited by the homogenous population. Moreover, although salivary measures have proven to be a reliable method, it is accepted that they only provide information pertaining to a single point in time. Therefore, the lack of additional evaluation of cortisol in 24-hour urine could be considered as a limitation of our study. A further limitation is that insulin resistance/HOMA-IR was only available in PCOS patients.

In conclusion, this prospective case-control study revealed the following main findings: there were no differences in laboratory stress biomarkers between PCOS women and controls. However, PCOS women suffered from higher perceived stress and a lower quality of life. A higher BMI and hirsutism seemed to influence perceived stress mainly in obese PCOS women. Last not least, perceived stress was positively correlated to prolactin levels in PCOS patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Medical University of Vienna, Vienna, Austria. The patients/participants provided their written informed consent to participate in this study.

Author contributions

M-LM and JO contributed to conception and design of the study and data acquisition. RM and CS contributed to the analysis and the interpretation of data. JO performed the statistical analysis. MM wrote the first draft of the manuscript. All authors wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1173422/full#supplementary-material

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Inflammation mediates the effect of adiposity and lipid metabolism indicators on the embryogenesis of PCOS women undergoing *in vitro* fertilization/ intracytoplasmic sperm injection

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Background: Polycystic ovary syndrome (PCOS) is a complex reproductive endocrine and metabolic disease affecting women of reproductive age. The low-grade chronic inflammation in PCOS is considered to be associated with obesity and dyslipidemia. We aim to investigate the potential mediating role of white blood cell (WBC) count, a representative inflammatory marker, in the effect of adiposity and lipid metabolism indicators on IVF/ICSI outcomes in PCOS women.

Methods: We conducted a retrospective cohort study of 1,534 PCOS women who underwent their first IVF/ICSI cycles with autologous oocytes at a reproductive center from January 2018 to December 2020. The associations between PCOS women's adiposity and lipid metabolism indicators and WBC count and IVF/ICSI outcomes were examined using multivariable generalized linear models. Mediation analyses were conducted to evaluate the possible mediating role of WBC count.

Results: We found significant dose-dependent correlations between adiposity and lipid metabolism indicators and IVF/ICSI outcomes (i.e., hormone levels on the ovulatory triggering day, oocyte development outcomes, fertilization, early embryo development outcomes, and pregnancy outcomes) (all $p < 0.05$), as well as between adiposity and lipid metabolism indicators and WBC count (all $p < 0.001$). Increasing WBC count was associated with adverse oocyte and embryonic development outcomes (all $p < 0.05$). Mediation analyses suggested that increasing serum TG and LDL-C levels and decreasing serum HDL-C level were significantly associated with reduced high-quality Day 3

embryo count in PCOS women, with 21.51%, 9.75%, and 14.10% mediated by WBC count, respectively (all $p < 0.05$).

Conclusions: We observed significant associations between lipid metabolism indicators and high-quality Day 3 embryo count in PCOS women, partially mediated by inflammation-related mechanisms, suggesting the potential intervention target for improving embryo quality in PCOS women.

KEYWORDS

PCOS, adiposity, lipid metabolism, inflammation, mediating effect, IVF/ICSI outcomes

Introduction

Polycystic ovary syndrome (PCOS) is a lifelong reproductive, metabolic, and psychological condition affecting 5% to 18% of reproductive-aged women (1). The primary clinical manifestations of PCOS are oligo-anovulation, hyperandrogenism (clinical or biochemical), and polycystic ovarian morphology (2). PCOS women have high risks of anovulatory infertility, dyslipidemia, obesity, impaired glucose tolerance (IGT), insulin resistance (IR), type 2 diabetes mellitus (T2DM), cardiovascular disease, gynecological cancers, and psychiatric disorders (1, 3–5). PCOS is a heterogeneous and complex multifactorial disorder resulting from the combined influences of genetic factors, environment, endocrine, dietary habits, and lifestyle (1, 5–7). Increasing evidence has shown that PCOS is closely related to obesity and its relevant metabolic disorders (3).

Obesity, affecting approximately 15% of women worldwide, is a chronic metabolic disease and has grown to epidemic proportions over the past few decades (8). The prevalence of obesity in PCOS women ranges from 50% to 80%, which is approximately three times higher than in women without PCOS (6). The significant association between body mass index (BMI) and PCOS characteristics, regardless of age, has been elucidated by the Northern Finnish Birth Cohort (NFBC) study group as early as the 1960s (9). Obesity promotes and amplifies all endocrine, metabolic, and reproductive outcomes in PCOS women, including biochemical and clinical hyperandrogenism, IR, IGT, hyperglycemia, hyperlipidemia, infertility, and adverse obstetric outcomes (7). Among infertile PCOS women seeking assisted reproductive treatment (ART), obesity and dyslipidemia have been reported to be associated with *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) outcomes. A BMI beyond the normal range has been reported to have adverse effects on ovarian response (10), retrieved oocyte count (10, 11), oocyte maturation (11), clinical pregnancy (12), and live birth (13, 14). BMI is also reported to be a risk factor for miscarriage in PCOS women undergoing IVF/ICSI (10, 13). Increased serum total cholesterol (TC) level has been found to decrease the chance of live birth in PCOS women undergoing IVF/ICSI (14, 15). The mechanism of obesity-related adverse ART outcomes of PCOS may be attributed to the inflammatory response, oxidative stress, and epigenetic alterations (16–19). However, few studies have directly analyzed the potential intermediate mechanisms by which obesity and dyslipidemia affect IVF/ICSI outcomes in PCOS women.

PCOS is considered to be a chronic inflammatory disorder associated with obesity (5, 16). Studies have shown elevated levels of inflammatory markers in PCOS women, including white blood cell (WBC) count, C-reactive protein (CRP), complement element 3 (C3), tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), interleukin 18 (IL-18), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1α (MIP-1α) (19, 20). In PCOS women, obesity, pro-inflammatory response, oxidative stress, IR, hyperinsulinemia, and hyperandrogenism interact to form a vicious cycle (7, 19). Inflammation has been elucidated to cause damage to oocyte and follicular quality (21). A uterine hyperinflammatory state of PCOS women may contribute to placental insufficiency and obstetric complications (22). Therefore, we assume the potential mediating role of inflammation on the association between adiposity and lipid metabolism indicators and IVF/ICSI outcomes in PCOS women.

In this study, we aimed to conduct mediation analyses to determine whether WBC count, the representative inflammatory marker, mediates the effect of adiposity and lipid metabolism indicators on IVF/ICSI early reproductive outcomes and pregnancy outcomes in PCOS women. This study follows the principles of the AGReMA guidelines (23).

Materials and methods

Ethical statement

Approval for this study was obtained from the institutional review board of Peking University Third Hospital (No. 2021SZ–011). Each woman in this study signed an informed consent.

Study population

A total of 1,534 PCOS women aged between 20 and 45 years old who underwent their first fresh IVF/ICSI cycles with autologous oocytes at the Reproductive Center of Peking University Third

Hospital setting between January 2018 and December 2020 were screened for eligibility. PCOS was diagnosed according to the 2003 Rotterdam criteria (2). All the women had at least two of the following three criteria and excluded other causes of hyperandrogenism and ovulation dysfunction (1): biochemical hyperandrogenism and/or hirsutism (2); oligo- or amenorrhea; (3) polycystic ovarian morphology on transvaginal ultrasound (≥ 12 antral follicles in unilateral or bilateral ovaries or ovarian volume > 10 cm^3). The exclusion criteria were as follows: (1) history of iatrogenic ovarian injury; (2) uterine abnormality; (3) history of adrenal diseases, thyroid disorders, T2DM, hyperprolactinemia, and Cushing's syndrome; (4) history of autoimmune disease and recurrent spontaneous abortion; (5) chromosomal abnormalities in either of the spouses; (6) receiving *in vitro* maturation (IVM) or pre-implantation genetic testing (PGT). We extracted the data on baseline characteristics and IVF/ICSI laboratory outcomes from the internal ART database derived from the electronic medical records. Well-trained follow-up staff collected the pregnancy outcomes by telephone interviews, including biochemical pregnancy, clinical pregnancy, and live birth.

IVF procedures

The ovarian stimulation, ovulatory trigger, oocyte retrieval, insemination, embryo culture, and embryo transfer were performed following standardized protocols at our institution as previously described (24, 25). The ovarian stimulation regimen and initial gonadotropin dose were decided according to each woman's BMI and ovarian reserve condition. The embryos were evaluated for fertilization on Day 1 and morphologically graded on Day 3 and Day 5. Up to two Day 3 embryos or Day 5 to 6 blastocysts were transferred by an experienced reproductive medicine specialist (25, 26).

Laboratory assessment

Serum total triglyceride (TG), TC, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured *via* an automatic biochemical analyzer (7170A, HITACHI, Japan) within 3 months before ovarian stimulation. Peripheral blood WBC count was determined *via* a fully automatic blood cell analyzer (Sysmex XN, Japan) within 1 week before ovarian stimulation.

Study outcomes

The study outcomes include serum peak estradiol (E2) level, serum luteinizing hormone (LH) level on the ovulatory triggering day, serum progestin (P) level on the ovulatory triggering day, retrieved oocyte count, metaphase II (MII) oocyte count, normally fertilized zygote count, normally cleaved embryo count, high-quality Day 3 embryo count, blastocyst formation count, biochemical pregnancy, clinical pregnancy, and live birth. An MII oocyte is a mature oocyte that converts into haploid gametes with the first polar body extruded. Normal fertilization involves two pronuclei (2PN) in the zygote. A high-quality Day 3 embryo is a five- to eight-cell embryo with less than 30% fragmentation and uniform cell size. Biochemical pregnancy is defined as positive serum β-hCG tested 2 weeks after embryo transfer. Clinical pregnancy is defined as the presence of a gestational sac with fetal heartbeats detected on the transvaginal ultrasound 3 to 4 weeks after embryo transfer. Live birth is defined as the delivery of a living fetus beyond 28 weeks of gestation. Multiple births are considered as one live birth. All pregnancy outcomes (i.e., biochemical pregnancy, clinical pregnancy, and live birth) were those after fresh-cycle embryo transfer.

Statistical analysis

All the data analyses were performed *via* "R" software (version 4.0.3). We summarized participants' baseline reproductive and cycle characteristics and presented them using median [interquartile range (IQR)] or *n* (%). Differences in baseline reproductive and cycle characteristics across the four BMI groups (underweight: <18.5 kg/m^2, normal weight: 18.5– 25 kg/m^2, overweight: 25 –30 kg/m^2, and obesity: ≥30 kg/m^2) were evaluated using Kruskal–Wallis tests for continuous variables and Chi-squared tests for categorical variables (or Fisher's exact test where appropriate). BMI, TG, TC, HDL-C, LDL-C, and WBC count were ln-transformed for tests that required a normal distribution due to right skewness.

Multivariate generalized linear models were constructed to assess the associations between adiposity and lipid metabolism indicators and IVF/ICSI outcomes, the associations between adiposity and lipid metabolism indicators and WBC count, and the effect of WBC count on IVF/ICSI outcomes in PCOS women to evaluate whether further mediation analyses were indicated. A normal distribution and identity link function were specified for serum peak E2, LH, and P levels on the ovulatory triggering day; a Poisson distribution and log link function were specified for retrieved oocyte count, MII oocyte count, normally fertilized zygote count, normally cleaved embryo count, high-quality Day 3 embryo count, and blastocyst formation count; and a binomial distribution and logit link function were specified for biochemical pregnancy, clinical pregnancy, and live birth. We examined the following covariates as potential confounders based on previous knowledge of biological relevance and statistical considerations: age (continuous), AFC (continuous), basal FSH (continuous), duration of infertility (continuous), infertility type (primary vs. secondary infertility), ovarian stimulation regimen (GnRH antagonist protocol, long GnRHa protocol, and others), insemination technique (IVF vs. ICSI), transferred embryos number (one vs. two), and the timing of embryo transfer (Day 3, Day 5, and Day 6). According to the Change-in-Estimate Method, these covariates remained in the final multivariate models if they caused an over 10% change in the effect estimates for the abovementioned associations (27).

Mediation analyses were performed to estimate the total, direct, and indirect effects *via* the R mediation package to determine whether WBC count is a potential mediator. When the hypothesis of the mediation analyses holds, the direct effect indicates the effect of adiposity and lipid metabolism indicators on IVF/ICSI outcomes in PCOS women after controlling for WBC count, and the indirect effect is the estimated effect of adiposity and lipid metabolism indicators mediated *via* WBC count. The proportion of mediation by WBC count was calculated as the ratio of the indirect effect to the total effect. Two-sided $p < 0.05$ were considered statistically significant.

Results

Baseline reproductive and cycle characteristics

This study included 1,534 PCOS women who had undergone their first cycles of IVF/ICSI. For comparing the baseline reproductive and cycle characteristics, these women were stratified into four groups according to the BMI classification of the World Health Organization: underweight (<18.5 kg/m^2, $n = 51$), normal weight (18.5– 25 kg/m^2, $n = 656$), overweight (25– 30 kg/m^2, $n = 489$), and obesity (≥30 kg/m^2, $n = 338$) (Table 1). Their age tended to increase with the increase of BMI class (all $p = 0.04$). Women with higher BMI tended to have higher AFC and lower basal FSH ($p < 0.001$). With BMI class increased, serum TG, TC, and LDL-C increased, and serum HDL-C decreased (all $p < 0.05$). There was no significant difference in AMH, infertility type, ovarian stimulation regimen, insemination technique, and timing of embryo transfer across the four BMI groups.

Adiposity and lipid metabolism indicators and IVF/ICSI outcomes in PCOS women

All the multivariable models were adjusted for age, AFC, and infertility type for consistency (Tables 2, 3). Table 2 displays the effects of adiposity and lipid metabolism indicators on hormone levels on the ovulatory triggering day, oocyte development outcomes, and fertilization in PCOS women. We found that BMI and serum TG were negatively associated with peak E2 level, retrieved oocyte count, MII oocyte count, and normally fertilized zygote count (all $p < 0.001$) and positively associated with serum P level on the ovulatory triggering day (all $p < 0.05$). Serum HDL-C was positively associated with peak E2 level and normally fertilized zygote count (all $p < 0.01$). Table 3 displays the effects of adiposity and lipid metabolism indicators on early embryo development outcomes and pregnancy outcomes in PCOS women. We found that increasing BMI and serum TG levels were associated with decreasing normally cleaved embryo count, high-quality Day 3 embryo count, and blastocyst formation count and caused lower chances of clinical pregnancy and live birth (all $p < 0.05$). We also found that serum HDL-C was positively associated with normally cleaved embryo count, high-quality Day 3 embryo count, and blastocyst formation count (all $p < 0.01$). Moreover, increasing serum LDL-C level was associated with decreasing normally cleaved embryo count and high-quality Day 3 embryo count (all $p < 0.05$).

Adiposity and lipid metabolism indicators and WBC count in PCOS women

The associations between adiposity and lipid metabolism indicators and WBC count in PCOS women are shown in Table 4. After controlling for age, AFC, and infertility type, BMI, serum TG, serum TC, and serum LDL-C were positively associated with WBC count (all $p < 0.001$). Serum HDL-C was negatively associated with WBC count (all $p < 0.001$).

WBC count and IVF/ICSI outcomes in PCOS women

The associations between WBC count and IVF/ICSI outcomes in PCOS women are presented in Table 5. After controlling for age, AFC, and infertility type, increasing WBC count was significantly associated with decreasing MII oocyte count, normally fertilized zygote count, normally cleaved embryo count, and high-quality Day 3 embryo count (all $p < 0.05$).

Mediation analyses

Given that WBC count was associated with both adiposity and lipid metabolism indicators and IVF/ICSI outcomes, we assumed that WBC count could be a possible mediator. Table 6 and Figure 1 show the result of mediation analyses. After controlling for confounders and WBC count, we found significant adverse effects of serum TG and LDL-C on high-quality Day 3 embryo count (all $p < 0.01$). A significant mediating effect by WBC count was observed for the impact of serum TG and LDL-C on high-quality Day 3 embryo count, with 21.51% and 9.75% proportion mediated, respectively (all $p < 0.05$). We also found significant positive associations between serum HDL-C and high-quality Day 3 embryo count ($p < 0.01$). A significant mediating effect by WBC count was observed for the association between serum HDL-C and high-quality Day 3 embryo count, with 14.10% proportion mediated ($p < 0.05$).

Discussion

In this retrospective study among PCOS women, we found significant dose-dependent associations between adiposity and lipid metabolism indicators and IVF/ICSI outcomes, including hormone levels on the ovulatory triggering day, oocyte development outcomes, fertilization, early embryo development outcomes, and pregnancy outcomes. We also found significant dose-dependent associations between adiposity and lipid metabolism indicators and WBC count, as well as between WBC count and IVF/ICSI outcomes. Further analyses elucidated that WBC count partially

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TABLE 1 Baseline reproductive and cycle characteristics of the PCOS women undergoing the first fresh IVF/ICSI cycles.

Characteristics	BMI, kg/m^2				p^a
	<18.5	18.5–25	25–30	≥30	
n = 1,534	*n* = 51	*n* = 656	*n* = 489	*n* = 338	
Age, years	30 (28, 33)	31 (29, 34)	31 (28, 34)	31 (28, 33)	0.04
AFC, *n*	13 (9, 19)	15 (9, 20)	15 (11, 20)	16 (12, 22)	<0.001
Basal FSH, mIU/ml	6.2 (5.3, 7.3)	6.4 (5.1, 7.8)	5.9 (4.7, 7.2)	5.7 (4.5, 7.0)	<0.001
AMH, ng/ml	5.6 (2.6, 8.4)	5.5 (2.7, 8.5)	4.9 (3.1, 7.5)	4.4 (2.9, 7.2)	0.14
TG, mmol/L	0.8 (0.6, 1.0)	1.0 (0.7, 1.5)	1.5 (1.0, 2.0)	1.5 (1.2, 2.3)	<0.001
TC, mmol/L	4.3 (4.0, 4.7)	4.5 (4.0, 5.0)	4.5 (4.0, 5.1)	4.6 (4.1, 5.1)	0.01
HDL-C, mmol/L	1.5 (1.3, 1.7)	1.3 (1.1, 1.5)	1.2 (1.0, 1.3)	1.1 (1.0, 1.3)	<0.001
LDL-C, mmol/L	2.5 (2.0, 2.8)	2.8 (2.4, 3.2)	2.9 (2.5, 3.5)	3.0 (2.6, 3.5)	<0.001
WBC count, $\times10^9$/L	6.6 (4.8, 8.1)	6.2 (5.1, 7.5)	7.0 (5.9, 8.4)	7.5 (6.3, 8.9)	<0.001
Duration of infertility, years	2 (1, 4)	3 (1, 4)	3 (2, 5)	4 (2, 6)	<0.001
Infertility type					0.67
Primary	38 (74.5%)	443 (67.5%)	327 (66.9%)	234 (69.2%)	
Secondary	13 (25.5%)	213 (32.5%)	162 (33.1%)	104 (30.8%)	
Ovarian stimulation regimen					0.05
GnRH antagonist	40 (78.4%)	521 (79.4%)	397 (81.2%)	269 (79.6%)	
Long GnRHa	7 (13.7%)	116 (17.7%)	87 (17.8%)	63 (18.6%)	
Others[b]	4 (7.8%)	19 (2.9%)	5 (1.0%)	6 (1.8%)	
Insemination technique					0.14
IVF	30 (58.8%)	476 (72.6%)	354 (72.4%)	252 (74.6%)	
ICSI	21 (41.2%)	180 (27.4%)	135 (27.6%)	86 (25.4%)	
Transferred embryos number					0.03
1	0 (0.0%)	4 (0.6%)	0 (0.0%)	2 (0.6%)	
2	44 (86.3%)	555 (84.6%)	438 (89.6%)	277 (82.0%)	
Timing of embryo transfer					0.07
Day 3	49 (96.1%)	620 (94.5%)	476 (97.3%)	314 (92.9%)	
Day 5	1 (2.0%)	24 (3.7%)	7 (1.4%)	13 (3.8%)	
Day 6	1 (2.0%)	12 (1.8%)	6 (1.2%)	11 (3.3%)	

Numbers are median ± IQR (range), except for percentages.
PCOS, polycystic ovary syndrome; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; BMI, body mass index; AFC, antral follicle count; FSH, follicle-stimulating hormone; AMH, anti-Mullerian hormone; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; WBC, white blood cell; GnRH, gonadotropin-releasing hormone; GnRHa, gonadotropin-releasing hormone agonist; IQR, interquartile range.
[a]p-values comparing the differences across the four BMI groups.
[b]Other protocols include the short GnRHa protocol, ultrashort GnRH antagonist protocol, and minimal stimulation protocol.

mediates the adverse effects of serum TG and LDL-C on high-quality Day 3 embryo count in PCOS women. We also found that WBC count partially mediates the association between serum HDL-C and high-quality Day 3 embryo count in PCOS women.

PCOS and obesity have an intense and complex association (28). Evidence from Mendelian randomization (MR) study suggested that an increase in body fat distribution indicators (i.e., BMI and waist-to-hip ratio) is causally related to PCOS (29). Obesity increases hyperinsulinemia and IR, subsequently increasing ovarian androgen production (30, 31). The excessive adipose tissue can aromatize these androgens to estrogens, which are then released into the circulation and interfere with the function of the hypothalamic–pituitary–ovarian (HPO) axis (28). Moreover, obesity increases inflammatory adipokines, which forms a vicious feedback cycle with hyperinsulinemia (7). These alterations exert a significant impact on PCOS-related symptoms. Obesity and

TABLE 2 β/RR (95% CI) in hormone levels on the ovulatory triggering day, oocyte development outcomes, and fertilization associated with adiposity and lipid metabolism indicators among PCOS women undergoing their first IVF/ICSI cycles based on generalized linear models (n = 1,534)[a].

Adiposity and lipid metabolism indicators[b]	Peak E2 levels[b], pmol/L		LH levels[b], mIU/ml		p levels[b], nmol/L		Retrieved oocytes, n		MII oocytes, n		Normal fertilization, n	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	RR (95% CI)	p	RR (95% CI)	p	RR (95% CI)	p
BMI, kg/m^2	−0.56 (−0.75, −0.38)	<0.001	0.10 (−0.20, 0.40)	0.52	0.18 (0.03, 0.33)	0.02	0.82 (0.74, 0.90)	<0.001	0.84 (0.76, 0.93)	<0.001	0.78 (0.69, 0.89)	<0.001
TG, mmol/L	−0.12 (−0.18, −0.07)	<0.001	−0.06 (−0.15, 0.03)	0.18	0.07 (0.02, 0.11)	<0.01	0.95 (0.92, 0.98)	<0.001	0.95 (0.92, 0.98)	<0.001	0.92 (0.89, 0.96)	<0.001
TC, mmol/L	−0.04 (−0.22, 0.13)	0.61	0.15 (−0.13, 0.43)	0.28	0.05 (−0.08, 0.19)	0.46	0.95 (0.87, 1.04)	0.26	0.95 (0.87, 1.05)	0.31	0.95 (0.85, 1.06)	0.32
HDL-C, mmol/L	0.21 (0.07, 0.35)	<0.01	−0.08 (−0.31, 0.16)	0.53	−0.07 (−0.18, 0.04)	0.23	1.06 (0.98, 1.14)	0.16	1.07 (0.99, 1.15)	0.09	1.18 (1.07, 1.29)	<0.001
LDL-C, mmol/L	−0.06 (−0.17, 0.06)	0.36	0.18 (−0.02, 0.37)	0.07	0.05 (−0.04, 0.14)	0.28	0.97 (0.91, 1.03)	0.25	0.97 (0.91, 1.03)	0.26	0.94 (0.87, 1.02)	0.13

[a]Adjusted for age (continuous), AFC (continuous), and infertility type.
[b]Transformed by natural logarithm.

dyslipidemia worsen all PCOS's clinical manifestations and reproductive outcomes, including menstrual disorder, biochemical and clinical hyperandrogenism, hyperglycemia, metabolic syndrome, infertility, miscarriage, gestational diabetes mellitus, and pregnancy-induced hypertension (7, 32–34). Obesity and dyslipidemia may also damage oocyte and embryo quality. Several pieces of evidence can support the negative impact of obesity on ART outcomes in PCOS women. Among PCOS women undergoing ART, obesity can lead to poor ovarian response and disturb oocyte maturation (10, 11). Adiposity and lipid metabolism indicators have also been frequently reported as risk factors for clinical pregnancy and live birth in PCOS women (12–15). In this study, we observed that adiposity and lipid metabolism indicators are negatively associated with PCOS women's IVF/ICSI outcomes, including ovarian response indicators, oogenesis outcomes, embryogenesis outcomes, and pregnancy outcomes. These results provide complementary evidence for the above-related studies.

Obesity and dyslipidemia are involved in the low-grade chronic inflammatory progress in PCOS women (5, 16, 19). The hematologic inflammatory markers in PCOS women have been reported to be closely related to obesity (19, 35, 36). Consistently, we found significant correlations between the PCOS women's lipid

TABLE 3 RR/OR (95% CI) in early embryo development outcomes and pregnancy outcomes associated with adiposity and lipid metabolism indicators among PCOS women undergoing their first IVF/ICSI cycles based on generalized linear models (n = 1,534)[a].

Adiposity and lipid metabolism indicators[b]	Normal cleavage, n		High-quality Day 3 embryos, n		Blastocyst formation, n		Biochemical pregnancy		Clinical pregnancy		Live birth	
	RR (95% CI)	p	RR (95% CI)	p	RR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
BMI, kg/m^2	0.79 (0.70, 0.89)	<0.001	0.79 (0.68, 0.91)	<0.01	0.64 (0.49, 0.84)	<0.01	1.84 (0.99, 3.43)	0.05	2.11 (1.13, 3.98)	0.02	3.00 (1.54, 5.88)	<0.01
TG, mmol/L	0.92 (0.89, 0.96)	<0.001	0.95 (0.91, 0.99)	0.01	0.85 (0.79, 0.92)	<0.001	1.13 (0.94, 1.36)	0.18	1.33 (1.10, 1.60)	<0.01	1.24 (1.02, 1.52)	0.03
TC, mmol/L	0.92 (0.82, 1.03)	0.14	0.91 (0.80, 1.04)	0.17	0.92 (0.72, 1.17)	0.50	1.15 (0.65, 2.03)	0.62	1.24 (0.70, 2.21)	0.46	1.25 (0.69, 2.30)	0.46
HDL-C, mmol/L	1.17 (1.07, 1.29)	<0.001	1.17 (1.05, 1.30)	<0.01	1.45 (1.18, 1.78)	<0.001	0.79 (0.49, 1.27)	0.33	0.75 (0.46, 1.21)	0.23	0.65 (0.39, 1.07)	0.09
LDL-C, mmol/L	0.92 (0.86, 1.00)	0.04	0.90 (0.82, 0.98)	0.02	0.88 (0.74, 1.04)	0.14	1.23 (0.83, 1.82)	0.30	1.24 (0.84, 1.85)	0.28	1.38 (0.91, 2.10)	0.13

[a]Adjusted for age (continuous), AFC (continuous), and infertility type.
[b]Transformed by natural logarithm.

TABLE 4 β (95% CI) in WBC count associated with adiposity and lipid metabolism indicators among PCOS women undergoing their first IVF/ICSI cycles based on generalized linear models (n = 1,534)[a,b].

Adiposity and lipid metabolism indicators	WBC count, $\times10^9$/L	
	β (95% CI)	*p*
BMI, kg/m^2	0.44 (0.35, 0.52)	<0.001
TG, mmol/L	0.13 (0.11, 0.15)	<0.001
TC, mmol/L	0.14 (0.06, 0.22)	<0.001
HDL-C, mmol/L	−0.25 (−0.31, −0.19)	<0.001
LDL-C, mmol/L	0.10 (0.04, 0.15)	<0.001

[a]Adjusted for age (continuous), AFC (continuous), and infertility type.
[b]Adiposity and lipid metabolism indicators and WBC count were transformed by natural logarithm.

TABLE 5 β/RR/OR (95% CI) in early reproductive and pregnancy outcomes associated with WBC count among PCOS women undergoing their first IVF/ICSI cycles based on generalized linear models (n = 1,534)[a].

Early reproductive and pregnancy outcomes	WBC count[b], $\times10^9$/L	
	β/RR/OR (95% CI)	*p*
Peak E2 levels[b], pmol/L	−0.07 (−0.18, 0.04)	0.21
LH levels[b], mIU/ml	−0.05 (−0.05, 0.13)	0.60
P levels[b], nmol/L	0.05 (−0.03, 0.14)	0.23
Retrieved oocytes, *n*	0.96 (0.90, 1.01)	0.13
MII oocytes, *n*	0.93 (0.88, 0.99)	0.02
Normal fertilization, *n*	0.91 (0.85, 0.98)	0.01
Normal cleavage, *n*	0.91 (0.85, 0.98)	0.01
High-quality Day 3 embryos, *n*	0.89 (0.82, 0.97)	<0.01
Blastocyst formation, *n*	0.86 (0.73, 1.01)	0.07
Biochemical pregnancy	1.30 (0.90, 1.87)	0.16
Clinical pregnancy	1.25 (0.86, 1.81)	0.24
Live birth	1.38 (0.93, 2.04)	0.11

[a]Adjusted for age (continuous), AFC (continuous), and infertility type.
[b]Transformed by natural logarithm.

TABLE 6 Mediation analyses investigating whether WBC count mediated the associations between adiposity and lipid metabolism indicators and high-quality Day 3 embryo count [a,b].

Mediators	Associations	Total effect (95% CI)	Mediated effect (95% CI)	Estimated proportion mediated (%)
WBC count	High-quality Day 3 embryo count and TG	−0.25 (−0.43, −0.07)**	−0.05 (−0.11, 0.00)*	21.51
	High-quality Day 3 embryo count and HDL-C	0.78 (0.28, 1.32)**	0.11 (0.00, 0.23)*	14.10
	High-quality Day 3 embryo count and LDL-C	−0.52 (−0.98, −0.10)**	−0.05 (−0.11, −0.01)*	9.75

[a]Adjusted for age (continuous), AFC (continuous), and infertility type.
[b]Adiposity and lipid metabolism indicators and WBC count were transformed by natural logarithm.
*$p < 0.05$.
**$p < 0.01$.

metabolism indicators and WBC count, a representative inflammatory marker. Studies have elucidated that excessive adipose tissue can cause the imbalance of the immune system and activate pro-inflammatory processes, characterized by high levels of TNF-α, pro-inflammatory interleukins, and chemokines (3, 37–39). The mechanisms triggering the inflammatory state in obese women have also been extensively studied, including cell enlargement and death in the obese tissue, imbalanced adipokine production, fatty

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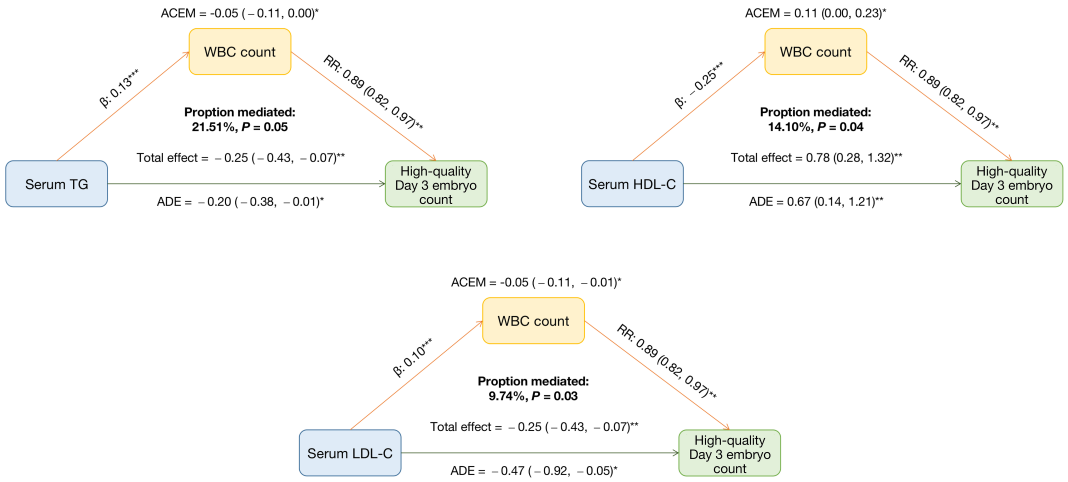

FIGURE 1
Mediating effects of WBC count on the associations between adiposity and lipid metabolism indicators and high-quality Day 3 embryo count. Adjusted for age (continuous), AFC (continuous), and infertility type. Adiposity and lipid metabolism indicators and WBC count were transformed by natural logarithm. ACME: average causal mediating effect. This represents the indirect effect, also known as the mediating effect of WBC count on the associations between adiposity and lipid metabolism indicators and high-quality embryo count. ADE: average direct effect. This represents the effects of adiposity and lipid metabolism indicators on high-quality Day 3 embryo count when WBC count is controlled. *$p < 0.05$. **$p < 0.01$.

acid dyshomeostasis, local hypoxia, mitochondria dysfunction, dysbiosis in the gut microbiome, mechanical stress, and endoplasmic reticulum stress (3, 19, 40). Moreover, epigenetic regulations, including DNA methylation, histone acetylation, and changes in noncoding RNA levels, have been reported to affect the inflammatory response and oxidative stress in PCOS women (16).

Obesity-induced excessive pro-inflammatory mediators may contribute to the occurrence and progress of PCOS by various mechanisms. Pro-inflammatory mediators may inhibit insulin receptors, reduce insulin sensitivity and aggravate IR, consequently worsening reproductive dysfunction (41, 42). Obesity can induce higher levels of inflammatory mediators in the ovary, which may cause irreversible damage to the oogenesis and ovary function (21, 43). Oxidative stress, closely related to inflammatory response, can also lead to oocyte apoptosis and ovarian dysfunction (44). The change in obesity-induced inflammation and oxidative stress pathways may alter the expression of genes related to oocyte quality, thus adversely affecting the subsequent embryogenesis (21). Furthermore, the NLRP3 inflammasome and the chemokine decoy receptor D6, two representative uterine inflammatory mediators, may disturb the maternal–fetal interface with the subsequent occurrence and progress of obstetric complications (22). Existing clinical studies have elucidated the adverse effects of inflammatory mediators on female reproductive outcomes. According to a meta-analysis of 22 studies, peripheral NK cells are associated with recurrent miscarriage (45). Increasing circulating CRP in the pre-implantation period is related to adverse ART outcomes, including folliculogenesis disorder, poor oocyte competence, abnormal embryo development, and poor endometrial receptivity (46). Moreover, hematologic inflammatory markers (i.e., WBC count and neutrophils) have been reported to be negatively associated with oocyte developmental competence in PCOS women (35). Mean platelet volume (MPV) has been reported to correlate significantly to the clinical pregnancy rate among PCOS women undergoing ART (35).

This study found that WBC count was a significant mediator for the associations between adiposity and lipid metabolism indicators (i.e., serum TG, HDL-C, and LDL-C) and high-quality Day 3 embryo count, suggesting that inflammation may mediate the adverse effect of obesity and dyslipidemia on embryogenesis. As we have discussed above, obesity and dyslipidemia have been illuminated to be associated with hematologic inflammatory changes in PCOS women and are the potential causality of PCOS's low-grade chronic inflammatory state (19). Studies also supported the adverse effect of obesity-induced excessive pro-inflammatory mediators on oogenesis and embryogenesis (21). However, the results of these mediation analyses need to be interpreted with caution since solid assumptions are required for the causal interpretation of the findings.

Although cautious interpretation is needed for the results of our mediation analysis, our findings explained the potential inflammatory response mechanism of obesity-induced embryo development impairment in PCOS women and provided potential therapeutic targets. Anti-inflammatory therapy was found to help alleviate PCOS. In a PCOS mouse model, as a kind of stem cell therapy, transplantation of bone marrow mesenchymal stromal cells (BM-MSCs) reduced serum malondialdehyde (MDA), TNF-α, and IL-6 concentrations and improved folliculogenesis (47). Another animal study found that inulin and metformin improved ovarian morphology and endocrine function *via* anti-inflammation and modulating gut microbiota (48). Saffron petal extract and saffron petal anthocyanins have been reported to ameliorate symptoms of PCOS *via* improving dysregulation of ovarian steroidogenesis, antioxidant enzymes, and inflammatory response (49). Resveratrol has been demonstrated to have anti-inflammatory

effects in PCOS women by suppressing the expression of NF-κB and NF-κB-regulated gene products (50). There has been considerable progress in the research of antioxidants in anti-inflammation and improving IVF outcomes in PCOS women. In clomiphene-citrate-resistant PCOS women, the combined application of coenzyme Q10 and clomiphene citrate improves ovulation and clinical pregnancy rates (51). A recent randomized controlled trial elucidated that quercetin consumption reduced serum TNF-α and IL-6 concentrations and improved oocyte quality, embryo grade, and the pregnancy rate in PCOS women (52). There are also some emerging therapies under investigation. Adipokine-targeted regimens (i.e., recombinant protein, therapeutic peptide, monoclonal antibody, adipokine receptor agonist, and adipokine receptor antagonist) can improve obesity, pro-inflammatory response, IR, and ovarian dysfunction, thus may be a novel therapeutic strategy for PCOS (3). Moreover, as PCOS is a chronic inflammatory state associated with obesity, lifestyle interventions focused on diet, exercise, and behavioral or combined treatments, and anti-obesity medications still need to be promoted and encouraged in PCOS patients (7).

This is the first study focused on the mediating role of inflammation in the associations between PCOS women's adiposity and lipid metabolism indicators and IVF/ICSI outcomes. However, some limitations in this study still warrant consideration. First, we conducted a retrospective study, thus possibly causing bias in data collection and making it difficult to determine causation. However, the objective nature and specific definitions of the indicators we studied may eliminate bias to some extent. Second, we did not consider adjunctive therapy (i.e., diet, exercise, or medication) before or during IVF/ICSI treatment. There are also some other adiposity and lipid metabolism indicators (i.e., waist circumference, hip circumference, waist-to-hip ratio, body fat percentage, skinfold thickness, lipoproteins, and apolipoproteins). Moreover, many other inflammatory markers have been proposed to be associated with PCOS (53). Future prospective studies can address these issues in a better experimental design. Third, serum lipid and peripheral blood WBC count testing was performed only once and may not be at the same time point. Moreover, the timing of laboratory assessment of some PCOS women may be long before ovarian stimulation. This affected the representativeness of the data. Future studies with more rigorous designs strictly specifying the timing of specimen collection and using repeated measures are still warranted. Fourth, we simultaneously performed multiple hypothesis tests to assess the correlations between adiposity and lipid metabolism indicators and WBC count. Therefore, the possibility that our findings were due to chance cannot be excluded entirely. Fifth, our study population was from a reproductive center, which limits the extrapolation of our results to the general PCOS population due to potential selection bias. However, these findings may have implications for PCOS women seeking infertility treatment, which accounts for 40% to 72% of PCOS women (1, 54).

In conclusion, we found that lipid metabolism dysfunction was associated with WBC count in PCOS women. We also found that WBC count was negatively associated with PCOS women's IVF/ICSI outcomes (i.e., MII oocyte count, normally fertilized zygote count, normally cleaved embryo count, and high-quality Day 3 embryo count). As a representative inflammatory marker, WBC count partially mediated the association between adiposity and lipid metabolism indicators (i.e., serum TG, HDL-C, and LDL-C) and high-quality Day 3 embryo count. These findings show the importance of the inflammation-related mechanism on the obesity- and dyslipidemia-induced abnormal embryogenesis in PCOS women.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by institutional review board of Peking University Third Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JQ and XL conceived and designed the research. LC and HJ collected the clinical data. HJ analyzed the data and wrote the draft. TT and HS provided statistical advice. JQ, XL, HC, RY, and NH critically revised the paper. All the authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Metformin combined with spironolactone vs. metformin alone in polycystic ovary syndrome: a meta-analysis

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Aims: Due to its high heterogenicity and unclear etiology, there is currently no specific treatment for polycystic ovary syndrome (PCOS). Metformin, as an insulin sensitizer, combined with spironolactone, an antiandrogen medication, may exert complementary effects on PCOS. We therefore performed a meta-analysis of trials in which metformin combined with spironolactone was applied to treat PCOS to evaluate the efficacy and safety of the combination therapy.

Methods: We retrieved the PubMed, Embase, Scopus, Cochrane Library, CNKI, CBM, Wangfang, and VIP databases for literatures published from their inception to December 16, 2022 on the effects of metformin combined with spironolactone in the treatment of PCOS. Inclusion criteria according to P.I.C.O.S criteria were: PCOS patients, metformin combined with spironolactone interventions, metformin alone control group, and randomized controlled trials with the following outcome data: body mass index (BMI), hirsutism score, luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone (TT), fasting blood glucose (FBG), Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), and side effects including nausea, vomiting, diarrhea and drug withdrawal.

Results: Our results revealed that metformin combined with spironolactone significantly reduced BMI and TT, but that it exerted no significant effects on hirsutism score, or on FSH or LH concentrations. Combined treatment also resulted in a significant diminution in FBG and insulin resistance using the HOMA-IR when the interventional time was greater than 6 months. In addition, the combination did not have a higher occurrence of adverse reactions than metformin alone.

Conclusion: Compared with metformin alone, metformin combined with spironolactone therapy may be more effective in reducing BMI and serum androgen levels, but the combination showed no significant effect on the hirsutism score or gonadotropin hormone levels, and was not associated with

an elevation in side-effects. Moreover, when the treatment course was greater than 6 months, combination therapy reduced FBG and improved insulin resistance more effectively than metformin alone. However, more research is needed to determine the most effective course of treatment.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42022355515.

KEYWORDS

polycystic ovary syndrome, metformin, spironolactone, combination, meta-analysis

1 Introduction

Polycystic ovary syndrome (PCOS) is a usual endocrine disease, affecting 5–20% of child-bearing period women. As the incidence of obesity has increased, the prevalence of PCOS is also increasing (1, 2). The Rotterdam diagnostic criteria are the most commonly used diagnostic criteria for PCOS, which must exclude the other causes and have at least 2 of the following three standards: 1) oligovulatory or anovulatory; 2) clinical or biochemical hyperandrogenism; and 3) polycystic ovaries under ultrasound (3). Due to an imbalance in female sex hormones, PCOS presents with a myriad of symptoms that include irregular menstrual cycles, anovulation, and hyperandrogenism. In addition to these classic symptoms, PCOS can also lead to metabolic disorders, including hypertension, cardiovascular issues, dyslipidemia, and increase the risk of endometrial cancer (4). Furthermore, previous study have shown that due to obesity, hirsutism and the decline in the quality of life, women with PCOS are at increased risk for anxiety and depression, and even more severe symptoms such as obsessive-compulsive disorder and somatization (5, 6).

As a result of the high heterogenicity of PCOS and unclear underlying cause(s), the treatment of PCOS is often symptomatic and individualized. At present, therapeutic options for PCOS range from pharmacologic treatments to surgery. Spironolactone, an androgen receptor blocker, plays an important role in the treatment of hyperandrogenism (7). Metformin, an anti-hyperglycemic agent, improves insulin resistance and enhances insulin sensitivity (8), has been proven to have a definite therapeutic effect on PCOS (9). Moreover, metformin has been also shown to be an effective treatment for pregnancy complications and obesity; it also improves menstrual cyclicity, but it is not effective for treating hirsutism (10). Some guidelines and reviews do not specifically describe the combination of metformin and spironolactone except when combined with other drugs such as combined oral contraceptive pills (COCPs) and clomiphene citrate (CC) (11).

Abbreviations: PCOS, polycystic ovary syndrome; BMI, body mass index; FGS, Ferriman-Gallwey score; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TT, total testosterone; FBG, fasting blood glucose; HOMA-IR, homeostatic model of assessment for insulin resistance; IR, insulin resistance.

In summary, when metformin is combined with spironolactone, a complementary effect may occur that better improves symptoms in PCOS patients. Using a meta-analysis, we herein summarized the current literature regarding the combined use of metformin with spironolactone in the treatment of PCOS, and examined the efficacy and drug safety of metformin combined with spironolactone when compared with metformin monotherapy in the treatment of PCOS.

2 Materials and methods

2.1 Data sources

2.1.1 Information sources

Two investigators searched the PubMed, Embase, Scopus, Cochrane Library, CNKI, CBM, Wangfang, and VIP databases for literatures published from their inception to December 16, 2022. The keywords we use were as follows: “polycystic ovary syndrome”, “PCOS”, “Metformin”, “Spironolactone”. Using a combination of MeSH and text words, the specific search strategy we used in PubMed was as follows: (((((((((metformin[MeSH Terms]) OR (Dimethylbiguanidine[Title/Abstract])) OR (Dimethylguanylguanidine[Title/Abstract])) OR (Glucophage [Title/Abstract])) OR (Metformin Hydrochloride[Title/Abstract])) OR (Hydrochloride, Metformin[Title/Abstract])) OR (Metformin HCl[Title/Abstract])) OR (HCl, Metformin[Title/Abstract])) AND ((((((((((((((((((((((((((((((Spironolactone[MeSH Terms]) OR (Spirolactone[Title/Abstract])) OR (Veroshpiron[Title/Abstract])) OR (Verospirone[Title/Abstract])) OR (Spiractin[Title/Abstract])) OR (Spirobeta[Title/Abstract])) OR (Spirogamma[Title/Abstract])) OR (Spirolang[Title/Abstract])) OR (Spirono-Isis[Title/Abstract])) OR (Spirono Isis[Title/Abstract])) OR (Spironone[Title/Abstract])) OR (Spirospare[Title/Abstract])) OR (Aldactone[Title/Abstract])) OR (Verospiron[Title/Abstract])) OR (Aldactone A [Title/Abstract])) OR (Aquareduct[Title/Abstract])) OR (Duraspiron [Title/Abstract])) OR (Espironolactona Alter[Title/Abstract])) OR (Espironolactona Mundogen[Title/Abstract])) OR (Flumach[Title/Abstract])) OR (Frumikal[Title/Abstract])) OR (Jenaspiron[Title/Abstract])) OR (Novo-Spiroton[Title/Abstract])) OR (Novo Spiroton[Title/Abstract])) OR (NovoSpiroton[Title/Abstract])) OR

(Practon[Title/Abstract])) OR (SC-9420[Title/Abstract])) OR (SC 9420[Title/Abstract])) OR (SC9420[Title/Abstract])) OR (Spiro L.U.T.[Title/Abstract])) OR (Spiro Von Ct[Title/Abstract])) OR (Ct, Spiro Von[Title/Abstract])) OR (Von Ct, Spiro[Title/Abstract]))) AND ((((((((((((((Polycystic Ovary Syndrome[MeSH Terms]) OR (Ovary Syndrome, Polycystic[Title/Abstract])) OR (Syndrome, Polycystic Ovary[Title/Abstract])) OR (Stein-Leventhal Syndrome [Title/Abstract])) OR (Stein Leventhal Syndrome[Title/Abstract])) OR (Syndrome, Stein-Leventhal[Title/Abstract])) OR (Sclerocystic Ovarian Degeneration[Title/Abstract])) OR (Ovarian Degeneration, Sclerocystic[Title/Abstract])) OR (Sclerocystic Ovary Syndrome[Title/Abstract])) OR (Polycystic Ovarian Syndrome[Title/Abstract])) OR (Ovarian Syndrome, Polycystic[Title/Abstract])) OR (Polycystic Ovary Syndrome 1[Title/Abstract])) OR (Sclerocystic Ovaries[Title/Abstract])) OR (Ovary, Sclerocystic[Title/Abstract])) OR (Sclerocystic Ovary[Title/Abstract])) OR (PCOS[Title/Abstract])).

2.1.2 Inclusion and exclusion criteria

The inclusion criteria according to P.I.C.O.S criteria: (a) patients with PCOS were diagnosed using standard criteria; (b) metformin combined with spironolactone interventions;(c) metformin alone control group; (d) relevant and complete data were provided; (e) randomized controlled trials (RCTs).

The exclusion criteria: (a) where the control treatment for PCOS was spironolactone or the combination therapy was metformin combined with other treatments; (b) no relevant outcome indicators; (c) non-randomized controlled trials (NRCTs), duplicate studies, case reports, cell or animal studies and other non-clinical controlled trials.

2.2 Data extraction

Two evaluators (HMZ and SKH) independently used Microsoft Excel to extract relevant data as follows: Basic information (e.g., sex, age, and country); intervention measures; control measures; BMI; hirsutism score; hormonal status (i.e., levels of FSH, LH, and total T); metabolic parameters such as fasting blood glucose and HOMA-IR); and medication side-effects including nausea, vomiting, diarrhea, and the need for drug withdrawal. All discrepancies will reach a consensus through negotiation.

2.3 Missing data

We would contact the corresponding author via email to obtain data to complete the missing data in the included literature. If still not available, the literature would be excluded.

2.4 Data items

The variables we had abstracted from including study were as follows: population (sample, age, ethnicity, diagnostic criteria); intervention and comparison (type, dose, frequency, cointervention); outcomes (BMI; hirsutism score; hormone status (FSH, LH, and total T); metabolic parameters (FBG and HOMA-IR); adverse effects (nausea, vomiting, diarrhea, and the need for drug withdrawal)).

2.5 Measures of treatment effects

Continuous data are represented by a mean difference (MD) or standardized mean difference (SMD), binary data are represented by odds ratio (OR), and a 95% confidence interval (CI) was used for effect size estimation. We kept the units consistent of measuring data for each RCT.

2.6 Quality assessment

Two reviewers (SKH and YYY) independently used the Cochrane Collaboration tool and the Jadad scale evaluate the quality of the included literatures. Each risk of bias (selection, performance, detection, churn, reporting, and other bias) was assessed as high, low, and unclear using the Cochrane collaboration tool (12). Three items on the Jadad scale were randomized (2 points), blinded (2 points), and dropped (1 point) to assess the methodological quality of each study. The total score ≤2 was classified as low-quality experiment, and the total score ≥3 was classified as high-quality experiment.

2.7 Quantitative synthesis

We used ReviewManager (RevMan) V.5.3 to analyze the outcomes from including literatures. The effect size is the mean/standard mean (MD/SMD) OR odds ratio (OR) with their 95% CI. All data were measured using a fixed effect model. The I2 value and Q test in the forest plots were used to analyze the heterogeneity of the included literatures. When I2 is greater than 50%, the heterogeneity is considered to be high. Subgroup sensitivity analyses were performed and one study was sequentially excluded per iteration to understand their impact on the results. We used Stata 12.0 for sensitivity analysis, and the difference was statistically significant when $p < 0.05$.

2.8 Registration trial

Two reviewers(YYY and LNH) submitted a research proposal to the PROSPERO(International Prospective Register of Systematic Reviews, PROSPERO)and applied for a registration code (PROSPERO CRD42022355515).

3 Results

3.1 Literature search

The systematic research totally obtained 658 pertinent literatures (PubMed 40, Embase 45, Cochrane Library 18, Scopus

105, CNKI 9, CBM 15, Wangfang 10, VIP 4, another database 2). By eliminating duplicate articles and preliminary screening of literatures, 25 literatures remained. After excluding conference presentations (n = 2), review and network-analysis (n = 3), and studies that did not report outcomes of interest (n = 2), seven articles were removed. Among the 18 articles, we further excluded 12 articles that contained other drug treatments such as SPIOMET (a combination of spironolactone, pioglitazone, and metformin), where the control was not metformin therapy, and where the studies had incomplete data. Six articles were ultimately retained (13–18). The screen process is detail in Figure 1 (19).

3.2 Quality assessment

The quality assessment applying the Cochrane Collaboration tool was as follows: random sequence generation was assessed as low risk in four articles (13–16), whereas allocation concealment was described in only two articles (13, 16). An elevated risk of blinding of participants and personnel and blinding of the outcome assessment appeared in two open-label RCTs (15, 16). Attrition bias and reporting bias were of low risk in four articles. However, reporting bias was noted to be of high risk in one article since the results of the report were inconsistent with the data and could therefore influence the significance of our results (17). The risk of other biases was mostly assessed as unclear due to insufficient information provided. Figure 2 illustrates the results of the risk-bias assessment.

Using Jadad scale, the total score of including studies was 1 to 4. Four studies were high-quality, but the low-quality studies were both from China. The Jadad scores of the included studies are displayed in Table 1.

3.3 Study characteristics

The principal characteristics of the six trials are presented in the Table below. In six clinical trials, a total of 717 patients, aged 14 to 40 years, were randomly assigned to combination therapy (n=236) and dimethylidyne (n=232), from different countries (China n=358, India n=240, Italy n=71, Egypt n=48). The duration of treatment maintained in clinical trials ranged from 3-12 months (mean 5 months, median 6 months), and most also combined a life intervention (diet/exercise). The dosage of metformin in clinical trials ranged from 850 to 2000 mg (median 1250 mg) and spironolactone ranged from 20 to 100 mg (median 32.5 mg). The diagnosis of PCOS is based on the Rotterdam and ESHRE criteria (Table 1).

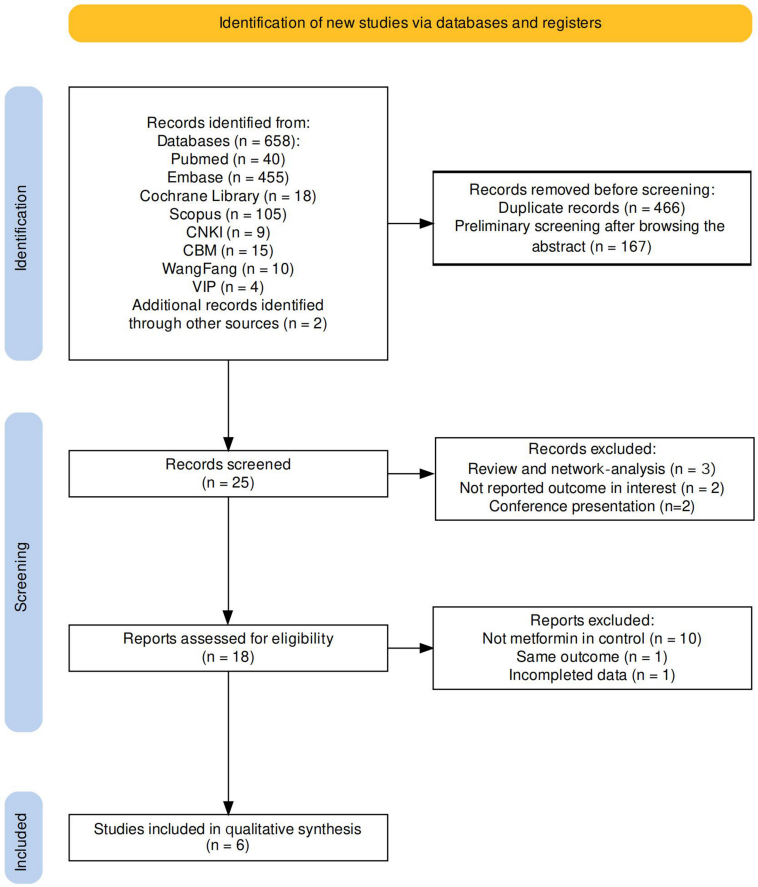

FIGURE 1
The flow chart of literature search is as follows.

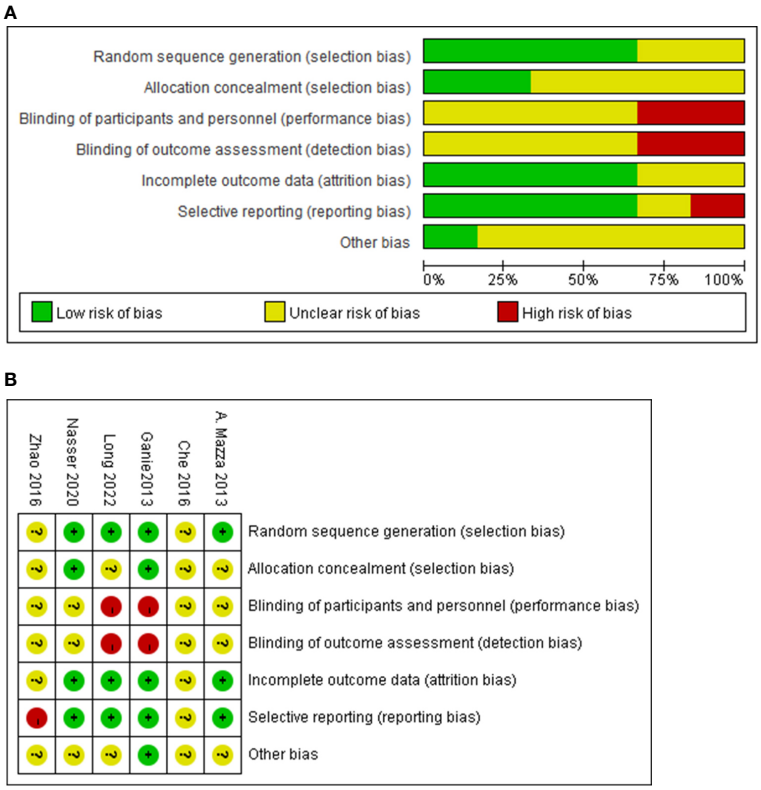

FIGURE 2
The quality of including studies by the Cochrane Collaboration tool. Summary of the risk of bias assessment **(A)**, and risk of bias **(B)**.

TABLE 1 Summary of study characteristics and Jadad score for quality assessment of included studies.

First author and year of publication	Country	Diagnostic criteria	Study duration including follow-up (month)	Sample size			Age (year)	Experimental	Control	Conintervention	Jadad score
				Total	Intervention	control		Dose / Frequency	Dose / Frequency		
A.Mazza 2014	Italy	The Rotterdam Diagnostic Criteria	6	71	26	26	–	First week: Metformin 425mg/ twice daily Spironolactone 25mg/ once daily After first week: Metformin 825mg/ twice daily Spironolactone 25mg/ once daily	First week: Metformin 425mg/ twice daily After first week: Metformin 825mg/ twice daily	Diet	3
Che 2016	China	The Rotterdam Diagnostic Criteria	6	90	45	45	–	Metformin 500mg/ three times daily Spironolactone 20mg/ once daily	Metformin 500mg/ three times daily	–	1
Ganie 2013	India	The 2006 Androgen Excess Society Criteria	6	240	62	107	14~39	Metformin 500mg/ twice daily Spironolactone 20mg/ once daily	Metformin 500mg/ twice daily	Diet + exercise	4
Long 2020	China	The Rotterdam Diagnostic Criteria	3	208	51	107	–	Metformin 1500mg/once daily Spironolactone 40mg/ once daily	Metformin 1500mg/ once daily	Diet + exercise	3

(Continued)

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TABLE 1 Continued

First author and year of publication	Country	Diagnostic criteria	Study duration including follow-up (month)	Sample size			Age (year)	Experimental	Control	Conintervention	Jadad score
				Total	Intervention	control		Dose / Frequency	Dose / Frequency		
Nasser 2020	Egypt	The Rotterdam Revised Criteria	6	48	22	21	20~28	First week: Metformin 500mg/ twice daily Spironolactone 25mg/ once daily After first week: Metformin 850mg/ twice daily Spironolactone 25mg/ once daily	First week: Metformin 500mg/ twice daily After first week: Metformin 850mg/ twice daily	Diet	4
Zhao 2016	China	The Rotterdam Diagnostic Criteria	3	60	30	30	25~35	Metformin 1000mg/once daily Spironolactone 40mg/ once daily	Metformin 1000mg/ once daily	Barrier method of contraception	1

3.4 Primary outcome

3.4.1 The effect of drug combination on BMI

Five studies showed BMI changes relative to combination treatment and included 446 patients. The forest plot indicated that the combination treatment was superior to the metformin alone in BMI (MD, −0.62; 95% Cl, −1.05 to 0.18; P = 0.005); and heterogeneity in BMI was low (I^2 = 12%) (Figure 3A).

3.4.2 The effect of drug combination on hirsutism score

The results of hirsutism score changes relative to combination treatment in five studies comprised 378 patients. The modified Ferriman–Gallwey score (mFGS) and Ferriman–Gallwey score (FGS) represent the hirsutism score. We noted no significant difference in hirsutism scores (SMD, −0.17; 95% Cl, −0.37 to −0.03; P = 0.10), and the heterogeneity in the hirsutism scores was low (I^2 = 33%) (Figure 3B).

3.4.3 The effect of drug combination on FSH

The results of FSH changes relative to combination treatment were reported for 311 patients in four studies. There was no significant difference in FSH (MD, −0.19; 95% Cl, −0.62 to 0.24; P = 0.39), and heterogeneity in FSH was low (I^2 = 0%) (Figure 4A).

3.4.4 The effect of drug combination on LH

Four studies showed results of LH changes relative to combination treatment in 311 patients. For LH reduction, the combination treatment was more effectively than the metformin alone and was statistically significant (MD, −0.65; 95% Cl, −1.19 to −0.11; P = 0.02), while heterogeneity was slightly higher (I^2 = 57%) (Figure 4B). When we excluded the study by Nasser published in 2020, the heterogeneity would decrease (I^2 = 0%), and there was still significant difference in LH (Figure 4C). The most probable reason for the result is that the patients in the clinical trial by Nasser were overweight/obese.

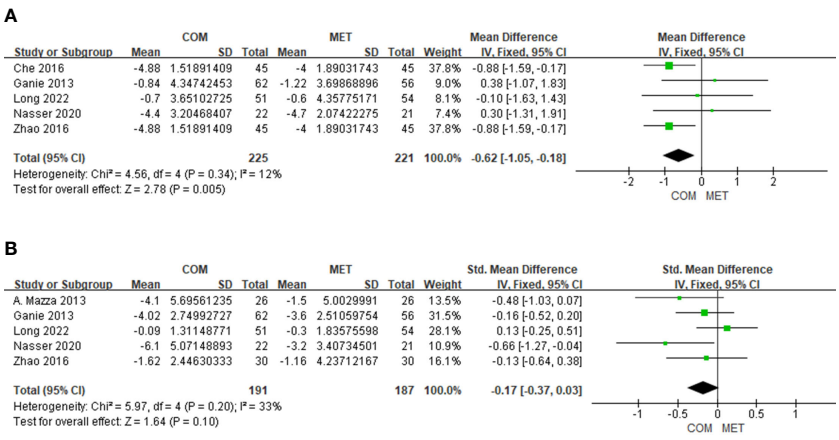

FIGURE 3
The forest plots of meta-analysis of BMI **(A)** and Hirsutism score **(B)**.

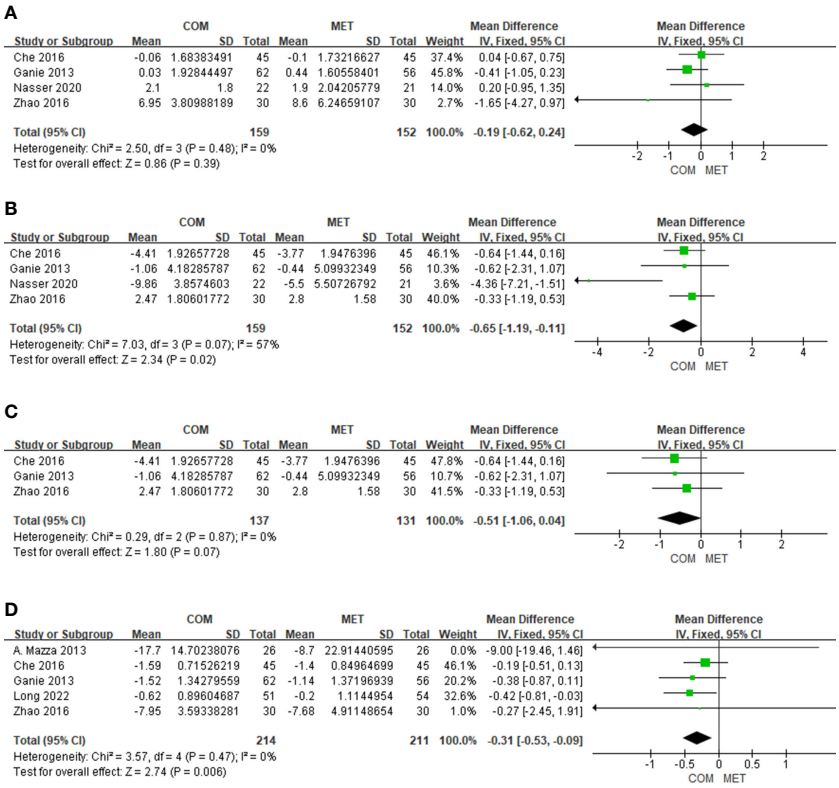

FIGURE 4
The forest plots of meta-analysis of FSH **(A)**, LH **(B)**, LH excluding Nasser 2020 **(C)**, and TT **(D)**.

3.4.5 The effect of drug combination on TT

Five studies reported the results of TT changes relative to combination treatment included 425 patients (Figure 4). With respect to lowering TT, the forest plot showed that the combination was more effective than metformin alone with low heterogeneity (I^2 = 0%) (Figure 4D).

3.4.6 The effect of drug combination on FBG

Six studies described results of FBG changes relative to combination treatment and included 468 patients. Combination therapy of metformin and spironolactone did not significantly improve FBG levels (P = 0.16), with mild heterogeneity uncovered (I^2 = 38%) (Figure 5A). We performed a subgroup analysis based on duration of treatment, and found that when intervention time was ≥6 months, the combination of metformin and spironolactone reduced FBG levels (MD, −0.22; 95% CI, −0.38 to −0.05; P = 0.01) with low heterogeneity (I2 = 0%) (Figure 5B).

3.4.7 The effect of drug combination on HOMA-IR

In six studies the results of HOMA-IR changes relative to combination treatment comprised 468 patients. We observed a significant difference in HOMA-IR (P = 0.006), with high heterogeneity detected (I^2 = 76%) (Figure 5C). When we conducted subgroup analysis where the intervention time was < 6 months, combination therapy did not improve HOMA-IR status (P = 0.12), and there was low heterogeneity (I^2 = 5%). However, when the intervention time was ≥ 6 months, combination therapy of metformin and spironolactone diminished HOMA-IR status (P = 0.0003), with high heterogeneity shown (I^2 = 74%) (Figure 5D). Through sensitivity analysis, Nasser's study showed significant heterogeneity compared with other studies. After removing Nasser's study, heterogeneity decreased (I^2 = 0%) and the overall effect still remained significantly different (P = 0.004) (Figure 5E). The high heterogeneity may be due to the fact that the subjects included in the Nasser's trial were overweight or obese.

3.4.8 The effect of drug combination on nausea

Three studies on the results of nausea events included 313 patients. There was no significant difference in nausea (OR, 0.95; 95% CI, 0.48 to 1.85; P = 0.87), and heterogeneity in nausea was low (I^2 = 0%) (Figure 6A).

3.4.9 The effect of drug combination on vomiting

Three studies on vomiting events comprised 313 patients. There was no significant difference in vomiting (OR, 1.00; 95% CI, 0.45 to 2.23; P = 0.99), and heterogeneity with respect to vomiting was low (I^2 = 0%) (Figure 6B).

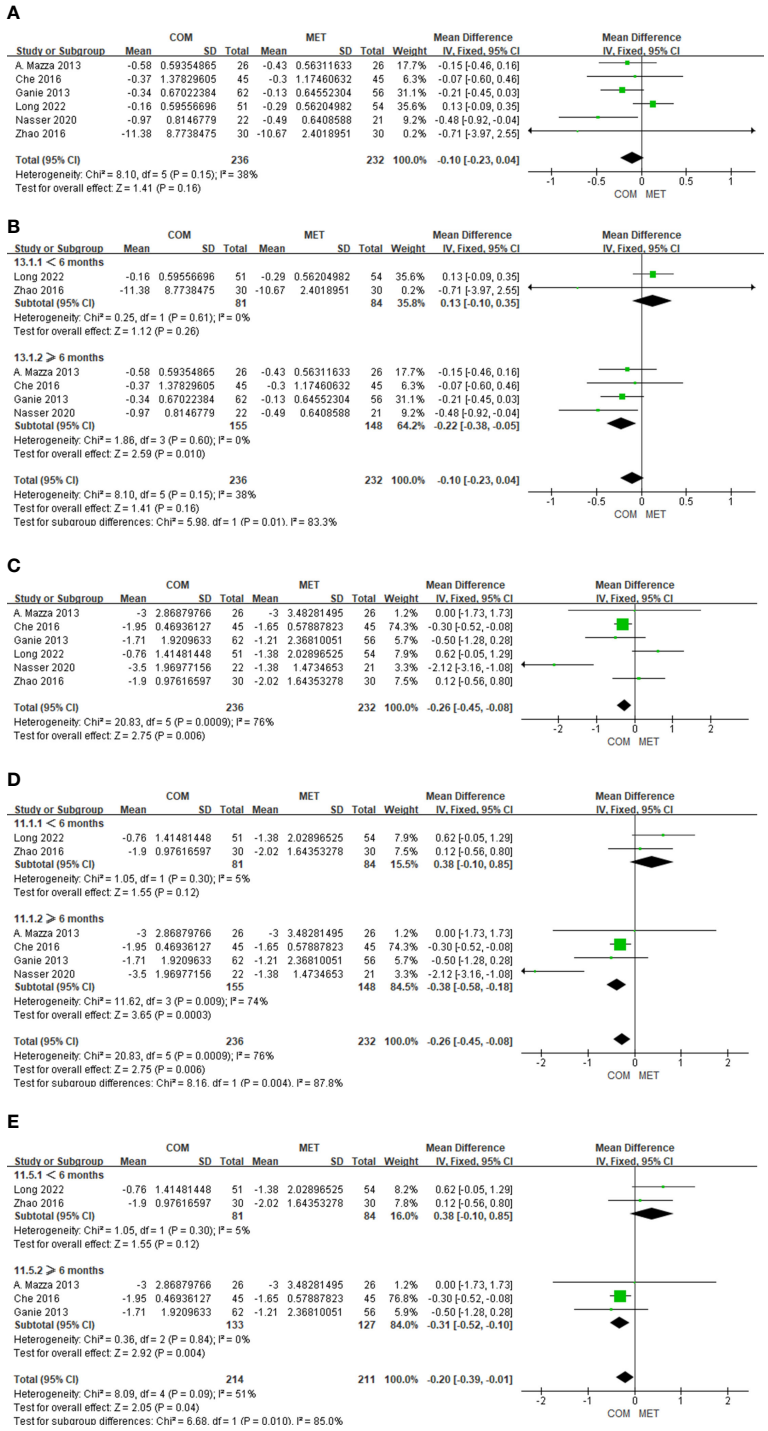

FIGURE 5
The forest plots of meta-analysis of FBG **(A)**, FBG according the study duration **(B)**, HOMA-IR **(C)**, HOMA-IR according the study duration **(D)**, and HOMA-IR excluding Nasser 2020 **(E)**.

3.4.10 The effect of drug combination on diarrhea

Three studies on diarrhea events included 313 patients. There was no significant difference in diarrhea (OR, 0. 87; 95% Cl, 0.47 to 1.64; P = 0.67), and heterogeneity in diarrhea was low (I^2 = 0%) (Figure 6C).

3.4.11 The effect of drug combination on drug withdrawal

Six studies depicted the results of drug withdrawal events and included 467 patients. The reason for drug withdrawal was primarily intolerance, with no other reasons given. There was no significant difference in drug withdrawal (OR, 0.64; 95% Cl, 0.18 to

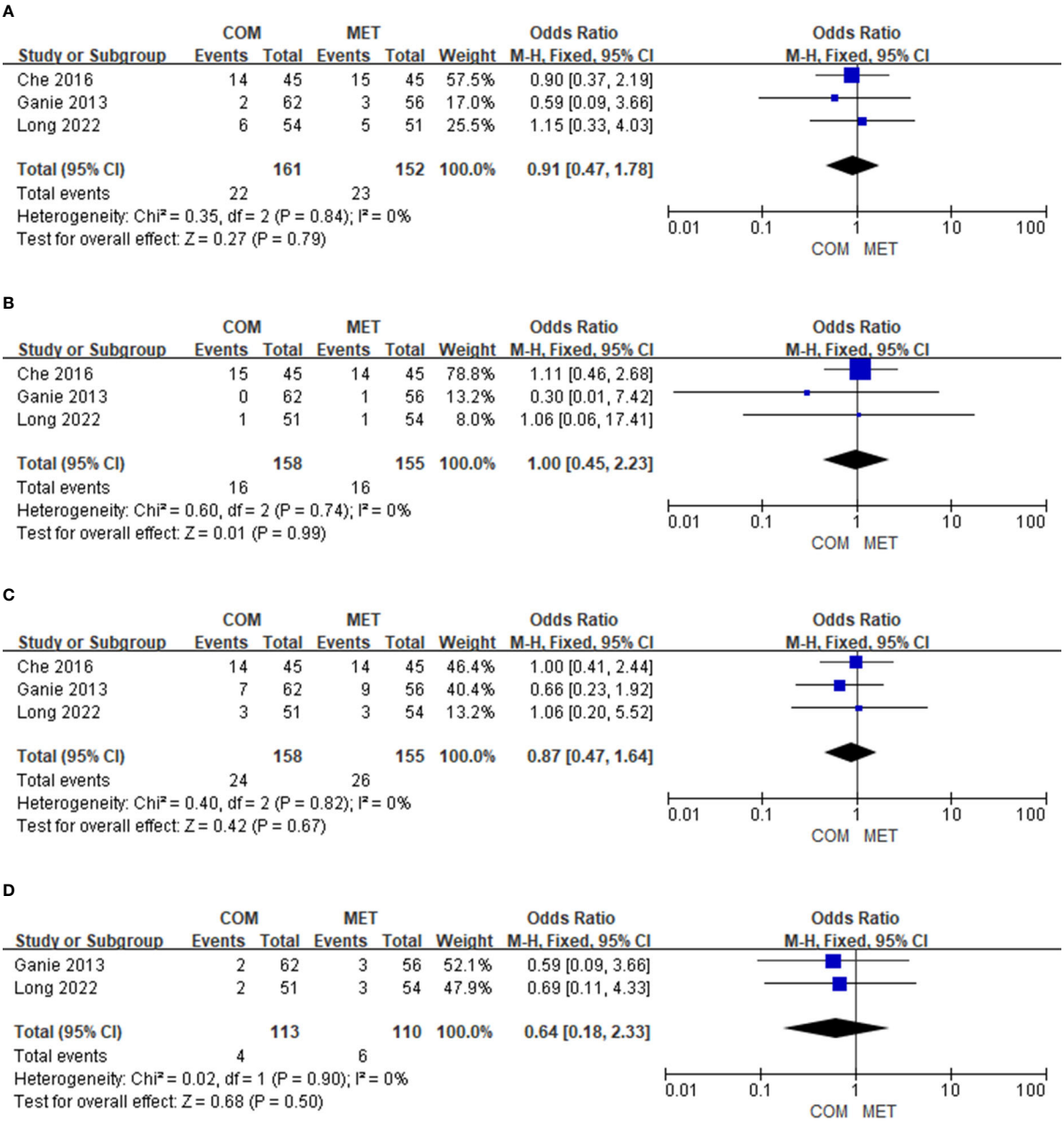

FIGURE 6
The forest plots of meta-analysis of nausea **(A)**, vomiting **(B)**, diarrhea **(C)**, and drug withdrawal **(D)**.

2.33; P = 0.50), and heterogeneity was low (I^2 = 0%). In addition, there were no reports of serious adverse effects such as hyperkalemia or of elevated levels of creatinine or urea nitrogen in the six included studies (Figure 6D).

4 Discussion

PCOS is an endocrine disease that can lead to metabolic syndrome and simultaneously a psychiatric disease that can harm women's health and their quality of life. The etiology of PCOS, however, is not currently understood. International evidence-based guidelines propose the following factors as potential causes of PCOS: hypothalamic-pituitary dysfunction, insulin resistance and hyperinsulinemia, hyperandrogenism, abnormal ovarian regulatory mechanisms, genetic predisposition, and the environment (11). Insulin resistance can interfere with the hypothalamic-pituitary-ovarian axis, and hyperinsulinemia amplifies the androgenic potential of the ovarian theca cells and enhances the secretion of androgens (20). The increase in androgen sensitivity and utilization and the decrease in its clearance all contribute to the hyperandrogenism, which then further inhibits the development and ovulation of follicles and causes polycystic ovaries and anovulation (11, 21). Therefore, improving insulin resistance and treating hyperandrogenemia constitute the focus of treatment for PCOS.

Metformin is an insulin sensitizer that can decrease glucose absorption in the gut lumen, increase glucose uptake and utilization by peripheral tissues, inhibit glucose production, improve insulin resistance, reduce body weight, and achieve euglycemia. It is the first-line treatment for type 2 diabetes mellitus (T2DM) and has also been shown to be effective in the treatment of PCOS (9). Spironolactone is an androgen receptor blocker, exerts antiandrogenic effects directly by blocking androgen receptors, stimulates aromatase, and partially blocks androgen synthesis (7). In addition, spironolactone is able to inhibit inflammation by preventing aldosterone from binding to mineralocorticoid receptors, and its positive effect on insulin resistance has also been demonstrated in clinical and experimental studies (22). Diamanti-Kandarakis et al. (23) demonstrated that metformin improved hirsutism, and Ganie et al.[15] showed that spironolactone improved insulin sensitivity, although to a lesser degree than metformin. Adeyanju et al. (24) also found that spironolactone improved IR in PCOS patients, most likely by suppressing the elevations in T, and that spironolactone exerted a protective effect. The combination of the two drugs in theory, then, should act in a complementary fashion.

Our study included six RCTs, four of which were of high quality (7). We ascertained that the combination of metformin and spironolactone was more beneficial to improve BMI and TT, but that it was not more effective on FGS, FSH, or LH than metformin alone. In addition, combination treatment resulted in a significant diminution in FBG and HOMA-IR when the duration of therapy was ≥ 6 months. However, in a meta-analysis of 28 RCTs that encompassed 1631 PCOS patients, Chen et al. determined that 1000 mg/day of metformin alone was needed for at least 25.5 weeks and that 1000 mg/day of metformin combination therapy was required for at least 58.6 weeks to produce better curative effects on body weight. The reason for the longer time period required to achieve effects by the combination therapy (e.g., metformin + simavastin, metformin + drospirenone/ethinyl estrdiol, metformin + ethinyl estradiol/norgestimate, metformin + flutamide, and metformin + rosiglitazone) might be the perceived synergistic actions between the two drugs, which are time-dependent. The reason for this may be that the weight gain caused by combination drugs, such as rosiglitazone and ethinylestradiol, counteracts the weight reduction effect of metformin, which prolongs the effective duration of weight reduction in combination therapy. These effects are also somewhat contradictory to the present study, which may be due to the dosages of metformin used (which ranged from 1000 to 1700 mg/day) in our included studies and the fact that insulin resistance (IR) changed earlier than BMI. In addition, previous studies have shown that IR resulting from abnormal insulin signaling and metabolic dysfunction in insulin-responsive tissue was elevated or that augmented IR was associated with increased weight gain (25–27). Similarly, Kirstin et al (28) investigated variations in insulin sensitivity and BMI and found that women with high BMI were at greater risk of impaired insulin sensitivity and elevated glucose levels during the luteal phase of their menstrual cycles. The findings of Song also suggested that a high IR resulted in low future weight gain because of a negative feedback mechanism by low glucose oxidation (26). Furthermore, Johnson (29) demonstrated in his 2014 analysis that both high BMI and insulin resistance contributed to the pathogenesis of PCOS. Therefore, metformin, as a common insulin sensitizer, was considered to be a priority option treatment for women with PCOS and insulin resistance. Noteworthy is that metformin exerts different effects on overweight/obese and non-obese women with PCOS. Yang et al. (30) indicated that HOMA-IR and insulin levels of overweight women decreased significantly after using metformin for 12 months and returned to the baseline levels for 24 months, whereas there was no discernible change in HOMA-IR and insulin levels in non-obese patients during 24 months. We interpret this to mean that the high heterogeneity of the Nasser study was due to the authors' inclusion of overweight/obese patients. Unfortunately, for the present study we could not obtain the original BMI data for subgroup analysis in order to further prove the efficacy of metformin in obese and non-obese women with PCOS. In addition, compared with the hyperinsulinemic-euglycemic clamp test, the gold standard of assessing insulin resistance, HOMA-IR, still has several limitations and may affect the accuracy of the study results (31).

On the one hand, spironolactone could improve insulin resistance by lowering androgens, reduce body weight and BMI. In addition, Mineralocorticoid receptors (MR) activation has been shown to trigger abnormal responses in various tissues, including adipose tissue. Spironolactone, as an aldosterone antagonist, can block the activation of MR, reduce the expression of inflammatory bodies and the inflammation of adipose tissue, decrease the levels of various inflammatory indicators (NF-κB, TNF-α, IL-6), improve the oxidative stress and antioxidant capacity, and protect the injured adipose cells (32). In addition, spironolactone acts only on the distal tubules and collecting ducts as diuretics, resulting in a potassium-sparing diuretic effect, simultaneously reduction in plasma volume would activate the neurohumoral systems (the renin-aldosterone-angiotensin system, sympathetic nervous system, and ADH secretion), leading to a relative increase in Na and water absorption. Therefore, spironolactone has a weak diuretic effect and is less likely to reduce the patient's body weight through Na and water excretion, which is not considered as the main reason for the reduction of BMI in combination therapy (22). In summary, spironolactone combined with metformin can reduce androgen, improve insulin resistance, promote the remission of lipolysis, and improve glucose uptake and energy homeostasis together with metformin, ultimately resulting in weight loss in patients. This may be the main mechanism of combination therapy.

Hyperandrogenism is characterized by the increase or over-activity of male hormones in the blood circulation, which can lead to the dysfunction of the hypothalamic-pituitary-ovarian axis and the imbalance of energy metabolism in women. Clinically, it mainly presents symptoms such as hirsutism, acne, obesity and irregular menstruation. Hirsutism can be quantified by the androgen level based on the hirsutism score (Ferriman-Gallwey/modified Ferriman-Gallwey) and is the most commonly used clinical diagnostic criterion for hyperandrogenism. Relevant study has shown that the severity of hirsutism is correlated with biochemical hyperandrogenism markers, and hirsutism score has

a stronger correlation with FT level, but a lesser extent to TT level (33). It is speculated that the severity of hirsutism depends mainly on the bioavailable circulating and FT. This may also be the reason why the combination treatment in this study effectively reduced TT level, but did not significantly improve the hirsute score. Unfortunately, FT data could not be collected in this study.

It should be noted that both metformin and spironolactone have side-effects. Maliha et al. demonstrated that the use of metformin was related to a high incidence of side-effects, particularly gastrointestinal (GI) reactions such as nausea, vomiting, diarrhea, and abdominal pain. While the most serious adverse effect of metformin is lactic acidosis, it is quite rare (34). The side-effects of spironolactone include hyperkalemia, transient polyuria, gastrointestinal discomfort, nausea, breast tenderness, allergic reactions, somnolence, headache, vertigo, and menstrual irregularity (35). However, these side-effects are generally mild and rarely lead to drug withdrawal. Intriguingly, regardless of which drug is used, the side-effects of metformin and spironolactone are both closely dose-related (36). Metformin is generally administered at a dosage of 500–2000 mg/day, and the dosage for spironolactone ranges from 25 to 400 mg daily depending upon the specific disease. In order to avoid adverse drug reactions, the dosage of metformin or spironolactone at the initiation of treatment usually begins at a low level. In our study, none of the six included studies reported serious adverse reactions, including hyperkalemia or elevated levels of urea nitrogen or serum creatinine. In only three studies (15–17) did the investigators record adverse reactions (mainly gastrointestinal reactions), and in one study (16) metrorrhagia was reported. We also found that the incidence rates of these side-effects and of drug withdrawal were quite low, and that there was no significant difference between metformin alone and metformin combined with spironolactone. Therefore, metformin combined with spironolactone will not increase the occurrence of side-effects compared with metformin therapy alone, and the combination is safe and tolerated by patients. However, our sample of included studies was small and the dosage of spironolactone was low (20–50 mg/day); therefore, additional clinical studies are needed to verify the apparent side-effects of therapy with metformin combined with spironolactone.

There were some limitations to our meta-analysis. There were only six RCTs included, and the majority involved small sample sizes, high attrition rates, and poor patient adherence. Moreover, due to the limited number of included studies, publication bias was not assessed. In addition, the duration of treatment was limited to 3 to 6 months, and parameters of the menstrual cycle and blood lipid metabolism were lacking. Moreover, other factors may have produced clinical heterogenicity, such as ethnic groups, age, baseline BMI, the dosages of metformin and spironolactone, and dietary and exercise guidelines.

5 Conclusions

Compared with metformin alone, we ascertained that metformin combined with spironolactone therapy was more effective in reducing BMI and serum androgen levels, but it exerted no significant effects on hirsutism score or hormone levels and did not produce more side-effects. Moreover, when the course of treatment was ≥ 6 months, combination therapy lowered fasting blood glucose and improved insulin resistance more effectively than metformin; however, additional studies need to be conducted to determine the most effective course of treatment.

Author contributions

Conception and design: HZ. Analysis and interpretation: HZ, SH, JW, WR, and LZ. Data collection: HZ, SH, and LZ, Article writing: HZ, SH, and LH. Critical revision of the article: HZ and SH. Final approval of the article: HZ, SH, JW, WR, LZ, LH, and YY. Statistical analysis: HZ, SH, JW, and WR. Overall responsibility: YZ.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Trends in metabolic dysfunction in polycystic ovary syndrome: a bibliometric analysis

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Polycystic ovary syndrome (PCOS) is a very common chronic disease and causes reproductive disorders in women of childbearing age worldwide. The cause of metabolic dysfunction in PCOS is unknown, and there is a lack of systematic bibliometric analysis for this disease. This study included 3,972 articles on metabolic dysfunction in PCOS published from 2012 to 2021. We applied the VOSviewer and the CiteSpace scientometric analysis software to analyze the data regarding the publication of the articles, countries, authors, institutions, scientific categories, cited journals, and keywords. Through this analysis, we determined the research efforts and their developing trends and anticipated the progress in understanding PCOS-related metabolic dysfunction.

KEYWORDS

polycystic ovary syndrome, metabolism, VOSviewer, CiteSpace, bibliometric analysis

1 Introduction

Polycystic ovary syndrome (PCOS) is a common reproductive and endocrine disease affecting 6–10% of women of reproductive age worldwide (1). This syndrome is mostly characterized by menstrual disorder, infertility, abnormal increase in the level of androgen, and a polycystic ovary (2). Many PCOS patients have metabolic disorders, including insulin resistance, obesity, and abnormal lipid metabolism (3). The current difficulty is that the detailed pathophysiological mechanisms of PCOS are unknown (4–6).

In particular, it is important to note that elucidating the intricate relationship between the gonadotropin pathway and steroidogenesis in PCOS is critical to understanding the underlying pathophysiological mechanisms of this disorder. The hypothalamic defect prevalent in PCOS patients can cause abnormal signaling from the hypothalamus to the pituitary gland and lead to the release of excessive gonadotropin-releasing hormone (GnRH). This increases the frequency at which luteinizing hormone (LH) is released and decreases the secretion of follicle-stimulating hormone (FSH) and abnormal signaling in the pathways between them. Therefore, most PCOS patients have an increased LH/FSH ratio during ovulation (7, 8). PCOS metabolic dysfunction is related to hyperandrogenemia caused by neuroendocrine dysfunction of the hypothalamic-pituitary-ovarian axis. Additionally, hyperandrogenemia and infertility caused by PCOS are closely related to

steroid production (9). Normally, steroid production occurs mainly in the ovaries or adrenal cortex, where cholesterol is converted to progesterone by different cholesterol-producing enzymes, which in turn can be converted to progesterone and androgens, which in turn can be converted to estrogen. These transformation processes require the participation of gonadotropins. For example, LH can regulate the transformation of cholesterol into pregnenolone, and FSH can affect the rate at which aromatase converts androgen into estrogen (10, 11). However, the neuroendocrine dysfunction of the hypothalamic-pituitary-ovarian axis in PCOS patients can affect the normal secretion of LH, FSH, and other hormones and thus, affect the normal production of steroids. Therefore, studies on metabolic dysfunction related-PCOS will help to understand the disease mechanism and develop novel therapies for its treatment.

Bibliometric analysis is based on mathematical and statistical methods to analyze all articles published on a research topic over a period of time, providing an overview of research categories or topics, co-authorship, keyword frequency and the most cited articles or journals, which is exactly what is needed to reveal the frontiers and hotspots of research into metabolic dysfunction in PCOS (12, 13). Previous articles have been published on different aspects related to PCOS, which are coronary heart disease, insulin resistance and infertility (14–16). This is the first bibliometric analysis of PCOS metabolic dysfunction in the last ten years (2012–2021) to investigate the current status of research in this field. First, a linear regression algorithm was innovatively introduced to analyze the number of publications in this field and predict the trend of future publications. We then discussed the collaboration between countries, authors, and institutions in-depth, analyzed the cited journals in a double-graph overlay, and introduced alluvial diagrams to analyze the scientific categories of the articles. In addition, we conducted a comprehensive analysis of keywords and co-cited literature networks.

During the course of this research, we discovered intriguing findings, including a substantial number of citations to articles in the field (80,408) and specifically in 2021 (19,302). Notably, prominent publications such as the Journal of Clinical Endocrinology & Metabolism, OBESITY, and Diabetes Care underscored the significance and relevance of our research topic. Therefore, the objective of this review is to gain a comprehensive understanding of the dynamic changes underlying metabolic dysfunction in PCOS, to assess the notable research advancements made over the past decade as well as the persisting challenges, and to offer valuable insights for future investigations.

2 Materials and methods

2.1 Data sources and retrieval strategies

In this study, we have used the Web of Science Core Collection (WoSCC), the world's most comprehensive and influential scientific literature database, to conduct a literature search on metabolic characteristics of polycystic ovary syndrome and collected related articles from the website published till December 25, 2021 (17). The keywords for searching the articles was: TS =((((PCOS)OR ("Polycystic ovary syndrome"))AND (((metabolic*) OR (metabolism*) OR(metabolite*)))). Since this study didn't include any animal or experiments, ethical consent was not required. The inclusion criteria were limited to "article" and "review", the language as "English", and the duration from 2012 to 2021, with the aim of selecting a specific subject and study purpose while also standardizing the language for analysis in the follow-up process. Other literature types, non-English articles, and articles outside this period were excluded. We completed relevant searches and investigated all retrieval records of articles in different formats in plain text format on the same day to generate source files for subsequent use by the different bibliometric analysis software. From the exported articles, we documented the name of the authors, study source, title, keywords, and cited references to avoid errors in retrieving articles at different times.

2.2 Methods and statistical analysis

We used the linear regression algorithm of SPSSPRO "Scientific Platform for Professional Statistical Services" to analyze the publication dates of all studies. SPSSPRO is a new online data analysis platform developed by a data analysis team in China, which is different from the traditional Statistical Product Service Solutions (SPSS) and Statistical Analysis System (SAS). Its benefits include simple operation, powerful data processing ability, and accessing various analysis algorithms. There is no record of researchers using SPSSPRO for analytical studies to date. A linear regression algorithm is a statistical analysis method that uses regression analysis in statistics to determine the interdependent quantitative relationship between two or more variables. It is widely used and expressed in the form of y = w'x+e, where e represents the normal distribution of errors with a mean value of 0. The regression analysis conducted in this study focuses on a single independent variable, namely the publication date, and one dependent variable, which is the number of literatures. The relationship between these variables can be approximated by a linear function, known as a univariate linear regression analysis. The specific algorithm employed is based on the method of least squares regression, ultimately resulting in the derivation of a linear regression model (18, 19). The results of the analysis showed that the fitting R^2 of the model was 0.949, which indicated that the model was excellent. Therefore, the model met the requirements of our study. For collinearity of variables, all large variance inflation factor (VIF) values were less than 10, suggesting that the model has no multicollinearity problem and the model was well constructed. The formula of the model is as follows: Y = −49172.036 + 24.582* year, Y represents the cumulative number of studies within a specific year.

We used VOSviewer1.6.16, Scimago Graphica1.0.15, and CiteSpace5.8.R1 software to analyze the author, source, title, keywords, cited references, and other details of the articles. VOSviewer is a scientific cartographic tool developed by Prof. Van Eck and Prof. Waltman from the Centre for Scientific and Technological Research of Leiden University, for visual bibliometric analysis, which is mainly used to analyze details such as co-authors, countries, and keywords (20, 21). Scimago Graphica, developed in

May 2021, is the latest software for browsing, filtering, and visualizing datasets with a simple drag-and-drop feature. Being a code-free tool, it is easy to use and has a fairly versatile application. The CiteSpace was developed by Professor Chaomei Chen of Drexel University in 2004. It can be used to analyze and measure the co-occurrence frequency of key information (keywords, author, region, and citation) in the articles and present the development trend of related studies (22). We used the VOSviewer version 1.6.16 to gather information about the places from where the studies were reported, keywords used in the studies, the total number of publications, quantity, and quality of cited documents, the collaboration between research workers affiliated with different institutions, and the clustering of studies' keywords. The results obtained from the VOSviewer were further processed using Scimago Graphica 1.0.15 to obtain more sensitive and comprehensive results. The CiteSpace 5.8.R1 was used to understand the pattern of appearance of the keywords and geographical time zones of the published articles. These software applications ensured that the research and development trends of metabolic dysfunction-related PCOS were analyzed from all perspectives.

3 Results

Bibliometrics is widely used in the biomedical field and provides a reliable basis for diagnosing and treating various diseases to reasonably predict their future development trend. In this study, through a comprehensive investigation and sorting of the field of PCOS metabolic dysfunction (2012–2021), we found that with an increase in the number of related publications in recent years, the United States has always been the leading contributor to this field, while the cooperation between authors and institutions is complicated. We also identified the four most stable scientific categories in the field over the last decade by introducing alluvial diagrams. Additionally, proteomics, metabolomics, and gut microbiota were the latest research hotspots in this field. Our results are summarized in detail below.

3.1 Literature analysis and prediction

We retrieved 3972 articles from WoSCC and used the linear regression algorithm of SPSSPRO. This statistical analysis suggested that the number of articles published each year, except for the years 2013–2014 and 2016–2017, where there was a slight downward trend, the number of published articles in the last 10 years had increased, especially in the last 5 years. Based on the actual number of articles published each year, the corresponding predicted values were assigned according to the linear regression model formula (Figure 1A). In addition, the number of publications for the next five years was predicted from the formula shown in Figure 1B. It was seen that the studies on PCOS metabolic dysfunction would continue to increase in the next five years. Although the prediction obtained from the linear regression model might deviate from the actual number of publications in the future, it fairly reflects the research importance on this syndrome. The Web of Science (WOS) citation report showed that the articles were cited 80,408 times; the citation frequency of articles in the past decade was increasing per year. Interestingly, studies published in 2021 were most frequently cited for 19,302 times (Figure Supplementary Image).

3.2 Contributions and cooperation among the top 15 producer countries

We retrieved 3972 articles published in 95 countries around the world. Among these countries, the United States was the topmost contributor with 809 papers; the least number was published by Iraq and Estonia (with only five articles each in the past decade).

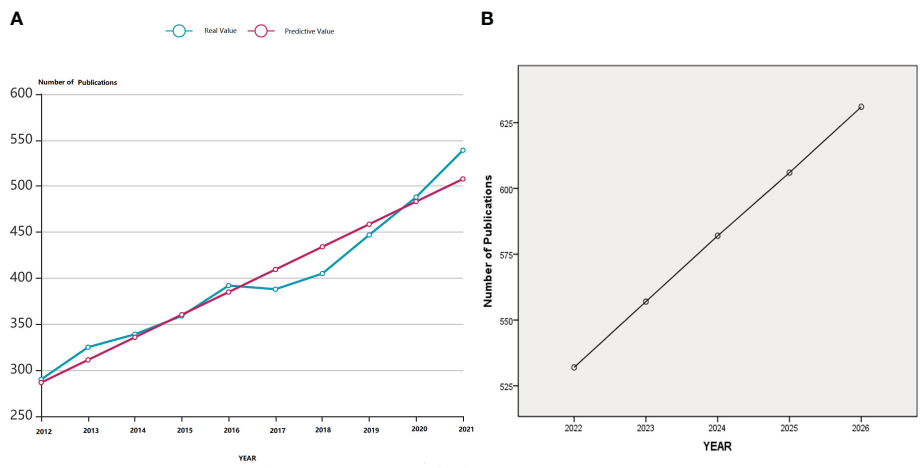

FIGURE 1
(A) A linear regression model showing the number of articles published and the number of articles that were predicted to be published in the last decade on metabolic dysfunction in PCOS. **(B)** Linear regression model for predicting the number of publications in the field of metabolic dysfunction in PCOS in the next five years.

VOSviewer and Scimago Graphica were used to select the top 15 countries that had collaborations to analyze their publication volume, the total collaborative work between them (Figure 2A), their citation frequency, and cooperation between countries (Figure 2B). In Figure 2A, each circle represents a country, and the size of the circle corresponds to the volume of its publications. This indicates that China (774), Italy (328), Britain (238), and Australia (201) have the most publications in the world after the United States. The color of the circle implies the total intensity of cooperation between the countries, with light blue and red colors representing the lowest and the highest intensity, respectively. Therefore, the total intensity of cooperation between countries increases clockwise from Poland (52) to the United States (342). In Figure 2B, the size of the circle signifies the total citations. The top 5 countries with maximum citations are the United States (27,613 times), Italy (10,623 times), China (10,201 times), the United Kingdom (9,281 times), and Australia (8,496 times). The color of each circle indicates the countries having several collaborations; blue indicates the fewest, and red indicates the most. Obviously, in the past decade, China (10) partnered least with other countries to study metabolic characteristics in PCOS, while the United States (14), Italy (14), Australia (14), Germany (14), the United Kingdom (14), and the Netherlands (14) had close cooperation.

3.3 Authors and institutions of relevant articles

About 16,088 authors were involved in these studies. Based on co-author analysis by VOSviewer, we defined "core authors" as those who have published at least 15 papers that were cited at least 700 times. Asemi and Zatollah, the two authors, contributed the most in this field

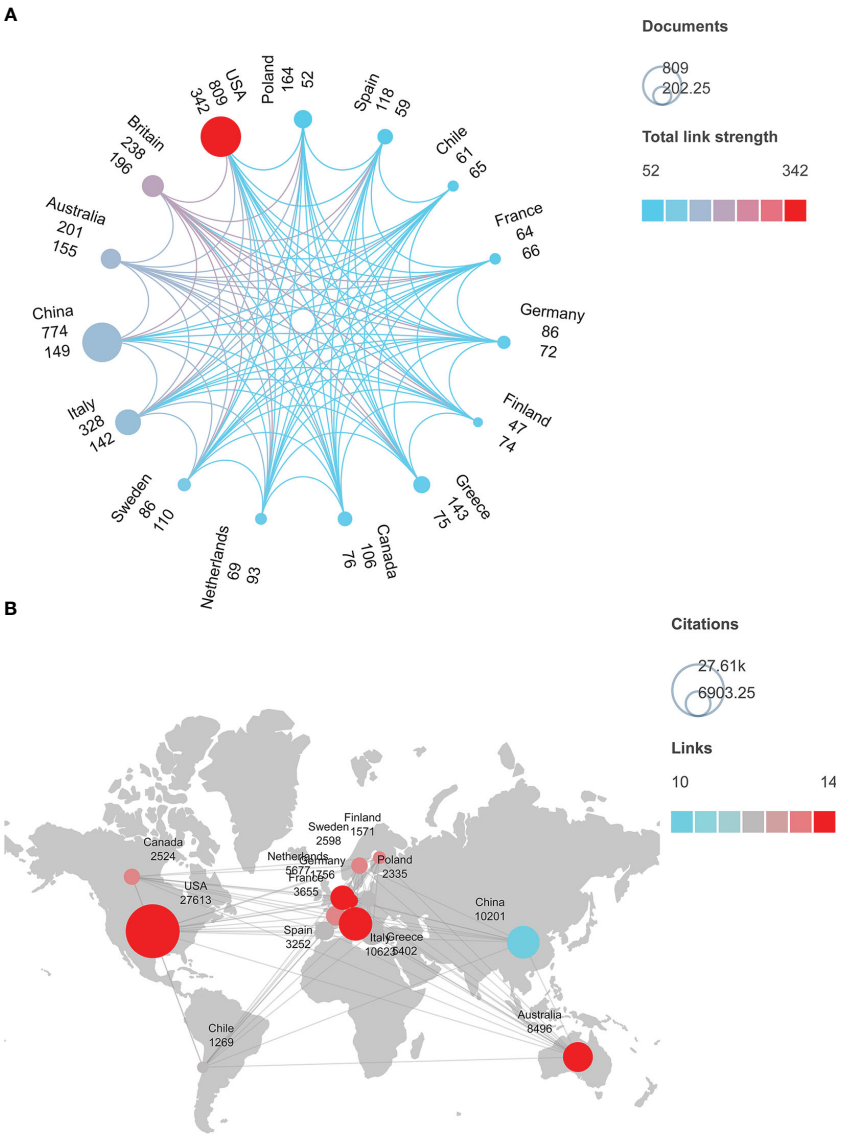

FIGURE 2
(A) Publication volume and cooperation intensity (collaboration network) of 15 countries in the field of metabolic dysfunction in PCOS. **(B)** Citation frequency and the number of collaborations among 15 countries that studied the metabolic dysfunction in PCOS.

with 49 articles published in the last decade; followed by Elisabet Stener-Victorin (44) and Helena J. Teede (39). The articles by Legro and S. Richard were the most frequently cited (3,527 times). The publications by Andrea Dunaif (2364) and Diamanti-Kandarakis and Evanthia (2138) ranked second and third, respectively. In addition, Teede, Helena J., and Moran, Lisa J. have the most cooperative relationship among them. Asemi, Zatollah, and Lerchbaum, Elisabeth are all independent authors without any cooperative relationship with the other 15 authors (Figure 3A). A total of 3,635 different institutions conducted studies on PCOS metabolic dysfunction. We used VOSviewer and Scimago Graphica to include only the institutions that had published at least 50 articles that were cited <1100 times; 10 different institutions were thus selected (Figure 3B). In Figure 3B, the size of the circle represents the number of published articles, and the color represents the frequency of their citations; ten institutions are shown with the average value of citations, and the intensity of the cooperation between institutions is indicated by the width and colors of the lines joining them. Monash University (97) had the most published articles, followed by Shanghai Jiao Tong University (85) and the University of Athens (61). The articles published by the University of California, Los Angeles, were the most cited ones (4,272), while Monash University had the highest average citations (145.0479). Monash Univ and Monash Hlth had the closest cooperation, while Kashan University of Medical Sciences was the only one among the ten institutions that did not collaborate with other institutions (Figure 3B).

3.4 Cited journals and scientific category analysis

We assessed the impact of highly cited journals in this research field through CiteSpace. The publications, from January 2020 to December 2021 and January 2012 to December 2021 were analyzed; the first map included the articles published in journals in 1-year out

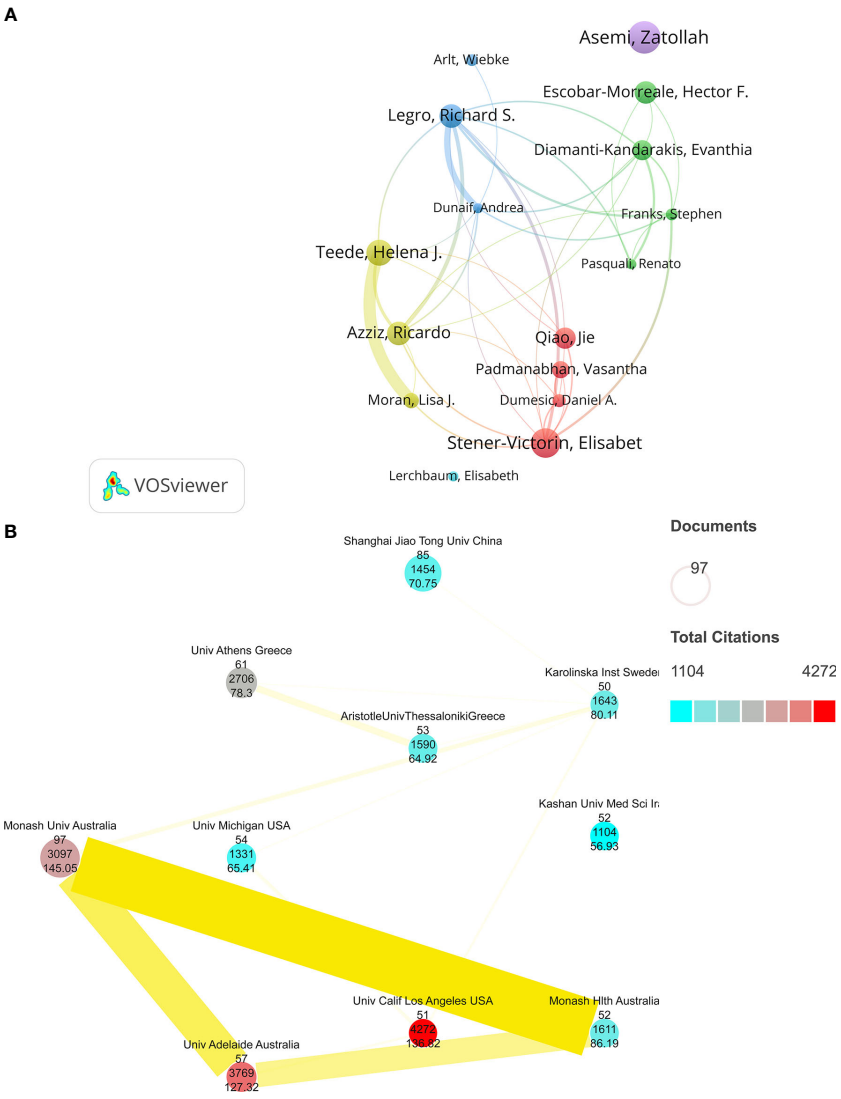

FIGURE 3
(A) A collaborative network of co-authors to study the effect of metabolic dysfunction in PCOS. **(B)** A collaborative network of national institutions on metabolic dysfunction in PCOS.

of which we chose the top 50 most cited journals, and the second map included the keywords used for each graph clustering, and finally, the two maps were superpositioned (Figure 4A). Each node represents a periodical, the size of the circle represents the number of times the journal was cited, purple halo represents the journal has better centricity (> 0.1). The connection between the two nodes indicates that the two journals under a topic cluster had cooperated for the study; the thick red line attachment suggests the last two years still included the clustering of the theme of the journal. CiteSpace grouped the 50 cited journals into 28 clusters and showed the 8 clusters that collaborated on the study (Figure 4A). The #0 indicates that metabolic biomarkers were the largest cluster group, which was also the most concerned topic cluster in many journals in the last two years. The most cited journal was The Journal of Clinical Endocrinology & Metabolism (3471 times). The cluster containing the least number of journals was OBESITY (7#), with only three journals. Obesity and International Journal of Obesity were the only two journals focused on obesity in the last two years. The topic cluster that received the least attention within all journals in the past two years was cardiovascular disease risk (#3), and only Diabetes Care covered this topic (Figure 4A). The classification of an article in the Science category was based on the SC field of the Web of Science text set; the Science category can help researchers readily learn the latest work in this research field (23). To show the evolution of scientific categories in the research field related to the metabolic abnormalities in PCOS in the past decade and find out which scientific categories were the most suitable for this field, the alluvial diagram of scientific categories was drawn according to the time trend by CiteSpace. Starting with the relevant science category topics in 2012, the impact map evolves into a pattern of multiple streams flowing smoothly over time through different splintering and merging of science category topics each year until it ends in 2021. In this process, different science categories were merged again. For example, after four years of relatively stable flow evolution, science-technology and other topics were interrupted during the middle years and were finally merged into multidisciplinary sciences. Four of the oldest but continued scientific categories were identified. Over the past decade, the divisions and mergers of each category were color-coded——blue for ZOOLOGY, orange for IMMUNOLOGY, purple for TOXICOLOGY, rose-red for RESPIRATORY SYSTEM. Among these, ZOOLOGY, IMMUNOLOGY, and TOXICOLOGY showed many overlapping splits and mergers over time, proving that the three scientific categories are closely related (Figure 4B).

3.5 Multi-angle analysis of keywords used in the published articles and co-citation network

To assess the main research contents and the change in research perspective over time about the metabolic dysfunctions in PCOS, VOSviewer and CiteSpace were used to draw different visual clustering maps of keywords used in the published articles. After removing redundant keywords such as “PCOS”, “women”, and “patients”, the clustering network visualization and frequency heat map of keywords were created on VOSviewer (Figures 5A, B).

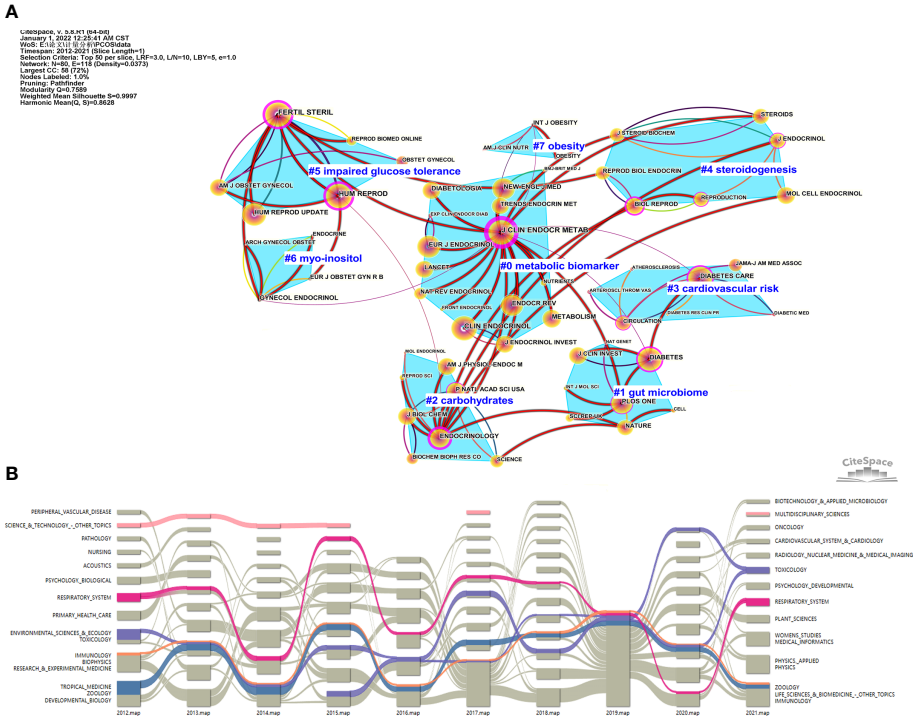

FIGURE 4
(A) Double graph overlay of journals that published articles on metabolic dysfunction in PCOS. **(B)**. The impact map of scientific categories related to the metabolic dysfunction in PCOS.

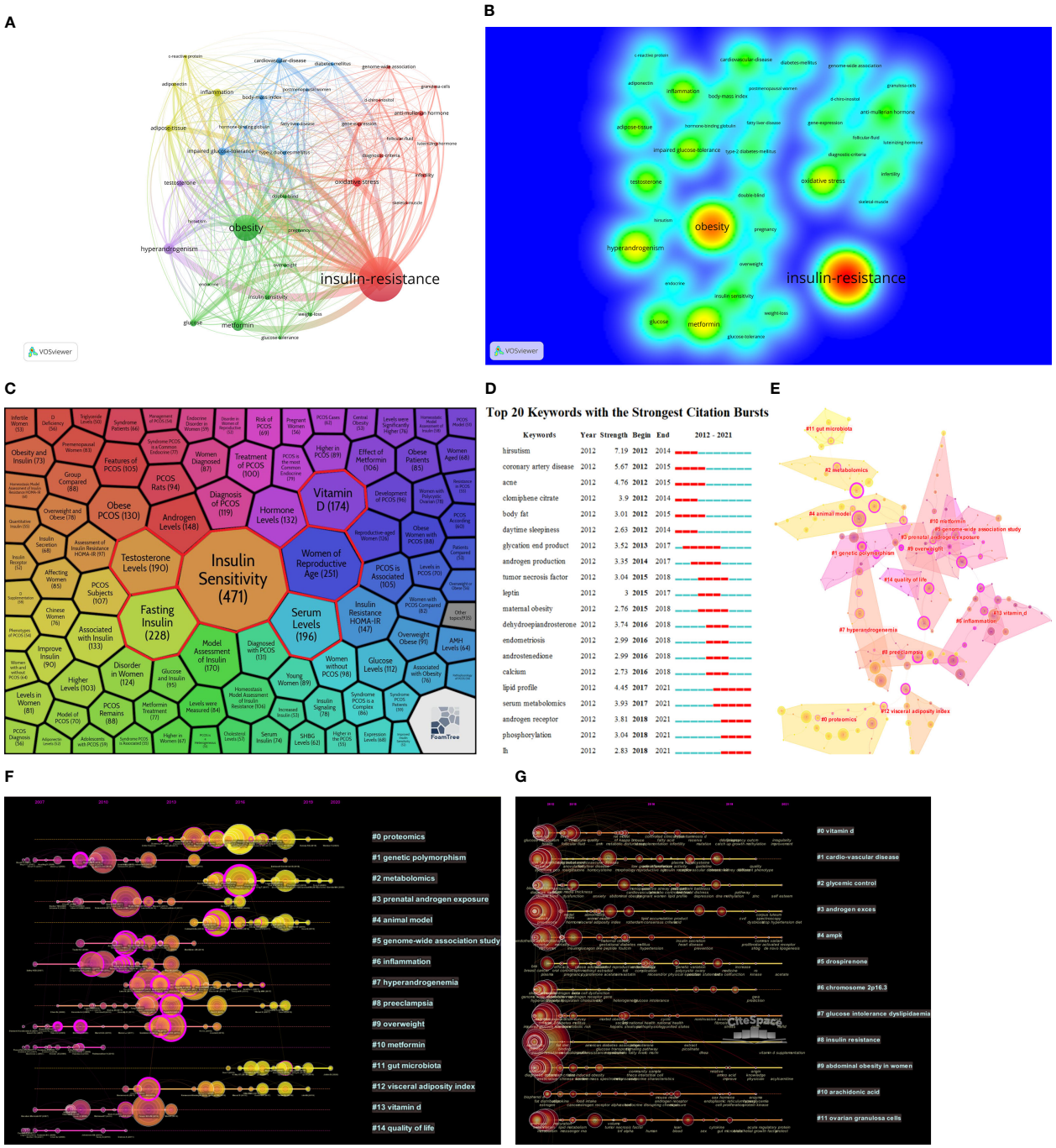

FIGURE 5
(A) Network visualization of keywords used in the published articles related to the topic of metabolic dysfunction in PCOS. **(B)** Heat map of keywords among the published articles on metabolic dysfunction in PCOS. **(C)** Keywords used in the articles in the field of metabolic dysfunction in PCOS. **(D)** Keywords' appearances in articles related to the study on metabolic dysfunction in PCOS. **(E)** Co-cited network cluster analysis in the domain of metabolic dysfunction in PCOS. **(F)** Co-cited network time zones on metabolic dysfunction in PCOS. **(G)** Time zone map of keywords used in articles on metabolic dysfunction in PCOS.

CiteSpace is connected to the carrot2 system to analyze the key topics and associated top six frequently occurring words; these are highlighted in red boxes and are as follows: Insulin Sensitivity, Women of Reproductive Age, Fasting Insulin, Serum Levels, Testosterone Levels, and Vitamin D (Figure 5C). CiteSpace software was used to complete the analysis of the appearance of keywords used in the studies of metabolic dysfunction in PCOS (Figure 5D).

CiteSpace software was used to analyze the cluster diagram and time area diagram of co-citation. Total cited articles published over time appear at the top of the image. Different nodes at each level represent the same category; the length of the horizontal line and fore and aft ends are the duration for that category. Each node represents the number of citations, and the size of the peripheral node (purple circles) represents the intermediate centricity (in

terms of numerical value). In short, the beginning, popularity, and decline periods of research categories in this field can be obtained by observing the time area chart (24). The co-citation analysis in this research field is divided into 14 different categories (Figures 5E, F). Genetic polymorphism (#1) was the most persisting one, which means that the research on metabolic dysfunction during PCOS was mostly based on genetics. Proteomics (#0), metabolomics (#2), and gut microbiota (#11) are the three key topics that were the interest of active research. According to the time zone map of keywords (Figure 5G), related studies were conducted continuously in the past decade for other categories except for #1 cardiovascular diseases, #6 chromosome 2p 16.3, and #10 arachidonic acid. This indicates that the pathogenesis of PCOS remains unclear, and further studies from different aspects are needed.

4 Discussion

The article analysis software can do scientometric analysis. All the documents on a particular subject under a limited period that contains a variety of effective information are analyzed and quantified in an intuitive way to summarize this effective information. It reveals whether a scientific topic was a hot spot for a certain period in the past, and it is then used to predict the next development trend on that topic. The valid information includes the number of publications and citations and the impact of publications based on the involvement of the countries, institutions, authors, journals, scientific categories, and keywords.

4.1 General information study

The comprehensive results indicate that the number of publications and citation frequency on the subject of metabolic dysfunctions in PCOS is on the rise, and it is predicted that it will continue to be an active research topic for the next five years (Figures 1A, B; Figure Supplementary Image). The United States, China, and Italy are prominent contributors to this research field, accounting for 48.11% of the total number of papers published. The papers published by the United States are the most cited, and they had the most number of research collaborations with other countries, suggesting that the United States had conducted in-depth research in this field (Figures 2A, B). Among the top 10 selected institutions, American institutions cooperated mostly with the Australian research institutions; only one institution was from a developing country, while the rest were from developed countries, implying that developing countries started delayed research in this field and need to improve their research outputs (Figure 3B).

The assessment and ranking of cited journals can help researchers quickly find the journal suitable for their article submission. It will also become easy to find the topics that most journals in this field are interested in publishing, such as metabolic biomarker was the most discussed topic in this field from 2020 to 2021. Biomarker studies are breakthrough studies that can explore the pathogenesis at the molecular level and provide early warning for diseases, including PCOS (a chronic endocrine disorder) and the investigation of its pathogenesis (Figure 4A). The results of the scientific category analysis of the published articles showed a dynamic evolution process of all the scientific categories in the last ten years, and we found the four most stable and suitable scientific categories in the field of PCOS, which were also the focus of the difficult long-term research in this field (Figure 4B).

4.2 Emerging trends, hotspots, and frontiers

The utilization of literature co-citation networks and keyword clustering can unveil the underlying research structure in the field of metabolic dysfunction associated with PCOS. Through careful examination of these analyses, a wealth of valuable information can be gleaned, including but not limited to luteinizing hormone, serum metabolomics and proteomics, insulin resistance, steroids, multiple complications and therapeutic options. These findings allow for identification of emerging trends and research hotspots within the study of PCOS metabolic dysfunction. In subsequent sections we will discuss their profound significance for this area of research as well as potential implications for future directions.

4.2.1 Gonadotropin research

Abnormal gonadotropin secretion, as one of the common characteristics of PCOS, is a key reference for the clinical diagnosis of the disease. Among them, LH is usually elevated in PCOS, while FSH is mostly at a low level, and its intrinsic pathogenesis is mostly related to neuroendocrine defects (25). The synthesis of LH and FSH is closely related to the GnRH pulse frequency. Experiments on ovaries have shown that the hourly exogenous GnRH pulse frequency can accelerate the secretion of LH, while a 3–4 h pulse frequency is beneficial to the secretion of FSH (26, 27). Other studies on patients with PCOS found that LH increased in response to GnRH, but FSH had no significant response, which was the same as the spontaneous LH pulse frequency increase in PCOS patients (23). Therefore, finding an effective treatment to improve the gonadotropin abnormalities in PCOS patients is necessary. In the latest pharmacological experimental study, saffron petal extract and petal anthocyanin were confirmed to reduce luteinizing hormone and androgen levels in PCOS mice (28). It is confirmed that there is dysregulation of metabolism and oxidative phosphorylation pathways in oocytes of PCOS patients, and metformin can improve such dysregulation and enhance the developmental ability of oocytes in patients with PCOS (29, 30).

4.2.2 Proteomics and metabolomics research

Among 14 different clusters co-cited in studies and were hot topics among researchers accounted #0 from the proteomics field, #2 from metabolomics (Figures 5E, F). Various “omics” studies emerging in recent years are tools that analyze large-scale data to better interpret biological research (31). Notably, proteomics and metabolomics have been successively applied to the study of various diseases (32). Proteomics is mainly used to quantitatively analyze protein data obtained from the cells or body fluids using the mass spectrometry method (33). Proteomics applied in the study of

metabolic disorders in PCOS was largely to screen out biomarkers (proteins) and understand this disease's pathological mechanism (34, 35). Metabolomics, a scientific approach, used non-invasive and specific methods to identify potential biomarkers for PCOS in recent years and also elucidated disease pathology (36, 37). Compared with proteomics, which can only show the existence of metabolic disorders in the organism, metabonomics can detect the slightest biochemical changes in the pathophysiology of PCOS, improving the diagnosis methodology for PCOS (38, 39).

4.2.3 Insulin resistance and oxidative stress research

Carrot2 and VOSviewer are also used to identify relevant keywords and find connections between them (Figures 5A, C). Prominent words that appear in the articles related to PCOS include insulin resistance, obesity, and androgen overload. The cause of insulin resistance has not been accurately determined, but it may be related to the accumulation of certain lipid metabolites (diacylglycerol and/or neuramide) in skeletal muscle and the oxidative stress-induced pro-inflammatory state (caused by reactive oxygen species). Previous bibliometric analyses have suggested that oxidative stress may exacerbate PCOS and insulin resistance by impairing glucose uptake in musculoskeletal muscle and insulin secretion in pancreatic β-cells, ultimately leading to elevated androgen levels and disruption of the follicular intracellular environment (15). Oxidative stress is caused by excessive production of reactive oxygen species when the balance between pro-oxidant and anti-oxidant in the human body is impaired. Excessive production of reactive oxygen species can cause DNA damage, endothelial damage, and apoptosis of ovarian epithelial cells (40–42). Oxidative stress, induced by ovarian torsion/detorsion (T/D), damages ovarian tissue, including a reduction in follicles, apoptosis of granulosa cells, and an increase in atretic bodies. Excess levels of reactive oxygen species in the ovarian torsion/detorsion process are accompanied by an increase in the Bcl2-Associated X (Bax) protein and a decrease in the B-cell lymphoma-2 (Bcl-2) protein, resulting in ovarian tissue damage (43). Other studies have confirmed that oxidative stress can reduce superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity and result in ovarian T/D, damaging ovarian tissue (44, 45). Additionally, cyclophosphamide can induce apoptosis by increasing reactive oxygen species and causing oxidative stress in ovarian cells, thus, inducing them to initiate programmed cell death and resulting in ovarian tissue damage (46, 47). High levels of various oxidative stress markers were also observed in PCOS, suggesting that oxidative stress might be involved in the pathophysiology of PCOS (48). Some studies have shown that an increase in the level of advanced glycation end (AGE) products in women with PCOS can increase the production of reactive oxygen species, thus, causing oxidative stress in ovarian cells and leading to various negative effects on cell metabolism (49, 50).

4.2.4 Pathological steroid research

As a naturally occurring stereoisomer of inositol, D-chiro-inositol has the potential to safely mitigate multiple metabolic conditions such as PCOS-associated insulin resistance (51–53). The abnormal ratio of D-chiro-inositol to inositol in the ovaries is a common feature of PCOS, and this abnormal ratio might lead to the formation of pathological steroids in PCOS (54). The higher the ratio, the higher the aromatase activity for producing estrogen, while a lower ratio leads to the production of androgens, which are closely related to the generation of PCOS steroids (55). D-chiro-inositol can directly affect the regulation of the steroidase gene in ovarian granulosa cells. This is achieved by reducing the Cytochrome P450 side-chain cleavage (CYP450scc) genes and the aromatase CYP19A1 (56). Studies on steroidogenesis of PCOS have focused on genetics and genes, especially the correlation between ovarian steroids and hyperandrogenemia, mainly including the CYP11A1 gene, the CYP17 gene, the CYP19 gene, and the androgen receptor (AR) gene. These genes mainly encode enzymes or regulate androgen levels in ovarian steroid production (57). Some studies have shown that some loci are related to steroid production in genome-wide association studies (58). DENND1A is not only closely related to PCOS hyperandrogenemia but also influences steroid production by affecting the transcription of CYP11A1 and CYP17 (59). Luteinizing hormone chorio gonadotropin receptor (LHCGR) overstimulates LH by enhancing the expression of the LH receptor, thus, affecting PCOS steroid production (60). Additionally, the elevated level of AGE products in PCOS patients might cause steroid production (49). The specific effects of advanced glycation end products on PCOS steroid production are under investigation. However, its interaction with the membrane receptor late glycation end product receptor (RAGE) can affect the mRNA expression levels of acute regulation of steroidogenesis (StAR), CYP17A1, and 3β-hydroxysteroid dehydrogenase (3β-HSD). The AGE-RAGE signals can affect the formation of PCOS steroids (49).

4.2.5 Research on multiple complications and potential treatment regimens

In a comprehensive evaluation of endometrial function in PCOS, obesity and insulin resistance along with endometrial disorders can result in miscarriage, pregnancy complications, and affect glucose transport; lifestyle changes and taking metformin can improve the function of gestational endometrium in PCOS patients. As for the severely obese person, bariatric surgery is an optimal option (61). Testosterone level indicates hyperandrogenemia; hypertrichosis is the most used clinical diagnostic criterion for hyperandrogenism (62, 63). Excessive androgen affects the metabolic function in PCOS, resulting in long-term metabolic complications, mainly type 2 diabetes, non-alcoholic fatty liver disease, and cardiovascular diseases (64, 65). Adiponectin is a circulating protein produced by adipocytes that are negatively correlated with metabolic disorders. Recently, its collateral homolog C1q tumor necrosis factor-related protein 6 (C1QTNF6) has been shown to intervene in the pathogenesis of PCOS by affecting the inflammatory response in granulosa cells (66, 67).

In addition, several studies have shown that the intestinal microbial community in PCOS patients is different from that of healthy people, especially in their metabolic functions (68–70). The health status of an individual's metabolism depends on the quantity and diversity of gut microorganisms (71, 72). Thus, metabolic dysfunction in PCOS patients can be treated by improving their gut microbiome. For example, improving intestinal flora, regulating

bile acid metabolism, or increasing IL-22 levels can be used for the treatment of PCOS (73). Currently, therapies that can significantly improve the metabolic dysfunction in PCOS patients include fecal microbiota transplantation (61), probiotics (74), and prebiotics (75). It should be noted that while research on the gut microbiota of PCOS patients has been conducted, it remains in its early stages and much of the data and findings are derived from rodent studies (76–78). Therefore, there is currently insufficient evidence to fully elucidate the human gut microbiota and pathogenesis of PCOS, necessitating further human-based studies in this area to better understand the pivotal role played by gut microbiota in PCOS patients. It is necessary to conduct more detailed and systematic studies in this field, focusing on various potential complications of PCOS and the underlying etiology. This can improve the accuracy of early diagnosis and the effectiveness of the treatment of PCOS.

4.3 Practical implication

The practical significance of this study encompasses three main aspects: (1) Junior researchers can efficiently acquire essential knowledge on PCOS metabolic dysfunction by studying the research findings of prominent authors and institutions presented in this paper, thereby enhancing their scientific research proficiency and capabilities. (2) By utilizing journal overlay analysis, we can identify relevant research hotspots and suitable journal titles, which will greatly facilitate future scholars working in the same field. (3) Through co-citation analysis and keyword co-occurrence analysis, we can effectively identify the current research trends and unresolved issues related to PCOS metabolic dysfunction. This enables us to formulate targeted policies as future research goals and allocate financial resources towards practically addressing the challenges faced within this research domain.

4.4 Limitation

Although this study provides the first comprehensive bibliometric analysis of metabolic dysfunction in PCOS, there are several limitations that may impact its findings. Firstly, the data used in this article were obtained solely from the WOSCC database, which may have excluded some valuable information. Secondly, only articles and reviews were included while political and social publications such as editorials and books were not considered. Thirdly, although our search strategy was designed to be comprehensive, it is possible that other relevant keywords were overlooked which could affect our results. Lastly, due to the vast vocabulary involved when manually combining synonyms errors are inevitable. However, given the large amount of data generated by numerous publications included in this study any potential bias should be minimal.

5 Conclusion

Several bibliometric analysis software was used to analyze the status of the field of research on metabolic dysfunction in PCOS from different perspectives. This study not only contains information on countries, journals, authors, institutions, and scientific categories but also specifically illustrates the internal relationship between keywords in different clustering groups and their influence on this field. Furthermore, we found that the main research topics to be investigated in the future for a better understanding are lipid profile, androgen receptors, phosphorylation, luteinizing hormones, proteomics, metabolomics, and gut microbiota. This bibliometric analysis might provide more answers to researchers involved in this field.

Author contributions

Conceptualization: TC and YX. Validation: JR, YX, and TC. Data curation: YX and ZC. Writing—original draft preparation: YX. Writing—review and editing: TC. Visualization: JR. Supervision: JR and YX. Project administration: JR and TC. Funding acquisition: JR and TC. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1245719/full#supplementary-material

SUPPLEMENTARY FIGURE
Trends in citation frequency of articles related to metabolic dysfunction in PCOS over the last decade.

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A novel model based on necroptosis to assess progression for polycystic ovary syndrome and identification of potential therapeutic drugs

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Background: Polycystic ovary syndrome (PCOS), a common endocrine and reproductive disorder, lacks precise diagnostic strategies. Necroptosis was found to be crucial in reproductive and endocrine disorders, but its function in PCOS remains unclear. We aimed to identify differentially diagnostic genes for necroptosis (NDDGs), construct a diagnostic model to assess the progression of PCOS and explore the potential therapeutic drugs.

Methods: Gene expression datasets were combined with weighted gene co-expression network analysis (WGCNA) and necroptosis gene sets to screen the differentially expressed genes for PCOS. Least absolute shrinkage and selection operator (LASSO) regression analysis was used to construct a necroptosis-related gene signatures. Independent risk analyses were performed using nomograms. Pathway enrichment of NDDGs was conducted with the GeneMANIA database and gene set enrichment analysis (GSEA). Immune microenvironment analysis was estimated based on ssGSEA algorithm analysis. The Comparative Toxicogenomics Database (CTD) was used to explore potential therapeutic drugs for NDDGs. The expression of NDDGs was validated in GSE84958, mouse model and clinical samples.

Results: Four necroptosis-related signature genes, IL33, TNFSF10, BCL2 and PYGM, were identified to define necroptosis for PCOS. The areas under curve (AUC) of receiver operating characteristic curve (ROC) for training set and validation in diagnostic risk model were 0.940 and 0.788, respectively. Enrichment analysis showed that NDDGs were enriched in immune-related signaling pathways such as B cells, T cells, and natural killer cells. Immune microenvironment analysis revealed that NDDGs were significantly correlated with 13 markedly different immune cells. A nomogram was constructed based on features that would benefit patients clinically. Several compounds, such as resveratrol, tretinoin, quercetin, curcumin, etc., were mined as therapeutic

drugs for PCOS. The expression of the NDDGs in the validated set, animal model and clinical samples was consistent with the results of the training sets.

Conclusion: In this study, 4 NDDGs were identified to be highly effective in assessing the progression and prognosis of PCOS and exploring potential targets for PCOS treatment.

KEYWORDS

polycystic ovary syndrome, diagnostic model, therapeutic drugs, necroptosis, gene signature

1 Introduction

Infertility affects 8-12% of couples of reproductive age and has become a global problem, among which female infertility accounts for 60-70% (1). Polycystic ovary syndrome (PCOS), a common endocrine reproductive disorder, affects 5-20% of women of reproductive age. It is characterized by various reproductive, endocrine, and metabolic features such as oligoovulation, infertility, hyperandrogenemia, obesity, hyperinsulinemia, type 2 diabetes mellitus and cardiovascular diseases (2).

As a highly heterogeneous metabolic syndrome, PCOS is affected by multiple factors, such as race and living environment, and the clinical phenotype varies (3). Recently, Qiao et al. reported the important role of gut microorganisms in the development and treatment of PCOS (4). Another study explored the classification and etiology of PCOS from a genomic perspective (5). Moreover, some groups attempted to investigate the PCOS bio-markers and pathogenic mechanisms using multi-omics and bioinformatics (6–8). Despite the efforts made in recent decades, the etiology of PCOS is not yet fully understood due to its complex pathogenesis and variability. Hence, the diagnosis of PCOS is principally based on the classical Rotterdam criteria and clinical symptoms (9). There is still a lack of precise diagnostic criteria, and many patients often suffer from misdiagnoses or missed diagnoses (10). Given this, it is essential to discover new biomarkers to facilitate timely diagnosis and intervention for PCOS.

Studies have shown that apoptosis of ovarian granulosa cells (GCs) could lead to oocyte apoptosis and follicular atresia, which may be one of the significant factors contributing to the development of PCOS (11–13). Oxidative stress is one of the major causes of GCs apoptosis and ovarian atresia. This process generates sustained high levels of reactive oxygen species, triggering an inflammatory response (14). Inflammation can induce necroptosis in GCs, which may be responsible for the development of PCOS (15).

Necroptosis, a new type of cell death, has recently been considered a potential factor for GC death in the preovulatory follicle (16). Necroptosis, also known as programmed necrosis, is a regulated necrotic cell death mediated by receptor-interacting protein kinase (RIPK) 1 and RIPK3. Features of necroptosis include early loss of cytoplasmic membrane integrity, leakage of cell contents, and swelling of organelles (17). RIPK1 and RIPK3 act as stress sensors to promote necroptosis of GCs (18, 19). It has been shown that the reticulophagy receptor CCPG1 mediated STAT1/STAT3-(p) RIPK1-(p) RIPK3-(p) MLKL pathway could trigger the necroptosis of GCs and be involved in the development of PCOS (12, 20). Moreover, high levels of ROS and immunoinflammatory factors could increase the expression of RIPK1 and RIPK3 in GCs (15). Furthermore, dysregulation of GCs and immune cells in PCOS patients also accelerates anovulation (21). Based on these findings, necroptosis may be a helpful diagnostic tool and therapeutic target for PCOS. However, the gene signatures associated with necroptosis and its mechanism in PCOS remain unclear.

In recent years, the combination of big data analytics and clinical data has become an effective approach for identifying diagnostic markers, exploring pathogenesis, and developing drugs (22). In this study, we utilized machine learning combined with clinical samples to identify necroptosis-related genes, constructed a diagnostic model to assess the progression of PCOS and explored the potential therapeutic drugs for PCOS. This work provides a theoretical basis for identifying new diagnostic markers and therapeutic targets for PCOS.

2 Materials and methods

2.3 Gene expression profile acquisition and differentially expressed genes screening

We searched the GEO database using the keywords "PCOS, Homo sapiens" and obtained 5 expression datasets: GSE95728, GSE114419, GSE106724, GSE137684, and GSE84958 (23). GSE95728, GSE114419, GSE106724, and GSE137684 were used as the training set and GSE84958 as the validation set. As the training

Abbreviations: PCOS, polycystic ovarian syndrome; GEO, gene expression omnibus; WGCNA, weighted gene co-expression network analysis; LASSO, least absolute shrinkage and selection operator; NDDGs, differentially diagnostic genes for necroptosis; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; GSEA, gene set enrichment analysis; PPI, protein-protein interactions; ROC, receiver operating characteristic curve.

set was from different batches and platforms, it was merged using the sva package version 3.36.0 in R version 3.6.1 to remove batch effects (24). The validation set was normalized with the voom function provided by the limma package (https://bioconductor.org/packages/release/bioc/html/limma.html) version 3.34.7 for model validation analysis (25). Details of the dataset are shown in Table 1.

The samples were divided into control and PCOS groups based on their source information and screened for differentially expressed genes using the limma package in R (version 3.6.1). False discovery rate (FDR)< 0.05 and $|\log_2 FC|>0.5$ were used as the threshold for differential screening.

2.2 WGCNA screening for PCOS-related modules and genes

Weighted Gene Co-Expression Network Analysis (WGCNA) could identify modules with similar expression patterns, analyze the linkage between the modules and the phenotype of the samples, map the regulatory network between the modules and identify key regulatory genes. To identify synergistic variation in gene sets, a modular clustering analysis of all genes was performed using the R package WGCNA (Version 1.61, https://cran.rproject.org/web/packages/WGCNA/) (26). In the WGCNA algorithm, the elements of the gene co-expression matrix were defined as the weighted values of the correlation coefficients of the genes. The weights were chosen so that the connections between the genes in each gene network follow a scale-free network distribution. The weighted value here was the softPower. Firstly, by setting a series of powers and calculating the squared correlation coefficients of the connectivity k and p (k) and the average connectivity for each power value, selecting the appropriate power value to make the connections between genes in the network obey the scale-free networks distribution. Secondly, based on the clustering and dynamic pruning method, the parameters (minModuleSize=50: each module contained at least 50 genes; MEDissThres=0.3: modules with similarity > 0.7 will be merged) were set to aggregate highly correlated genes into modules. Finally, calculating the module-phenotype correlation, where the phenotype referred to the disease status of the sample, and the modules closely associated with PCOS were selected as disease-associated genes.

2.3 Acquisition of differentially expressed genes for necroptosis

We obtained 159 necroptosis genes from published literature and intersected them with differentially expressed genes and PCOS modular genes to obtain differentially expressed genes for necroptosis (27). The Pearson correlation coefficients and significance between the differentially expressed genes were calculated based on the correspondence of the PCOS samples.

2.4 Functional enrichment analysis

Gene Ontology (GO) was used to analyze the biological function, pathway or cellular localization of differentially enriched genes. Kyoto Encyclopedia of Genes and Genomes (KEGG) was a functional enrichment database for identifying the pathways in which a gene set (multiple genes) might be significantly concentrated. Gene-Set Enrichment Analysis (GSEA) could determine whether gene sets differed significantly between two biological states. GO and KEGG enrichment analysis was performed with DAVID 6.8. Enrichment results were considered significant for each enriched item containing at least 2 genes and p<0.05. The top 20 items were selected for presentation (28–30). Moreover, the "cluster Profiler" package (Version 1.2.1) was used to conduct GSEA. Significant p-values were obtained using the "BH" correction, with p.adjust<0.05 considered a significant enrichment (31).

2.5 Protein-protein interaction network construction with GeneMANIA database

The GeneMANIA database (http://genemania.org/) was used to perform a PPI analysis of model genes and their 20 interacting genes to predict correlations between co-localization, shared protein structural domains, co-expression and pathways (32).

2.6 Diagnostic model construction

The Least Absolute Shrinkage and Selection Operator (LASSO) logistic regression model was used to further screen for PCOS-

TABLE 1 Gene expression profile information.

Dataset	Database	Sample information	Data source
GSE95728	GEO	7 controls and 7 PCOS patients	GPL16956
GSE114419	GEO	3 controls and 3 PCOS patients	GPL17586
GSE106724	GEO	4 controls and 8 PCOS patients	GPL21096
GSE137684	GEO	4 controls and 8 PCOS patients	GPL16956
GSE84958	GEO	23 controls and 15 PCOS patients	GPL16791
Necroptosis related genes	Published article	159 Necroptosis-related genes	

associated necroptosis genes with the glmnet package (version 2.0-18) in R3.6.1 language (33). The Riskscore model was constructed based on the gene regression coefficients and expression levels. The Riskscore was calculated as follows:

$$\text{Riskscore} = \sum \beta_{gene} \times Exp_{gene}$$

β_{gene} represents the LASSO regression coefficient of the gene and Exp_{gene} represents the expression level of the gene in each sample. To validate the model accuracy, the riskscore values for each sample in the validation dataset were calculated to plot the diagnostic ROC curves, box plots of the distribution of RiskScore and heat maps of the expression of the model genes, following the Riskscore formula and using the same regression coefficients.

2.7 The construction and visualization of nomogram

Nomogram was established based on multifactor regression analysis, which integrated multiple predictors and plotted them on the same plane at a certain scale to show the interrelationships of variables in the predictive model. Nomogram was constructed using the rms package Version 5.1-2 in the R (34). Calibration curves were used to assess the predictive power, decision curve analysis (DCA) to assess the clinical utility and the Concordance index (C-index) to evaluate the predictive power of the nomogram.

2.8 Immune microenvironment analysis

The ssGSEA algorithm was used to calculate individual immune cell enrichment scores using the R package GSVA (version 1.36.2). Using the Wilcoxon test and Spearman analysis, immune cells with a $p< 0.01$ were considered significantly correlated with PCOS (35).

2.9 Drugs prediction

To explore chemical drugs associated with diagnostic genes, we searched for the targeting drugs (Chemical Interactions) of the key genes as described above in the online CTD database (https://ctdbase.org/) (36). Drug-gene relationship pairs were selected to be supported by at least two references and PPI network construction was performed using Cytoscape software (version 3.4.0, http://chianti.ucsd.edu/cytoscape-3.4.0/) (37).

2.10 PCOS patients' samples collection and mouse model construction

The details of GCs collection from PCOS patients and controls are described in a previous publication (13). Briefly, follicular fluid was centrifuged at 250 x g for 10 minutes after removing the oocytes. The GCs layer was pipetted into a new centrifuge tube, washed, resuspended in PBS, centrifuged at 250 x g for 5 minutes and the cell was collected.

The PCOS mouse model construction details were described in a previous publication (13). Briefly, 3-week-old female mice were injected subcutaneously with DHEA (6 mg/100 g body weight) daily and controls were injected with an equivalent dose of sesame oil. Twenty-eight (28) days later, blood was collected from the eyes and ovarian tissue was collected for testing and analysis (28).

2.11 Gene expression validation with *real-time quantitative PCR*

Gene expression with qPCR has been previously described (12, 38). Briefly, mouse ovarian tissue and GCs from PCOS patients were extracted using the TRIzol method and reverse transcribed using a cDNA kit. The hub gene expression was measured using qPCR using Glyceraldehyde phosphate dehydrogenase (GAPDH) as an internal control. The mRNA expression levels were determined using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are listed in Table S1.

3 Results

3.1 Acquisition of differentially expressed genes for necroptosis

The four sets of gene expression profiles were combined into one dataset by removing the batch effect (Supplementary Figure 1, Figure S1). A total of 453 upregulated and 187 down-regulated genes were obtained (Figures 1A, B).

We performed WGCNA analysis using the full gene expression matrix to screen for genes associated with PCOS. Based on clustering and dynamic pruning methods, highly correlated genes were clustered and eventually integrated into 14 modules (Figures S2, 1C). As shown in Figure 1D, the negative correlation coefficients between the brown and cyan modules and PCOS are significant. In contrast, the positive correlation coefficients between the green and turquoise modules and PCOS are remarkable, so the four module genes (4421 genes in total) were considered closely related module genes for PCOS (Figure 1D).

A total of 12 differentially expressed genes for necroptosis were obtained by taking the intersection of necroptosis genes with differentially expressed genes and PCOS module genes (Figure 1E). The correlation coefficients between two of the 12 genes were further calculated and heat maps were created (Figure 1F).

3.2 Functional enrichment analysis

GO and KEGG enrichment analyses were performed to determine the functions and related pathways of the differentially expressed genes for necroptosis. We found 102 GO items and 31 KEGG pathways were enriched. The GO analysis showed enrichment of positive regulation of inflammatory response, defense response to virus, apoptotic process, regulation of interleukin-6 production, regulation of MHC class II biosynthetic process, positive regulation of tumor necrosis factor production,

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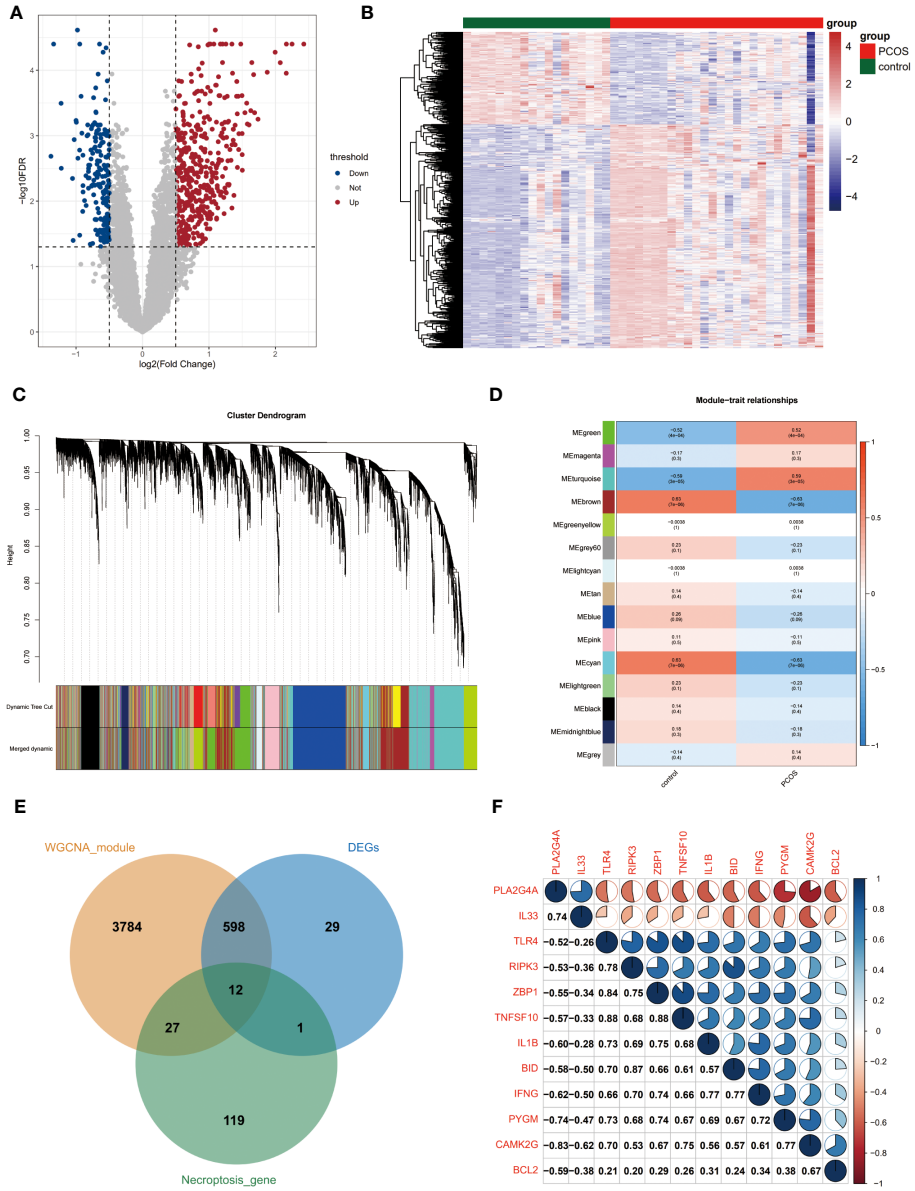

FIGURE 1
Acquisition of differentially expressed genes for necroptosis. **(A)** Volcano plot of differentially expressed genes. Red and blue dots indicate upregulated and down-regulated genes, respectively, and gray is the non-significant gene. **(B)** Heat map of differential gene expression. Blue is low-expressed genes and red is high-expressed genes. **(C)** Systematic clustering tree of genes and gene modules generated by dynamic shearing method. Different colors represent different gene modules. **(D)** Correlation analysis of WGCNA modules with the disease status of the samples. Numbers outside parentheses indicate correlation coefficients and those inside the parentheses indicate p-values. **(E)** The intersection of necroptosis genes with differentially expressed genes and PCOS module genes. **(F)** Correlation analysis of PCOS-associated differential necroptosis genes. Red sectors indicate negative correlations, while blue sectors indicate positive correlations. The larger fan area indicates the larger absolute value of the correlation coefficient. Numbers in the lower left corner indicate specific correlation coefficients. WGCNA, weighted gene co-expression network analysis.

and positive regulation of necroptotic process in biological process. Moreover, we observed an enrichment of cell membranes, cytoplasm in cellular components. Furthermore, cytokine activity and identical protein binding were enriched in molecular functions (Figure 2A). Most importantly, the KEGG mainly focused on necroptosis, HIF-1 signaling pathway, NOD-like receptor signaling pathway, inflammatory mediator regulation of TRP channels, NF-kappa B signaling pathway, and Natural killer cell-mediated cytotoxicity (Figure 2B).

3.3 LASSO algorithm to screen for differentially diagnostic genes for necroptosis (NDDGs)

Applying the LASSO Cox regression algorithm, we obtained four NDDGs BCL2, IL33, PYGM, and TNFSF10 in the training set, which was used as a necroptosis signature (Figures 3A, B). Furthermore, we constructed RiskScore model with the corresponding regression coefficients of NDDGs. The receiver operating characteristic curve

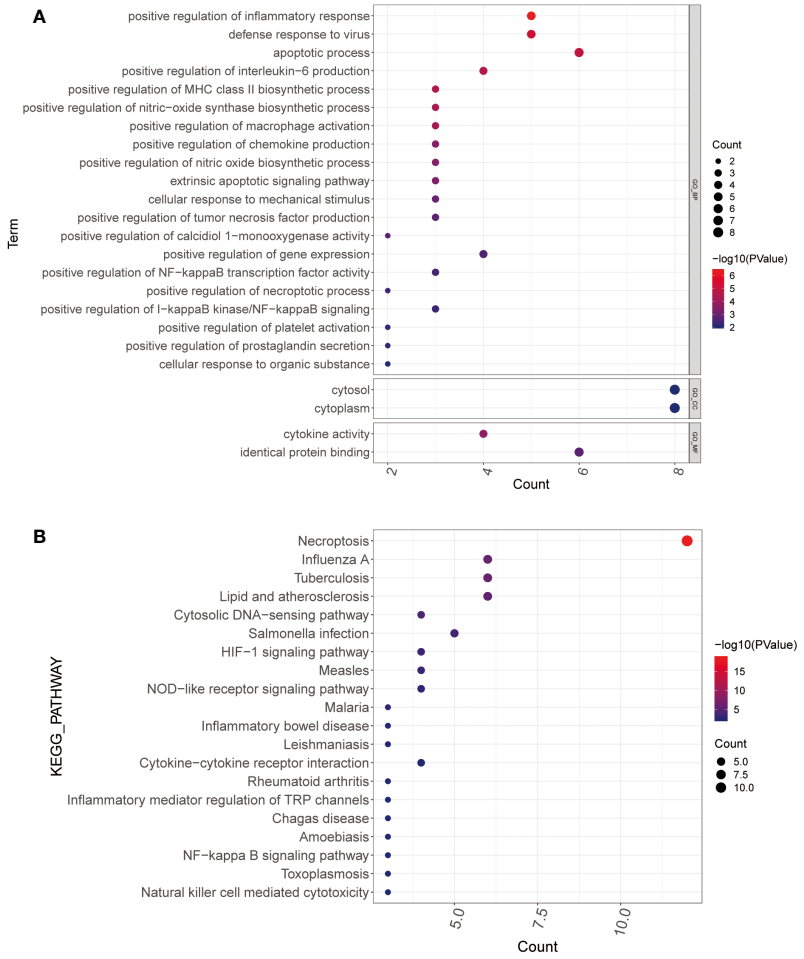

FIGURE 2
Functional enrichment analysis. **(A, B)** GO **(A)** and KEGG **(B)** analysis of PCOS-associated differential necroptosis genes. The color blue to red indicates the significance from less to more, and the bubble size indicates the number of enriched genes GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes.

(ROC) showed that the area under the curve (AUC) was 0.94, indicating an excellent disease prediction effect (Figure 3C). Moreover, the RiskScore was significantly higher in PCOS group than in control, which evidenced the accuracy of the model (Figure 3D). Furthermore, we have illustrated the relationship between the expression of the NDDGs and the RiskScore by using a heat map (Figure 3E). More importantly, we re-confirmed this model's accuracy with the same method in the validation set (Figure S3). All the results proved that the model has a good prediction effect.

3.4 The construction of a nomogram and ROC curve

To validate the diagnostic ability of the 4 NDDGs for PCOS, we constructed a nomogram and used calibration curves to assess its predictability (Figure 4A). The calibration curve showed a minor error with a C-index of 0.878 between the actual PCOS risk and the predicted risk, indicating that the nomogram has a high PCOS predictive accuracy (Figure 4B). Moreover, the decision curve analysis (DCA) showed a favorable clinical benefit for the nomogram of patients (Figure 4C). To better assess the clinical effect of the nomogram, a clinical impact curve was plotted based on the DCA curve. As shown in Figure 4D, the "Number high risk" curve is in proximity to the "Number high risk with event" curve for high-risk thresholds from 0.7 to 1, suggesting that the nomogram has a desirable performance in prediction.

To further evaluate the prognosis of NDDGs in PCOS patients, we conducted ROC curve analysis in both the training and validation cohorts. The results showed that NDDGs have high accuracy in predicting patient outcomes in both two cohorts. (Figures 4E, F)

3.5 PPI construction and GSEA enrichment analysis of NDDGs

We constructed a PPI network and performed functional analysis for the 4 NDDGs with their 20 reciprocal genes using the GeneMANIA database. As shown in Figure 5, NDDGs were involved in apoptotic signaling pathways and mitochondria-related functional pathways.

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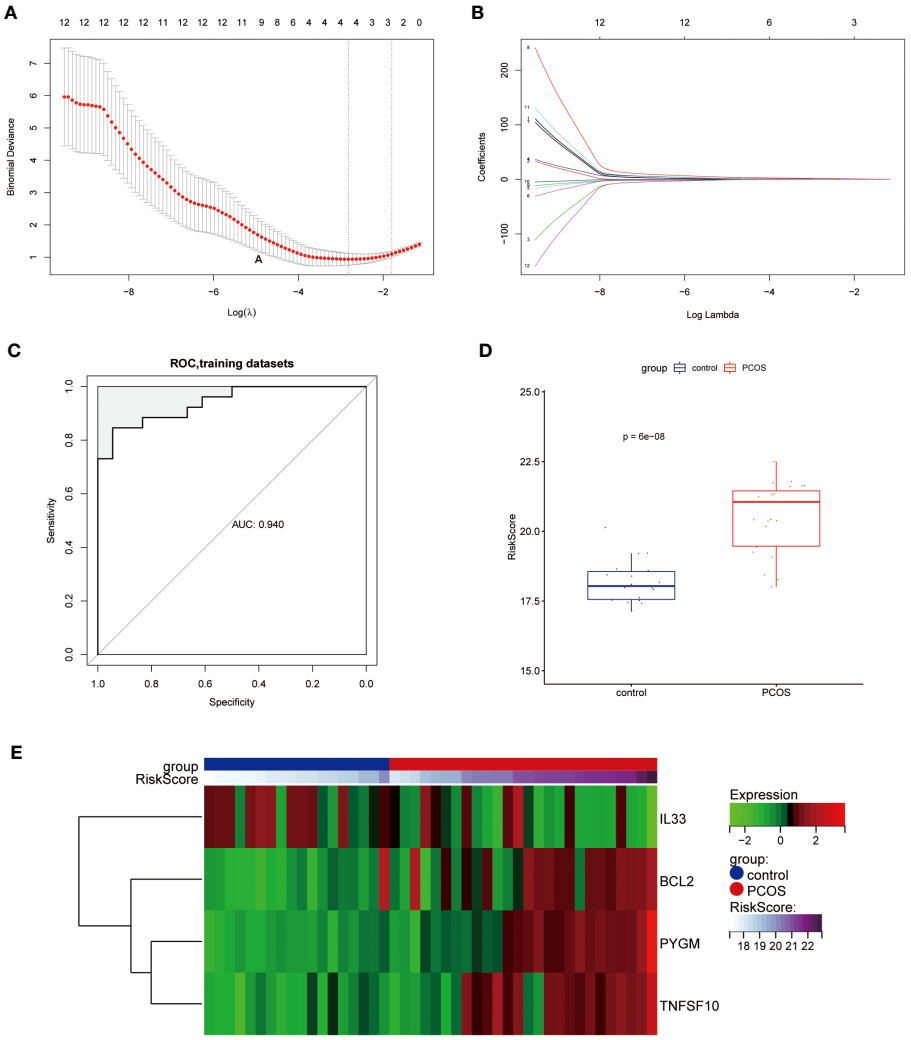

FIGURE 3
Construction of the diagnostic scoring model **(A, B)** LASSO logistic regression algorithm used to screen key genes. **(C)** ROC curves of the diagnostic model in the training set. **(D)** Distribution of RiskScore in control and PCOS groups. **(E)** Heat map of the expression of the NDDGs in each sample. LASSO, least absolute shrinkage and selection operator; ROC, receiver operating characteristic curve; NDDGs, differentially diagnostic genes for necroptosis.

Furthermore, GSEA enrichment analysis was used to mine the mechanism of KEGG pathway for NDDGs Figure 6. The present results demonstrate the positively correlated top 6 KEGG pathways for each gene. The findings show that the pathways were significantly enriched to T cells as natural killer cell-related pathways, which implied the crucial roles of the immune microenvironment in PCOS.

3.6 Relationships between the NDDGs and the immune microenvironment

In the GSEA analyses, we found that NDDGs were highly enriched in immune-related pathways. We further employed ssGSEA algorithm to evaluate the association between necroptosis and the immune microenvironment in PCOS (Figure 7A). Among 28 immune cells, we identified 13 immune cells that differed significantly between control and PCOS and were noticeably enriched in the PCOS group.

Furthermore, we estimated the correlation efficiency between NDDGs and the immune microenvironment. Among them, PYGM and TNFSF10 were predominantly favorably correlated with the immune cells while IL33 was primarily negatively correlated with natural killer cell, memory B cell, mast cell, immature B cell, effector memory CD8 T cell, CD56dim natural killer cell, activated dendritic cell (Figure 7B).

3.7 Validation of NDDGs and drug prediction

To validate the expression of NDDGs in PCOS, we first tested the expression of NDDGs using the validation set GSE84958, which showed significant differences in BCL2 (Fold change (FC) = 1.5),

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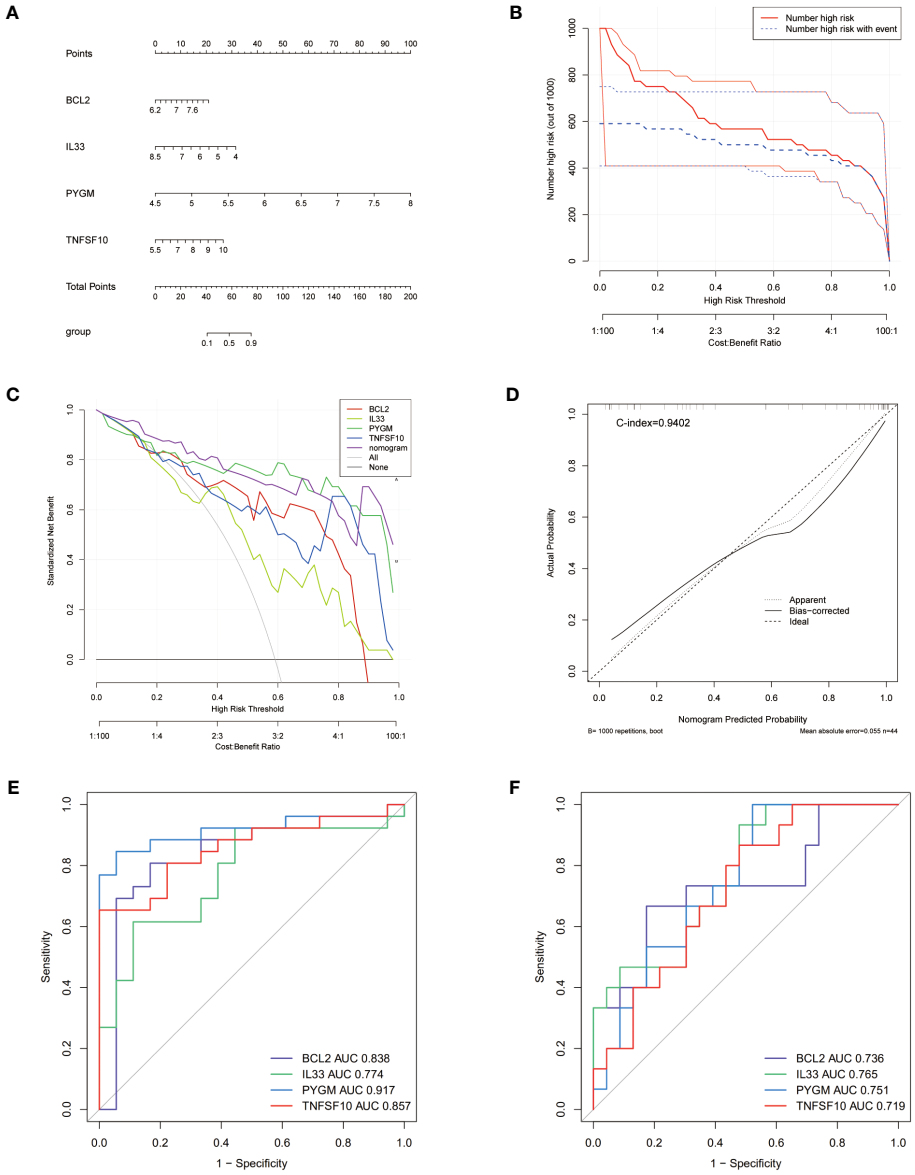

FIGURE 4
Nomogram for PCOS patients and ROC curves. **(A)** Nomogram for PCOS patients. **(B)** Calibration curve to assess the predictive power of the nomogram. **(C)** DCA curve to assess the clinical value of the nomogram. **(D)** Clinical effects curve based on DCA curves to assess the clinical impact of the nomogram. DCA, decision curve analysis. **(E, F)** ROC curves analysis of training set **(E)**, testing set **(F)**.

PYGM (FC = 1.87) and IL33 (FC = 1.76) (Figure S4). More importantly, we collected clinical samples and constructed a PCOS mouse model for further validation. The results exhibited that the expression of BCL2 (FC = 1.35 & 1.52), PYGM (FC = 1.38 & 2.08) and TNFSF10 (FC = 1.42 & 1.73) was significantly higher in the GCs from PCOS patients and ovarian tissues of PCOS mice compared with controls. In comparison, the expression of IL33 (FC = 1.27 & 1.32) was notably lower (Figure 8). In general, these results were consistent with the analysis of the training set.

To predict the corresponding drugs of NDDGs, we searched the CTD and constructed a network diagram. We discovered 58 drug-gene relationship pairs in the database containing 4 NDDGs and 45 drug molecules (Figure 9).

4 Discussion

PCOS is one of the most common endocrine disorders in women of reproductive age. PCOS patients are often misdiagnosed or underdiagnosed due to the lack of precise diagnostic criteria. Discovering new and effective biomarkers may help facilitate timely diagnosis and intervention for patients with PCOS. In this study, we construct a risk model based on necroptosis to assess the progression of PCOS. Based on machine learning and algorithmic analysis, we identified four necroptosis-related signature genes, IL33, BCL2, PYGM, and TNFSF10, and further determined the signaling pathways, immune microenvironment and targeted drugs.

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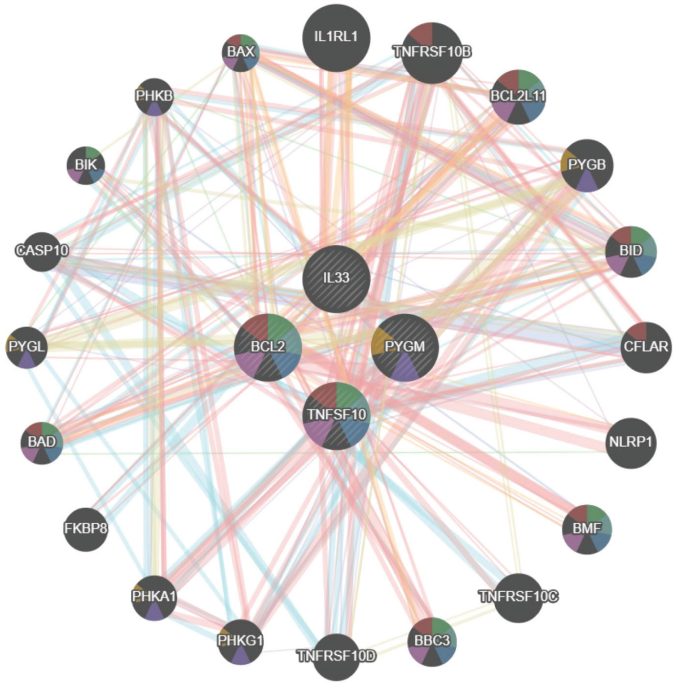

FIGURE 5
Construction of PPI network with GeneMANIA database.

In PCOS patients, the prevalence of insulin resistance is around 50-70% (39). Ovarian GCs are energy-consuming cells providing estradiol and nutrients to the oocyte (40). Consequently, insulin resistance or apoptosis in GCs can impair oocyte development and ovulation, an essential cause of PCOS (41, 42). PI3K/AKT-mediated insulin signaling pathway was closely associated with metabolic abnormalities and reproductive disorders in PCOS (43). Insulin could activate AS160, GSK-3β and FOXO1 through PI3K/AKT to promote GLUT4 transport and glucose uptake and to regulate gluconeogenesis and glycogen synthesis (44). Insulin resistance impairs glucose metabolism and imposes a hyperfunctional state on the ovaries, which increases the response to gonadotropins, leads to abnormal steroid hormone synthesis and secretion, and results in excessive follicular recruitment and development (44). In PCOS patients, insulin resistance in GCs leads to PI3K/AKT signaling inhibition, contributing to GCs apoptosis and degeneration, subsequently leading to follicular atresia (45). Besides, Insulin resistance is an important cause of hyperglycemia in women with PCOS. Hyperglycemia-derived ROS led to the activation of NF-kB and the production of pro-inflammatory factors such as TNF or IL-6 (46, 47). High glucose caused the inhibition of antioxidant enzymes, reducing the ability of GCs to remove ROS (48). The accumulation of ROS impairs the function of GCs and causes GCs apoptosis and follicular atresia. As such, dysregulation of insulin signaling and high glucose production leads to inflammation and GCs apoptosis, which might be responsible for antral follicular atresia and the development of PCOS (49).

PYGM is a muscle glycogen phosphorylase reported to have a primary role in providing energy for muscle contraction. It is also expressed in tissues other than muscle, such as the brain, lymphoid tissue, blood and ovaries (50, 51). PYGM was reportedly involved in insulin and glycogen signaling pathways, insulin resistance and necroptosis (51). Based on the increased expression of PYGM in GCs reported in this study, we speculate that PYGM may influence PCOS development via insulin resistance regulation in GCs. Besides, PYGM was also proven to regulate the immune function of T cells (52). Our immune infiltration results revealed a significant correlation between PYGM and various T cells. Hence, PYGM may influence the inflammatory status of PCOS by regulating the immune infiltration of T cells. However, the

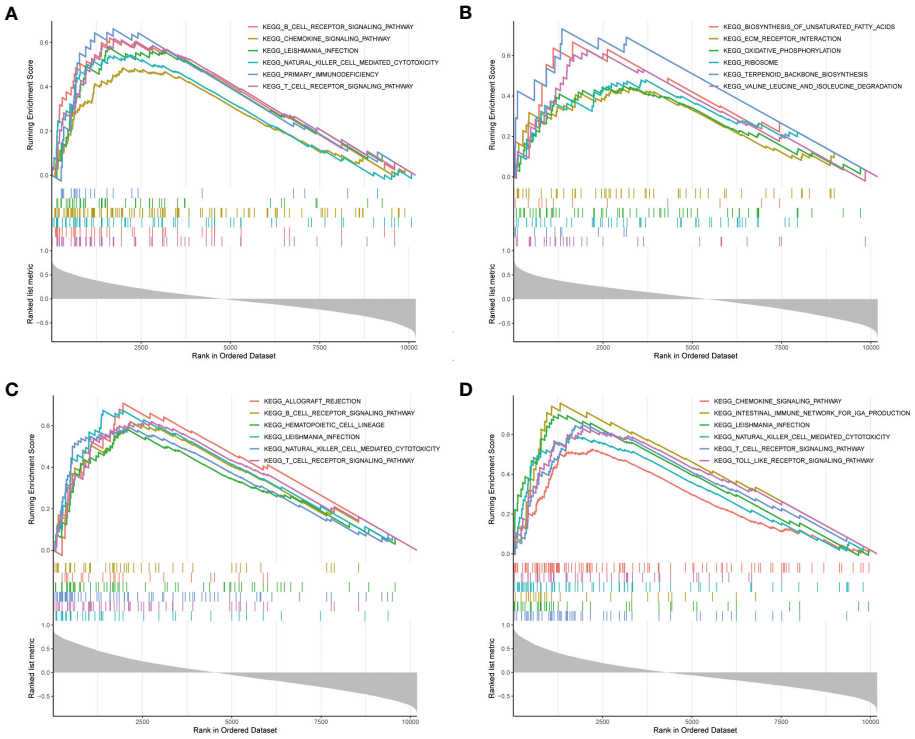

FIGURE 6
GSEA enrichment analysis **(A–D)** GSEA enrichment analysis of NDDGs of BCL2 **(A)**, IL33 **(B)**, PYGM **(C)** and TNFSF10 **(D)**. GSEA, gene set enrichment analysis.

mechanisms underlying these biological functions need to be thoroughly investigated.

TNFSF10 belongs to a family of tumor necrosis factor (TNF) ligands that induce apoptosis by binding to their receptors and triggering the activation of MAPK8/JNK, caspase 8 and caspase 3 (53). It was reported that TNFSF10 could be a marker of necroptosis in various diseases, such as ischemic cardiomyopathy and cancers (54, 55). This study is the first to identify TNFSF10 as a diagnostic marker of necroptosis in PCOS, and its specific function in the development of PCOS needs to be further investigated. BCL2 has been reported to be involved in the development of PCOS (56). Also, BCL2 was involved in necroptosis in various diseases (57, 58). In this study, it acted as one of the diagnostic markers of necroptosis in PCOS, consistent with the previous reports (59).

IL33 is a new member of the IL1 cytokine family and encodes a cytokine protein. It can function either extracellularly as an inflammatory factor or intracellularly as a nuclear factor with transcriptional regulatory functions (60). IL33 is mainly involved in the maturation of immune cells such as Th2 cells, the activation of mast cells and natural killer cells. One study found that IL33 expression was elevated in PCOS patients compared to controls (61). Another showed a significant decrease in IL33 levels in the ovaries of PCOS rats treated with omega-6 fatty acids (62). These results implied that IL-33 might be involved in the development of PCOS as a pro-inflammatory factor. However, the decreased expression of IL-33 in GCs from PCOS patients found in the public database in the present study was not consistent with the reports. The reason for this may be the method of analysis and the different sources, types, and size of the samples. Instead of using the serum of patients and ovarian tissue from rats, we obtained the results using several databases to analyze PCOS GCs. More importantly, we verified the expression of IL33 in the validation set GSE84958, clinical sample and mouse model. Despite this, the reasons for the discrepancy in results require further analysis.

The GO category "positive regulation of inflammatory response, apoptotic process, positive regulation of interleukin-6 production, positive regulation of macrophage activation, positive regulation of tumor necrosis factor production, positive regulation of necroptotic process, T cell homeostasis" indicated that the genes were involved in processes of immune regulation, which fits well with the concept of PCOS as a chronic inflammatory disease. KEGG enrichment analysis was significantly linked to necroptosis. The enriched GSEA pathways involve inflammatory, immune, and apoptotic signaling pathways. These pathways have included the T cell receptor signaling pathway, natural killer cell-mediated cytotoxicity, toll-like receptor signaling pathway, B cell receptor signaling pathway, and cytokine receptor interaction, critical in the inflammatory response to PCOS.

By analyzing the correlation between diagnostic genes and immune cells, we found that PYMG and TNFSF10 were positively correlated with immature B cells, memory B cells, effector memory CD8 T cell, regulatory T cell, T follicular helper cell and IL33 showed a negative correlation with immature B cell, memory B cells. Both T helper cell 17 (T17) and Treg cells belong to CD4+T lymphocytes. Chronic low-grade inflammation was considered a key factor in the pathogenesis of PCOS (63). The imbalance of T cell subsets and the abnormal cytokine concentrations exist in the ovary of women with PCOS (64). PYGM is also expressed in T lymphocytes, where it plays

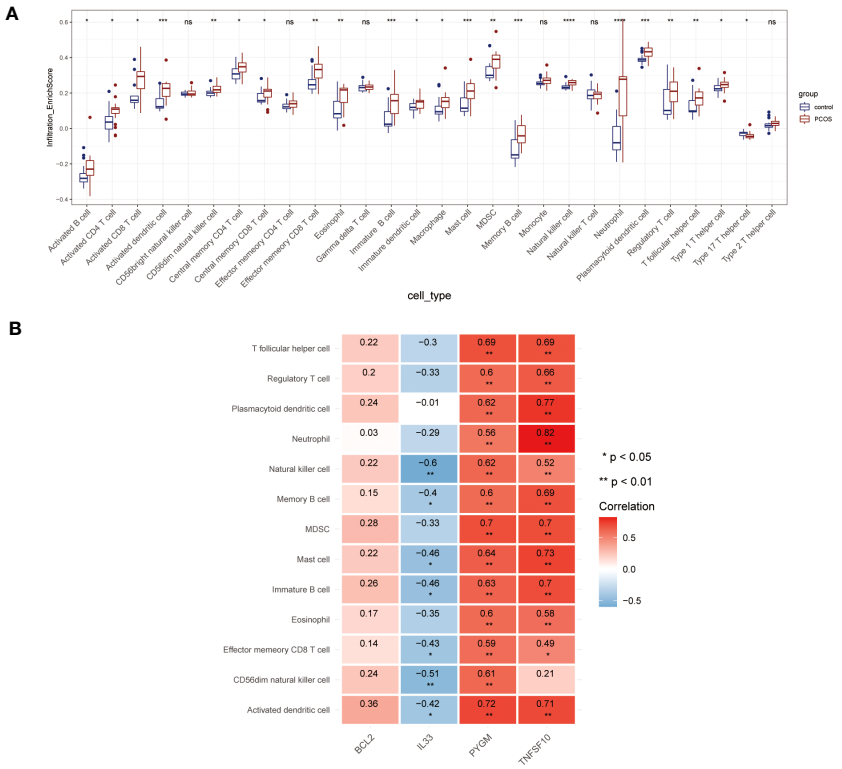

FIGURE 7
Immune microenvironment analysis. **(A)** The distribution of 28 immune cells in PCOS and control group. **(B)** Correlation analysis of 4 diagnostic necroptosis genes with 13 immune cell species. (* p< 0.05, ** p< 0.01, *** p< 0.001, **** p< 0.0001, ns indicates not significant).

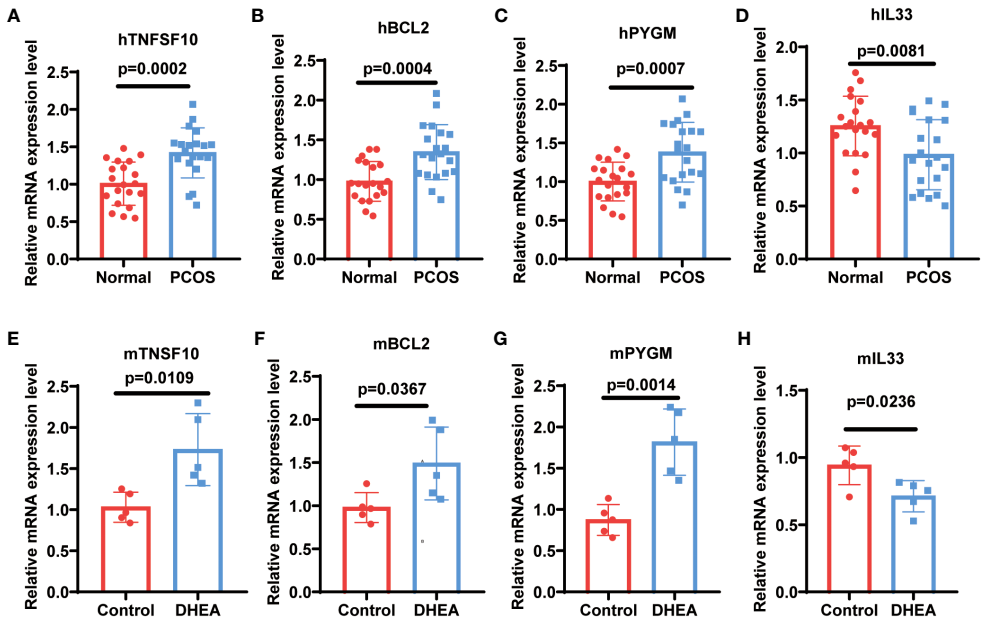

FIGURE 8
Validation of NDDGs in PCOS patients and mouse model. **(A–D)** The expression level of TNFSF10, BCL2, PYGM and IL33 in granulosa cells from PCOS patients and controls. **(E–H)** The expression level of TNFSF10, BCL2, PYGM and IL33 in the ovarian tissues of PCOS mice and controls.

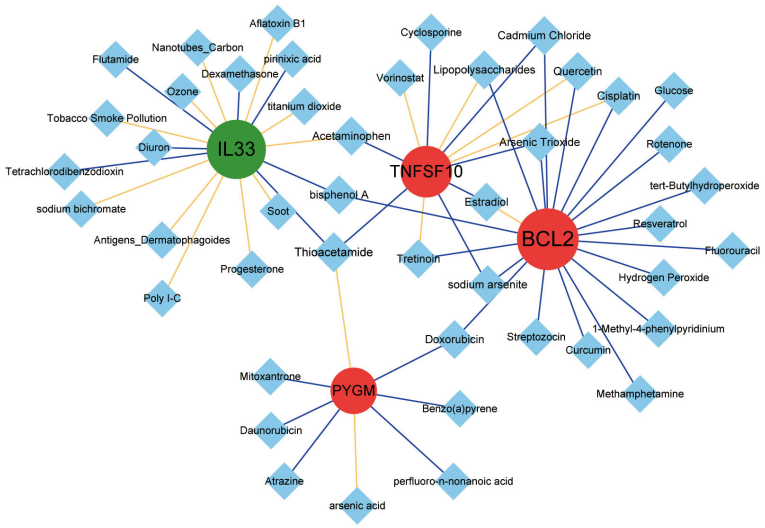

FIGURE 9
Drug prediction for the NDDGs. Red circles indicate upregulated genes, green circles indicate downregulated genes, and blue diamonds indicate drug small molecules. The yellow line represents a drug that increases the expression of a gene or protein, and the blue line represents a drug that decreases the expression of a gene or protein. The node size denotes the connectivity size.

a crucial role in the control of IL2–stimulated T-cell migration and proliferation in an EGFR-dependent manner (52, 65). EGF/EGFR signaling was reported to affect the proliferation of cumulus GCs, oocyte maturation and meiosis and play a potential role in the pathogenesis of PCOS (66). We hypothesize that EGFR may activate PYGM to regulate T cell migration and proliferation in PCOS cases. TNFSF10, also known as TRAIL, regulates immune responses and cell homeostasis via an apoptosis-independent pathway (67). TRAIL/TRAIL-R interaction regulates $CD4^+$ T cell activation and directly suppresses T cell activation via inhibiting TCR signaling, indicating that TRAIL-R is a novel immune checkpoint in T cell responses (68). In the present study, TNFSF10 was observed to have a strong positive correlation with CD4+ T cells, suggesting that it may contribute to immunomodulatory effects in PCOS by modulating the T-cell response.

As reported, CD19+ B cells could contribute to the pathogenesis of PCOS (69). The peripheral proportion and activity of CD19+ B cells were increased in women with PCOS. DHEA-induced morphological changes to mouse ovaries could be prevented by CD19+ B cell depletion. Moreover, TNF-α-producing B cells are involved in the pathological process of PCOS (70). In this study, GSEA analysis showed that NDDGs were enriched in the B cell receptor signaling pathway. Immune cell analysis indicated immature B cells and memory B cell numbers were significantly higher in PCOS samples. Meanwhile, PYGM, TNFSF10 and IL33 were notably associated with immune infiltration of B cells. It has been found that TNFSF10 was demonstrated to be upregulated in B cells in primary Sjögren's syndrome, suggesting a regulatory role for TNFSF10 in B cells. Our subsequent concern may be how these genes regulate B-cell function in PCOS.

We predicted compounds corresponding to the four diagnostic genes from the CTD database and discovered several compounds, such as resveratrol, tretinoin, quercetin, curcumin, etc., which have been investigated as therapeutic drugs for PCOS (71–73). For instance, quercetin has been demonstrated to reduce inflammation in PCOS patients and DHEA-induced PCOS rats (74, 75). Another study showed that curcumin might be a safe and useful supplement to ameliorate PCOS-associated hyperandrogenemia, hyperglycemia, and hyperlipidemia (76, 77).

Overall, we found IL33, BCL2, PYMG, and TNFSF10 to be potentially necrotic apoptosis-associated diagnostic markers in PCOS. Nonetheless, this study has some limitations. The results were based on mining and analyzing the published database. There was still a lack of experimental evidence for the mechanism of these diagnostic markers in necroptosis of GCs and the development of PCOS. In particular, whether PYMG is involved in the development of PCOS by affecting insulin resistance and inflammation needs to be thoroughly investigated. Second, the cellular composition of the immune infiltrate was significantly different in PCOS patients compared with normal. Primarily, the activated dendritic cell, immature B cell, mast cell, MDSC, memory B cell, natural killer cell, neutrophil, and plasmacytoid dendritic cell may be associated with GCs necroptosis and the development of PCOS. Further studies of these diagnostic genes and immune cells may provide feasible directions for clinical diagnosis and immunotherapy of PCOS.

5 Conclusion

In this study, we constructed a risk model based on necroptosis to assess the progression of PCOS. Based on machine learning and algorithmic analysis, we identified four necroptosis-related signature genes, IL33, BCL2, PYMG, and TNFSF10, and further determined the signaling pathways, immune microenvironment, and targeted drugs. Furthermore, the expression of the four model genes was validated in a PCOS mouse model and clinical samples. This is the first necroptosis-related signature in PCOS and could be a valuable and non-invasive tool for diagnosing and evaluating the progression and prognosis of PCOS

patients. This study provides a theoretical basis and new insights into the pathogenic mechanism and therapeutic drug development for PCOS.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving humans were approved by The Ethics Committee of the Medical University of Bialystok. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The animal study was approved by The Ethics Committee of China Agricultural University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MW and KA contributed to the data collection, qPCR analysis and manuscript writing. RM contributed to the manuscript revising. JH contributed to data collection. HD contributed to the data analysis and writing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1193992/full#supplementary-material

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Exploration of the correlation of serum polychlorinated biphenyl levels with luteal phase hormonal parameters and infertility in women with or without polycystic ovary syndrome

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Introduction: Polychlorinated biphenyls (PCBs) are organic lipophilic pollutants that accumulate in the body. Previous research has linked PCBs with menstrual function; therefore, this study was undertaken to investigate the correlation of PCBs with luteal phase hormonal parameters of menstrual function at day 21 in a group of non-obese women prior to *in vitro* fertilization (IVF).

Methods: Fifty-eight non-obese Caucasian women from a UK academic center, 29 with polycystic ovary syndrome (PCOS) and 29 without, were recruited. PCOS women all had anovulatory infertility. Non-PCOS women: five with unexplained infertility, the remainder with male factor infertility (n=14) or tubal problems (n=10). Blood was withdrawn at day 21 of the menstrual cycle for non-PCOS women, at the time of mock embryo transfer. PCBs were measured using high resolution gas chromatography.

Results: Only PCB118, PCB153, PCB138 and PCB180 were detected in all samples, and levels did not differ between PCOS and non-PCOS subjects. In non-PCOS subjects, PCB153, PCB138 and PCB180 inversely correlated with estradiol ($p<0.05$); PCB118 and PCB138 inversely correlated with follicle stimulating hormone (FSH) ($p<0.05$); PCB118 ($p<0.05$), PCB153, PCB138 and PCB180 (all $p<0.01$) inversely correlated with luteinizing hormone (LH). Control women without PCOS with unexplained infertility showed higher levels of PCB118, PCB153, PCB138 and PCB180 ($p<0.05$) compared to those control women without PCOS with tubal or male factor infertility, though other hormonal parameters did not differ other than that FSH that was lower in the unexplained group ($p=0.01$). The only correlation observed in PCOS women with anovulatory infertility was that between PCB180 and progesterone ($p<0.05$).

Conclusion: PCBs correlated with luteal phase menstrual cycle hormones in control women without PCOS and may contribute to the mechanism of unexplained infertility; in PCOS women, no correlations of the PCBs were seen for estradiol, LH or FSH.

KEYWORDS

polychlorinated biphenyls, organic pollutants, pcos, infertility, *in vitro* fertilization, reproductive health

Introduction

Polychlorinated biphenyls (PCBs) are organic pollutants that persist in the environment due to their resistance to biotransformation and high lipophilicity (1). Their accumulation is correlated to age (time of exposure) and obesity (2), with dietary consumption being the main route of exposure in humans (3). PCBs are classified as endocrine disrupters due to their observed thyroidogenic, estrogenic and antiandrogenic action (4) and are reported to affect the epigenome (5). Highly chlorinated PCB congeners (PCB118, PCB138, PCB153 and PCB180) reflect long term contamination, in contrast to PCB28, a low chlorinated volatile PCB that is degraded relatively fast and thus reflects relatively acute contamination (6). PCBs have been associated with infertility, higher levels associating with miscarriage (7). PCBs have been associated with endometriosis that causes pelvic pain and infertility (8); however, in a large cross-sectional study of self reported outcomes, whilst higher total PCB levels associated with fewer lifetime pregnancies, they did not correlate to the prevalence of infertility and pregnancy outcomes (9), though the infertility issues were not defined. Others report a health survey that suggested a prolonged time to pregnancy and reduced fertility in women previously exposed to PCBs (10); however, no study has looked at correlations between PCBs and causes of infertility in a defined population.

Polycystic ovary syndrome (PCOS), associated with menstrual dysfunction, infertility, hirsutism, acne, obesity and metabolic syndrome (11), is the most common endocrine disorder among women of reproductive age, with a reported prevalence of 6-10% (12). PCOS is a proinflammatory state with increased insulin resistance and elevation in the inflammatory marker C-reactive protein (CRP) (13), and is a complex multigenetic heterogenous disorder with evidence of epigenetic and environmental influences resulting in varied phenotypes, clinical manifestations and metabolic consequences (14).

We hypothesised that PCBs would be unlikely to be associated with infertility causes, but may relate to hormone levels in the luteal phase of the menstrual cycle; therefore, this pilot study was undertaken in two cohorts of women preparing to undergo IVF: firstly, women with PCOS and anovulatory infertility and, secondly, control women without PCOS in whom the luteal phase was carefully defined at the time of mock embryo transfer, and in whom the hypothalamo-ovarian axis would have been considered to be normal given the causes of infertility being male factor infertility, tubal problems or unexplained infertility.

Materials and methods

The study design was a case-control study. Participants were sequentially recruited in 2015 from the Hull *In Vitro* Fertilization (IVF) Unit, UK, following ethical approval from The Yorkshire and The Humber NRES ethical committee, UK (approval number 02/03/043). Participants with known immunological disease, diabetes, renal or liver insufficiency, acute or chronic infections, inflammatory disease, age <20 or >45 years, body mass index (BMI) >30kg/m^2 and those not undergoing IVF treatment, were excluded from the study. PCOS was diagnosed using the revised 2003 Rotterdam criteria (15). All PCOS subjects had hypogonadotropic anovulatory infertility (n=29; mean age 30.9 ± 4.8 years, mean weight 72.5 ± 13.1 kg, mean BMI 26.0 ± 3.8 kg/m^2), whilst for the non-PCOS women the cause of infertility was determined to be male factor infertility (MFI, n=14), tubal problems (n=10) [MFI and tubal problems were combined as explained infertility (n=24; mean age 32.5 ± 4.5 years, mean weight 67.2 ± 12.0 kg, mean BMI 24.9 ± 3.5 kg/m^2)] or unexplained infertility (n=5; mean age 34.7 ± 3.3 years, mean weight 71.5 ± 12.9 kg, mean BMI 27.1 ± 3.6 kg/m^2). Of the 58 participants recruited (29 PCOS and 29 controls), written informed consent was obtained from all participants (16). No subject had been on any prescribed or over-the-counter medication, all were non-smokers and none consumed alcohol in the preceding six months, data that was collected in the full medical history.

Sample collection

At 21 days of the menstrual cycle, prior to IVF treatment, when no hormonal treatment had been given, mock embryo transfer was undertaken as part of normal clinical practice, when ovarian and endometrial ultrasound were also performed. Single fasting blood samples were taken from each subject at the time of mock embryo transfer using vacutainers (Becton Dickinson, New Jersey, USA): ethylenediaminetetraacetic acid (EDTA) for glycosylated hemoglobin A1c (HbA1c) measurement in whole blood; sodium fluoride for glucose measurement; no additive for serum

measurement of all other parameters. Serum was prepared by centrifugation at 3500×g for 15 min at 4°C and stored at −80°C. Fasting blood glucose (FBG) was measured using a Synchron LX20 analyzer (Beckman-Coulter, High Wycombe, UK). Serum insulin was measured by competitive chemiluminescent immunoassay (DPC Immulite 2000 analyzer, Euro/DPC, Llanberis, UK). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated using the formula ((Insulin x glucose)/22.5) (17). Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone were measured using a chemiluminescent microparticle immunoassay technology (Abbott Diagnostics, Maidenhead, UK). C-reactive protein (CRP), total cholesterol (TC) and triglycerides (TG) were measured enzymatically (Synchon LX20 analyzer, Beckman-Coulter, High Wycombe, UK). Total serum lipid (TSL) was determined using the formula ((2.27 x TC) + TG + 62.3 mg/dL) (18). Anti-müllerian hormone (AMH) was measured using an immunoenzymatic assay (Beckman-Coulter, High Wycombe, UK). HbA1c was measured using ion-exchange chromatography. Estimated glomerular filtration rate (eGFR) was calculated using the formula (175 x (SCr, mg/dL)-1.154 x (age, years)-0.203 x 0.742) (19). Androgens were measured by liquid chromatography tandem mass spectrometry (LC/MS/MS; Acquity UPLC-Quattro Premier XE-MS, Waters, Manchester, UK). An immunometric assay with fluorescence detection (DPC Immulite 2000 analyzer, Siemens, Camberley, UK; upper limit 2.0 nmol/l) was used to measure sex hormone binding globulin (SHBG). The formula ((testosterone/SHBG) x 100) was used to calculate free androgen index (FAI). An Abbott Architect i4000 immunoassay analyzer (Abbott Diagnostics Division, Maidenhead, UK) was used to measure thyroid hormone levels.

Polychlorinated biphenyl analysis

Samples were analyzed for 7 indicator PCBs: PCB28, PCB52, PCB101, PCB118 (a dioxin like PCB), PCB138, PCB153 and PCB180. 5mL of serum was spiked with 5ng of each of 13C12-labelled PCBs (Wellington Laboratories, Guelph, Ontario, Canada) in 50mL Falcon tubes. Extraction and clean-up were performed using a previously described protocol (20). Clean extracts were evaporated to near-dryness, reconstituted in 50µL hexane containing 2.5 ng 13C12-PCB-141 as a recovery standard, and transferred to inserted autosampler vials prior to analysis. PCBs were determined using high resolution gas chromatography (Thermofisher TRACE 1300, Loughborough, UK) coupled with high resolution mass spectrometry (HRGC/HRMS, Thermofisher DFS, Loughborough, UK) with quality assurance checks using previously described methods (21). A sum PCB (∑PCB) variable was calculated by adding the molar concentrations of PCB congeners analyzed.

Statistical analysis

There are no published studies to base a power calculation on; therefore, this was an exploratory pilot study on which a power calculation could be determined based on the results observed. Descriptive data are presented as mean ± standard deviation (SD) for continuous data. Serum PCB concentrations are expressed as geometric mean ± SD. PCB levels, metabolic outcomes and hormone concentrations were assessed for normality and Independent T or Mann-Whitney U tests were used to compare means/medians, as appropriate. Potential correlations between PCBs and metabolic and fertility parameters were examined using exploratory Pearson's correlations. A p-value of <0.05 was considered indicative of statistical significance. Statistical analysis was carried out using Graphpad Prism version 9.5.1 (San Diego, CA, USA).

Results

Demographics, metabolic outcomes, and hormone levels

PCOS and non-PCOS women did not differ in age, BMI, insulin, HOMA-IR or TSL (Table 1), nor were there any differences in these variables for unexplained, tubal and anovulatory infertility. PCOS women had higher FAI (3.1 ± 2.9 vs 1.7 ± 3.4, p=0.002), testosterone (1.1 ± 0.5 vs 0.8 ± 0.4 nmol/L, p=0.01), androstenedione (4.1 ± 1.5 vs 2.5 ± 1.2 nmol/L, p<0.001), AMH (56 ± 14 vs 24 ± 14 ng/mL, p<0.001), LH (14.9 ± 14.3 vs 6.0 ± 8.3 iU/L, p=0.01) and triglycerides (1.3 ± 0.7 vs 1.0 ± 0.5 mmol/L, p=0.03) compared to control women without PCOS. FBG and SHBG were lower in PCOS women (4.5 ± 0.8 vs 4.8 ± 0.3 nmol/L, p=0.04 and 65 ± 51 vs 113 ± 83 nmol/L, p=0.008, respectively) (22).

Serum PCB levels, and correlations with metabolic outcomes and hormone levels

Geometric mean (GM) concentrations of frequently detected PCBs and the ∑PCB variable did not differ between PCOS and non-PCOS women (p=ns), as previously reported in this study cohort (23); non-PCOS versus PCOS (ng/g lipid): PCB118 (5.1 ± 1.4 vs 5.4 ± 1.5), PCB138 (11.6 ± 1.5 vs 10.1 ± 1.7), PCB153 (15.0 ± 1.6 vs 12.2 ± 1.8), PCB180 (13.5 ± 1.6 vs 10.7 ± 1.7), ∑PCBs (48.3 ± 1.5 vs 41.1 ± 1.6) (23). As reported before, using multivariable linear regression there were no associations between PCBs and blood glucose, HOMA-IR, insulin or HbA1c, nor with FAI, androstenedione or testosterone (23).

Gonadal hormones: in the control women without PCOS, PCB153, PCB138 and PCB180 inversely correlated with estradiol (p<0.05); there was no correlation between PCBs and progesterone in control women without PCOS.

Pituitary hormones: in the control women without PCOS, PCB118 and PCB138 inversely correlated with FSH (p<0.05); PCB118, PCB153, PCB138 and PCB180 inversely correlated with LH (p<0.05, p<0.01, p<0.01 and p<0.01, respectively) (Figures 1, 2).

Control women without PCOS with unexplained infertility showed higher levels of PCB118, PCB153, PCB138 and PCB180

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TABLE 1 Demographic data, together with metabolic and hormone level measurements for women with (n=29) and without (n=29) PCOS.

	Control women without PCOS (n=29)		Women with PCOS (n=29)		
	Mean	SD	Mean	SD	p
Age (Years)	33	4	31	5	0.10
BMI (kg/m^2)	25.4	3.6	26.0	3.8	0.49
Insulin (μIU/ml)	7.6	4.1	8.1	4.7	0.94
HOMA-IR	1.7	1.0	2.0	1.6	0.96
FBG (nmol/L)	4.8	0.3	4.5	0.8	0.04
TSL (mg/dL)	573	106	583	134	0.77
AMH (ng/mL)	24	14	56	14	< 0.001
eGFR (mL/min/1.73 m^2)	96	17	88.1	10.3	0.06
CRP (mg/L)	2.5	2.3	2.7	2.6	0.99
HbA1c (mmol/mol)	30.7	6.2	32.0	3.3	0.72
FAI	1.7	3.4	3.1	2.9	0.002
Testosterone (nmol/L)	0.8	0.4	1.1	0.5	0.01
SHBG (nmol/L)	113	83	65	51	0.008
Androstenedione (nmol/L)	2.5	1.2	4.1	1.5	< 0.001
TSH (mU/L)	2.2	1.1	2.6	3.0	0.86
Free-T3 (pmol/L)	4.8	0.7	4.8	0.7	0.9
Free-T4 (pmol/L)	11.2	1.3	11.5	2.2	0.61
Estradiol (pmol/L)	457	301	431	463	0.84
Progesterone (nmol/L)	15.0	15.4	7.1	9.1	0.08
FSH (iU/L)	3.3	2.2	4.1	2.3	0.18
LH (iU/L)	6.0	8.3	14.9	14.3	0.01
Cholesterol (mmol/L)	4.8	0.8	4.7	1.1	0.65
Triglycerides (mmol/L)	1.0	0.5	1.3	0.7	0.03

BMI, Body mass index; HOMA-IR, homeostatic model assessment for insulin resistance; FBG, fasting blood glucose; TSL, total serum lipids; AMH, anti-müllerian hormone; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; HbA1c, glycosylated hemoglobin A1c; FAI, free androgen index; SHBG, sex hormone binding globulin; TSH, thyroid stimulating hormone; Free-T3, free-triiodothyronine; Free-T4, free-thyroxine; FSH, follicle stimulating hormone; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; iU, international units; SD, standard deviation; sample size (n); significance at p=0.05 (p).

(p<0.05) compared to those control women without PCOS with tubal or male factor infertility (Figure 3), though other hormonal parameters did not differ between infertility causes, other than FSH that was lower in the unexplained group (p=0.01).

In women with PCOS, no correlations of the PCBs were seen for estradiol, LH or FSH. The only correlation observed in PCOS with anovulatory infertility was a positive correlation between PCB180 and progesterone (p=0.04) (Figure 2E).

Discussion

This study suggests that PCB concentrations may affect hormones of the luteal phase of the menstrual cycle that may then be reflected in infertility. It is recognized that PCBs and other endocrine disruptors may affect thyroid function and the actions of both estrogens and androgens (4). PCBs have been associated with infertility with a reported association with the development of endometriosis (8), and reduced fertility suggested by fewer lifetime pregnancies and prolonged time to pregnancy after PCB exposure (9, 10), but their relationship to individual causes of infertility has not previously been examined. In addition, PCBs have been linked to menstrual cycle irregularities, early menopause and miscarriage (7, 24), though that the PCBs are associated with effects on the menstrual cycle hormones is less reported. This study suggests that PCBs may be associated with the hormones of the menstrual cycle in the mid luteal phase, specifically FSH, LH and estradiol, but only in the women without PCOS. This is not surprising as, if PCBs are affecting the menstrual cycle, then anovulatory women with PCOS in this cohort who were not experiencing a menstrual cycle at the time of the study would not show any effect of their action unless the menstrual cycle is induced.

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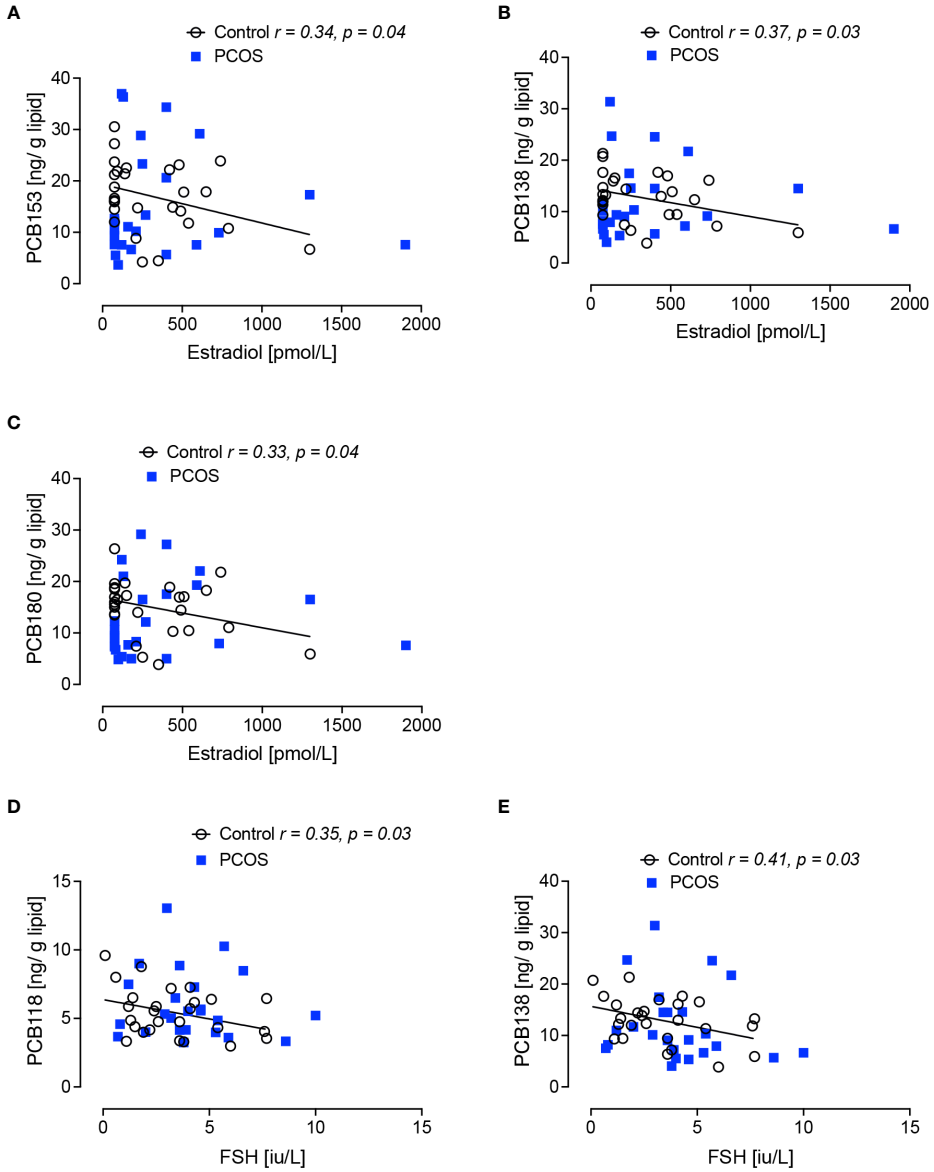

FIGURE 1
Correlations of serum concentrations of PCBs with serum estradiol and follicle stimulating hormone (FSH) levels in control women without polycystic ovary syndrome at 21 days of the menstrual cycle. Negative correlations of estradiol with PCB153 **(A)**, PCB 138 **(B)** and PCB180 **(C)**, and of follicle stimulating hormone (FSH) with PCB118 **(D)** and PCB138 **(E)** were found in women without polycystic ovary syndrome (black circles) but not in women with polycystic ovary syndrome (PCOS, blue squares).

Therefore, the correlations between PCBs (specifically PCB118, PCB153, PCB138 and PCB180) with the mid luteal phase only occurred in women exhibiting a regular menstrual cycle: PCB153, PCB138, PCB180 inversely correlated with estradiol, PCB118 and PCB138 inversely correlated with FSH; PCB118, PCB153, PCB138 and PCB180 inversely correlated with LH. Taken together, this suggests that the PCBs may act at the level of the hypothalamus (25) with an effect on the gonadotrophin releasing hormone pulse generator affecting LH, and potentially also at the level of the pituitary (25), with the correlation between estradiol and PCB being a secondary effect; thus, PCB180 in particular (26) may affect both LH and FSH secretion, thereby affecting estradiol. However, it cannot be excluded that PCBs may potentially also be acting at the level of the ovary and there is evidence of PCBs affecting follicular ovarian reserve (27) and follicular steroidogenesis (28). This suggests that PCB-induced perturbation of the menstrual hormones may contribute and potentially be additive to other factors causing infertility, and that PCBs are not related to anovulation as a mechanism for how endocrine disrupting chemicals may affect fertility.

Control women without PCOS with unexplained infertility showed higher levels of PCB118, PCB153, PCB138 and PCB180 compared to those control women without PCOS with tubal or male factor infertility, though other hormonal parameters did not differ other than FSH that was lower in the unexplained group. There are many etiologies for "unexplained" infertility and the contribution of

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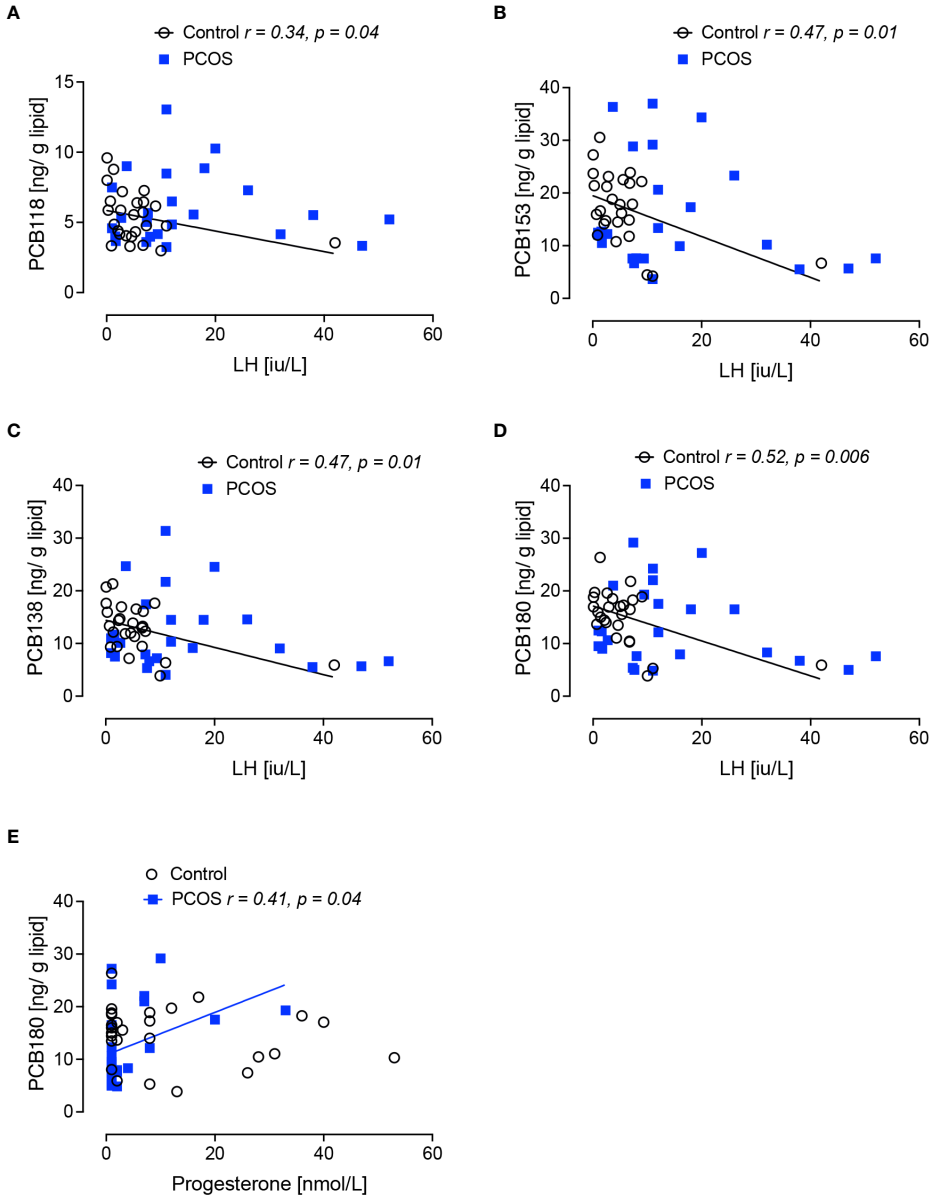

FIGURE 2
Correlations of serum concentrations of PCBs with serum lutenizing hormone (LH) and progesterone levels in women with polycystic ovary syndrome and control women without polycystic ovary syndrome at 21 days of the menstrual cycle. Negative correlations of lutenizing hormone (LH) with PCB118 **(A)**, PCB 153 **(B)**, PCB138 **(C)** and PCB180 **(D)** were found in women without polycystic ovary syndrome (black circles) but not in women with polycystic ovary syndrome (PCOS, blue squares). A positive correlation of progesterone with PCB180 **(E)** was found in women with PCOS but not in control women without PCOS.

PCBs is just one of many, including ovulatory dysfunction, sperm quality and quantity, fallopian tube function, endometriosis and immune factors (29). The possibility that the PCB association is an epiphenomenon for another contributory factor(s) must also be considered. There are several endocrine disruptor groups that have been associated with infertility, such as organochloride pesticides, heavy metals and dioxins (30); consequently, it may be that a combination of endocrine disrupting chemicals such as organochloride pesticides and polybrominated diethyl ethers among others (30) act in concert to affect fertility. The reported association of patient age and weight with PCB levels (31) could suggest that the PCBs are just a marker of another underlying process such as obesity. Clearly, this was not the case in this study, where all of the subjects were non-obese and not insulin resistant, thus suggesting that PCBs may have a more direct role than previously realized or represent a biological marker for unexplained infertility. With PCBs affecting hypothalamo-pituitary function resulting in effects on the menstrual cycle, this would suggest that in the evidence-based treatment of unexplained infertility (32) that early medical intervention rather than expectant management may be warranted in those with documented elevation of PCB levels.

The strengths of this study lie in the study design of differing causes of infertility in non-obese women with PCOS and control women without PCOS matched for age and BMI, together with the

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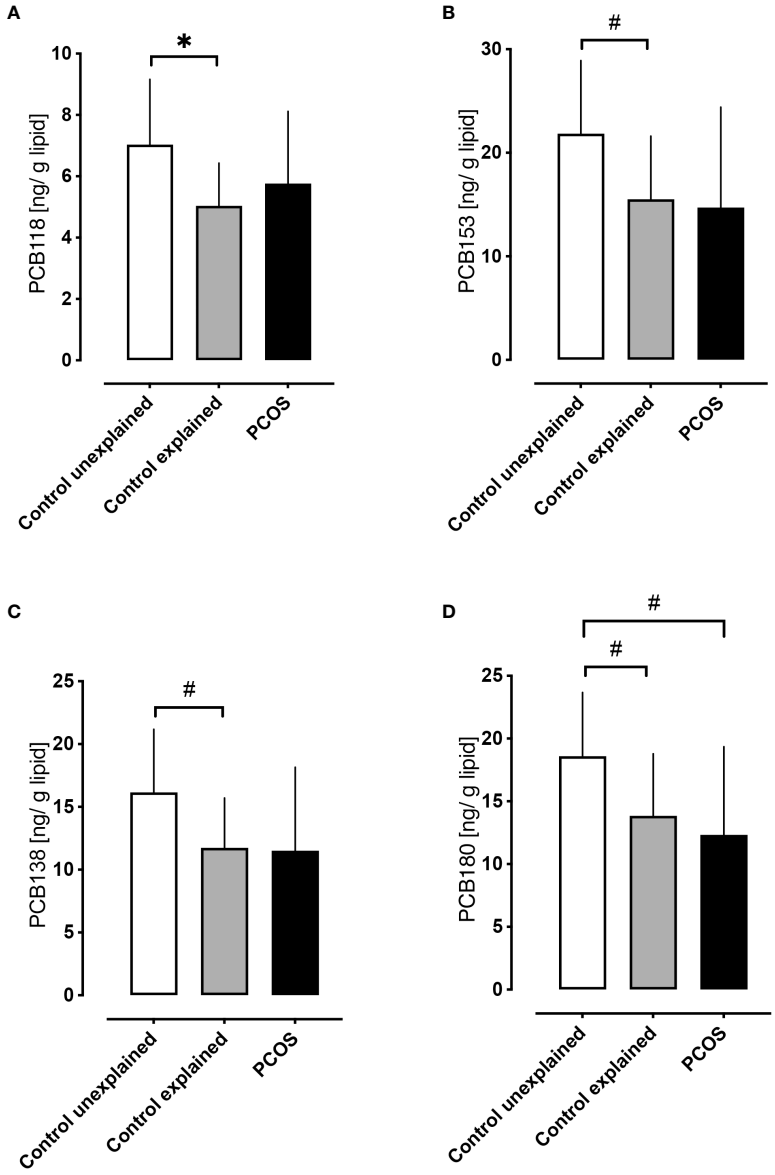

FIGURE 3
A comparison of serum concentrations of PCBs in control women without polycystic ovary syndrome with unexplained infertility versus control women without polycystic ovary syndrome with explained infertility (both at 21 days of menstrual cycle) versus women with PCOS. Higher levels of PCB118 **(A)**, PCB153 **(B)**, PCB138 **(C)** and PCB180 **(D)** were found in control women without PCOS with unexplained infertility (n=5) (white bars) versus control women without PCOS with explained fertility [due to tubal (n=10) or male factor infertility (n=14); gray bars]. Levels of PCB118 **(A)**, PCB153 **(B)** and PCB138 **(C)** in women with PCOS (n=29; black bars) did not differ from control women with either explained or unexplained infertility; only PCB180 **(D)** differed in women with PCOS versus control women without PCOS with unexplained infertility *p<0.01, $^{\#}$p<0.05.

measurement of relevant metabolic parameters and hormone levels. In addition, as these women were undergoing an IVF program for fertility, none had been on any hormonal contraception and they had all stopped any alcohol consumption, thus removing these confounders from the analysis. The limitations of the study include the small sample size and the potential lack of generalizability to ethnicities other than a Caucasian population. Comparison of the differing causes of fertility within the control group without PCOS needs to be interpreted with care as there were only 5 cases of unexplained infertility versus 24 in the explained fertility group. There is a need to determine the association of the endocrine disruptors to the menstrual cycle to ascertain causality and to perform a larger robust study incorporating the differing causes of infertility to determine the relationship to the differing endocrine disrupting chemicals. Of significance, much of the literature to date is based on animal studies that may not reflect what occurs in humans. It is likely that any contribution of the PCB effect on infertility may be multifactorial, possibly acting in combination with other endocrine disruptor chemicals, or contribute to pathophysiological processes such as obesity.

In conclusion, PCBs, specifically PCB118, PCB153, PCB138 and PCB180, are correlated with luteal phase menstrual cycle hormones in women without polycystic ovary syndrome. The serum levels of these PCBs were elevated in control women without PCOS with

unexplained infertility versus control women without PCOS with explained infertility and thus may contribute to the mechanism of unexplained infertility, though larger robust studies are needed to confirm these findings and to ascertain causality.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by The Yorkshire and The Humber NRES ethical committee, UK. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

AB: Conceptualization, Formal Analysis, Visualization, Writing – original draft, Writing – review & editing. EB: Writing – original draft, Writing – review & editing. DD: Investigation, Writing – review & editing. TS: Conceptualization, Methodology, Writing – review & editing. SA: Conceptualization, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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Stem cells and exosomes: as biological agents in the diagnosis and treatment of polycystic ovary syndrome (PCOS)

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A typical condition of the female reproductive system is polycystic ovary syndrome (PCOS). Hyperinsulinemia, insulin resistance, obesity, and hyperandrogenism are just a few of the metabolic abnormalities linked to this disease. Type 2 diabetes, hypertension, and cardiovascular disease are further issues related to PCOS. One consequence of this syndrome for which numerous treatment procedures have been developed is infertility. Metformin and clomiphene, two common allopathic medications used to treat PCOS, both have drawbacks and are ineffective. It is vital to seek novel therapeutic modalities to address these constraints. Exosomes (EXOs) are a particular class of extracellular vesicles that cells release, and they are known to play a significant role in mediating intercellular communication. A wide range of cargo, including lipids, proteins, mRNA, miRNAs, and numerous other noncoding RNAs, are contained in the nanoscale lipid bilayer exosomes. The cytokine effects of stem cells and EXOs derived from them enable the defense against metabolic diseases like PCOS. Moreover, EXO microRNAs can potentially be employed as biomarkers in the detection and management of PCOS. In this study, the potential of stem cells and exosomes are specifically investigated in the diagnosis and treatment of PCOS as one of the diseases of the female reproductive system.

KEYWORDS

polycystic ovary syndrome, exosome, stem cell, biomarker, miRNAs

1 Introduction

Increased androgen levels, ovulation issues, and morphological abnormalities are all symptoms of the disease known as polycystic ovarian syndrome (PCOS). The National Institutes of Health (NIH) defines PCOS as "hyperandrogenism with ovulation disorder." This condition occurs in at least 6% to 10% of women in the fertile phase, although the

frequency is suggested to be twice as high (1). The exact etiology and pathology of PCOS are not entirely known; however, a high ratio of luteinizing hormone (LH) to follicle-stimulating hormone (FSH) and an excess of gonadotropin-releasing hormone (GnRH) are recognized as its fundamental features. Evidence suggests that both internal and external factors, including genetics, epigenetics, hyperandrogenism (HA), insulin resistance (IR), and environmental factors, may be involved in the development of PCOS. It is also important to note that PCOS increases the risk of various illnesses, including type 2 diabetes, anxiety, heart disease, depression, and metabolic syndrome (2, 3). PCOS-positive women commonly exhibit low-grade chronic inflammation. Recent studies have shown that in lean, insulin-sensitive women with PCOS, the use of anti-inflammatory medication can reduce ovarian androgen release and promote ovulation. Interestingly, even in cases where insulin resistance is absent in PCOS, the data clearly indicate that inflammation acts as a crucial character in the underlying mechanism behind ovarian dysfunction (4, 5).

Mesenchymal stem cell (MSC)-based therapy bears promise as a viable therapeutic option for PCOS because of its capacity for self-renewal, differentiation potential, and immunomodulatory activities, particularly in diseases associated with inflammation. Numerous studies suggest that MSCs can potentially enhance and restore ovarian function through paracrine signaling pathways. Notably, the paracrine activity of MSCs is regulated by the RAP1/NFkB signaling pathway, which also influences immunological and inflammatory responses, making it particularly impactful on function (6). Research indicates that stem cells have the potential to improve the pathological changes associated with PCOS and reverse ovarian dysfunction. Certain pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interferon-gamma (IFN-γ) are down-regulated as part of this therapeutic effect. Additionally, fibrosis-related genes, e.g., connective tissue growth factor (CTGF), are also down-regulated to contribute to the restoration of ovarian function in females with PCOS (7, 8).

Exosomes (EXOs) are small vesicles, measuring 30 to 100 nm and protected by membrane packets, that are released by a variety of live cells under physiologically healthy or pathological circumstances. EXOs include a variety of regulatory substances, including proteins, lipids, mRNAs, and microRNAs (miRNAs). These substances have an impact on biological processes and can move between various cell types (9). These nanoparticles originate from different cells and can transport and release contents to target cells to act as intercellular mediators (10). All EXOs have membrane-associated proteins due to their endosomal origin, and these proteins can be used as biomarkers to identify EXOs. Tetraspanins, GTPases, heat-shock proteins, proteins involved in the formation of multivesicular bodies and antigen-presenting cells, and protein biomarkers including CD81, CD9, CD63, Alix, and TSG101 are some of the categories into which the biomarkers can be divided (11). Mesenchymal stem cell (MSC)-derived EXOs have recently demonstrated promise for use in treating various disorders because of their propensity to come from stem cells as well as the fact that they are more biologically stable and less immunogenic than MSCs. EXOs appear to have a therapeutic effect on female reproductive troubles, including the repair of injured endometrium, suppression of endometrial fibrosis, regulation of immunity and anti-inflammation, and inhibition of granulosa cell death in ovaries (12).

Despite the routine prescription of drugs for the treatment of PCOS, this disease remains a leading cause of female infertility in the world. New research is moving towards using the effects of stem cells and EXOs derived from them to treat this disease in the reproductive system of women. Studies have examined the effects of stem cells and EXOs on reproductive system diseases; however, the current research focuses on their potential for use specifically in the diagnosis and treatment of PCOS.

Considering the importance of fertility, it seems necessary to focus on the treatment of diseases leading to infertility such as PCOS. Studies related to this syndrome began seriously early in the 21st century. Although research into the benefit of using stem cells and EXOs in diagnosing or medicating PCOS began around the same time, it had not attracted a large amount of attention among researchers. Recent years have seen the rapid development of new technologies in the field of stem cells and EXOs isolation from them; accordingly, it is hoped that these elements will be successful in the treatment of this syndrome and be applied in clinical research. Figure 1 presents a history of studies on PCOS with and without the use of cell stem cells and their EXO derivatives.

2 Basics of PCOS and stem cells

PCOS is a prevalent metabolic and endocrine condition affecting women of reproductive age. It is a significant factor and the main reason for infertility among women of reproductive age (13–16). The prevalence of this heterogeneous familial disorder varies based on the population studied, but it generally ranges from 8% to 13% in adult women and around 6% in adolescent girls (17).

PCOS is a complex disease with an uncertain and specific etiology. It results from the interplay of various factors, including genetic susceptibility, intra- and extrauterine influences, as well as environmental factors (18). Some studies have reported that PCOS is characterized by chronic inflammation and an increase in cytokines such as interleukin-1β, interleukin-1 receptor antagonist, interleukin-6, interleukin-17, interleukin-18, and other factors like fibroblast growth, vascular endothelial growth, and pigment epithelium-derived factor play pathophysiological roles in the development of PCOS (14). One study showed that daughters of PCOS-afflicted mothers are also highly at risk for this disease. The factor for this predisposition is the Anti-Mullerian hormone (AMH)-coding gene, while other genes can also prove the genetic component of this disease (19, 20).

Luteinizing hormone (LH), FSH, AMH, and androgens all have an impact on the dynamic equilibrium between dormant and developing follicles in the ovary, which results in ovulation. In PCOS, the usual dynamic equilibrium breaks out. Androgen overproduction occurs in the ovary and/or the adrenal gland. The plethora of tiny follicles and incapacity to select the dominant follicle define the ovary's morphology. Ovarian dysfunction is correlated with alterations in kisspeptin, gonadotropin-releasing

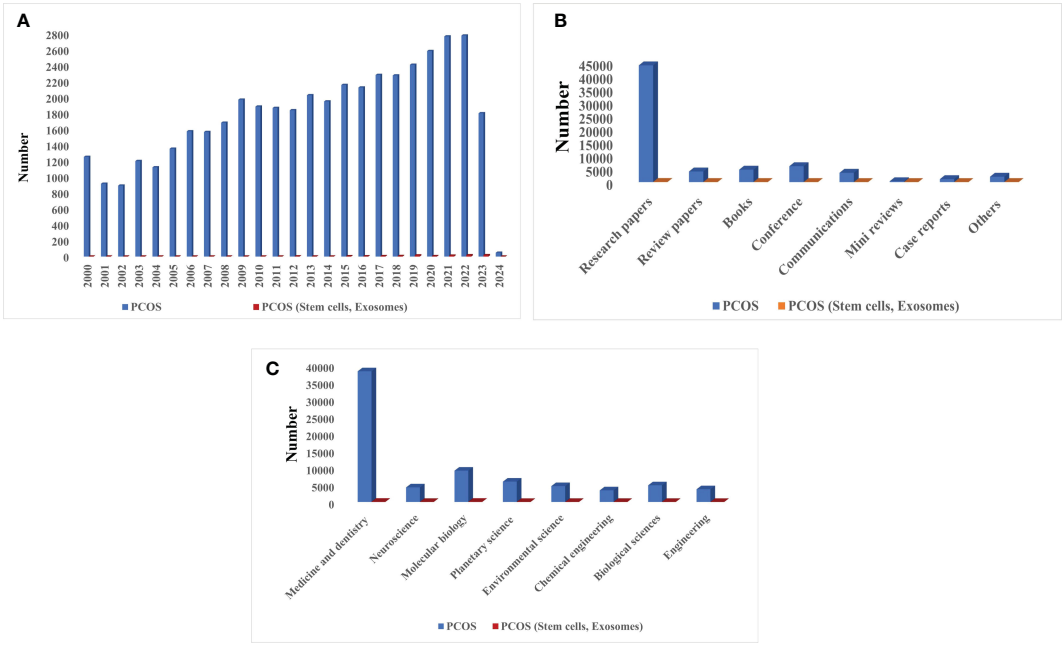

FIGURE 1
Scientific attention to PCOS based on Scopus, PubMed, and Web of Science reports: **(A)** Number of published articles on PCOS with and without stem cells and EXOs from 2000 to 2024, **(B)** Type of published literature on PCOS with and without stem cells and EXOs, **(C)** Statistics of scientific attention to different branches PCOS with and without stem cells and EXOs.

hormone, LH, and FSH output. It has been emphasized how important androgen activities are to neuroendocrine function. In addition to affecting egg quality, obesity is linked to an aberrant ovarian microenvironment (17).

To diagnose PCOS in adult women, the Rotterdam PCOS criteria are commonly employed, which require the presence of two out of three findings: clinical and/or biochemical hyperandrogenism, oligo-anovulation, and polycystic ovary morphology on ultrasound. Diagnosing PCOS in adolescents, however, can be challenging because of the significant overlap of its symptoms with normal changes during puberty, making accurate diagnosis difficult most of the time (17, 18, 21). PCOS goes well beyond being considered simply as an ovarian disorder; it is now recognized as a complex, polyfactorial, polygenic, inflammatory, dysregulated steroid state autoimmune, multi-systemic disease (20).

The comorbidities of PCOs can be classified into three major categories: metabolic, reproductive, and psychological. Irregular menses, subfertility, insulin resistance, obesity, cardiovascular disease, anxiety, depression, and altogether poor quality of life are the main comorbidities and consequences of PCOS (17, 18, 22). According to guidelines, the exact treatment for PCOS's underlying cause is not yet an available option, but symptoms therapy and preventing long-term morbidity associated with PCOS are prevalent treatments these days that can be achieved by 5-10% weight loss and/or a combination of oral contraceptives and ovulation stimulating drugs (such as letrozole, clomiphene, and metformin) for women who have not resumed ovulation (Figure 2) (16, 18, 23). International evidence-based PCOS recommendations that stress PCOS prevention, screening, and therapy across the reproductive lifespan were created as a result of the identification of pertinent difficulties in the treatment of adolescents and women with PCOS.

Stem cells are among the most significant and vital cells in the bodies of creatures with distinctive properties. Two important and main characteristics of stem cells are their differentiation into different forms of specialized cells and their unlimited division without differentiation as long as an organism is alive (24–28). These two characteristics of stem cells are an outcome of the types of divisions they undergo. Firstly, stem cells can perform symmetric division, which leads to the production of two daughter cells, each retaining the full stem cell potential. The second kind of division is known as asymmetric divide, where one stem cell is generated, which retains its stem cell properties, and a progenitor cell is produced, which has a reduced self-renewal potential (25–29).

Stem cells are categorized based on their source into four main types: embryonic stem cells (ESCs), fetal stem cells (FSCs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs). Each type has distinct characteristics and differentiation potentials, making them valuable for various research and medical applications (28–31). ESCs are created from the derivatives of the epiblast layer in the inner layer of the blastocyst, which can transform into the three primary germ layers of ectoderm, endoderm, and mesoderm (25, 29, 32–34).

FSCs are a category of stem cells derived from three main cell lines: fetal hematopoietic stem cells, neural stem cells (NSCs), and fetal-MSCs. These FSCs possess the ability to undergo division, proliferation, and differentiation into specialized cell types. These cell lines originate from fetal tissues and exhibit the capacity to divide, proliferate, and differentiate into specific cell types as needed

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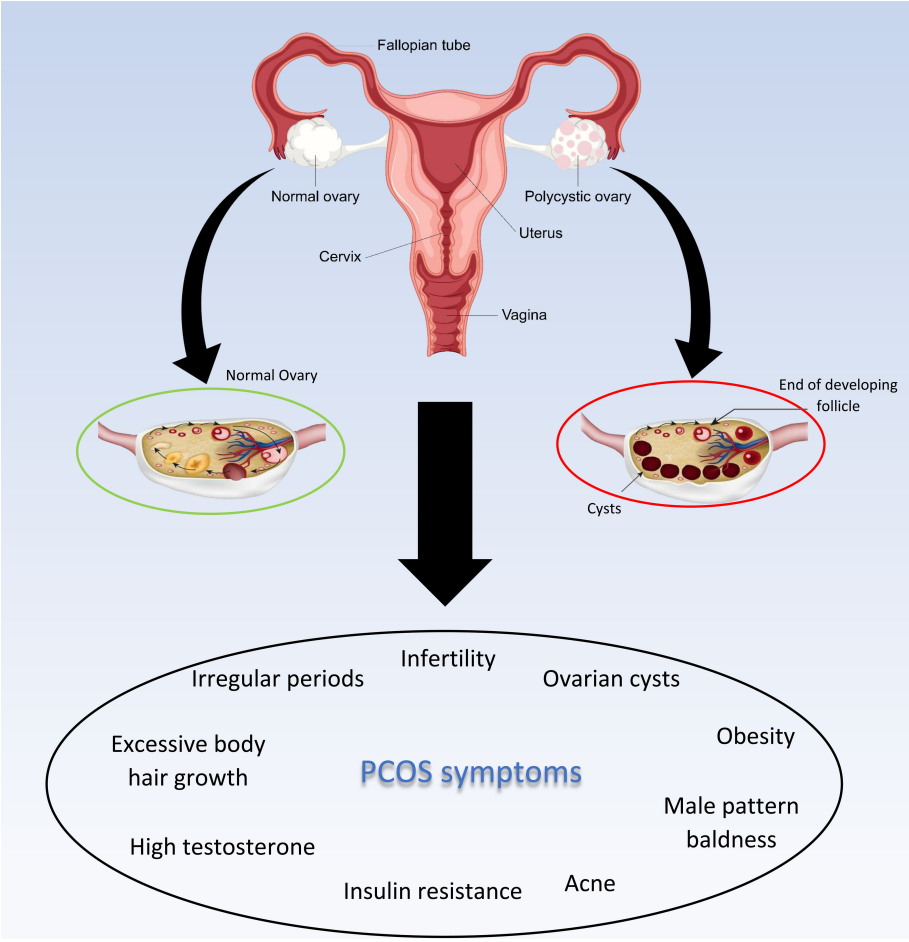

FIGURE 2
PCOS and its symptoms.

(25, 29). ASCs or somatic stem cells are cell lines that exist in post-natal adult tissues and can be either uni- or pluripotent. This less differentiated cell line is the origin of the production of other types of cells and can be classified into several cell types, i.e., epidermal stem cells (EDSCs), NSCs, MSCs, and hematopoietic stem cells (HSCs) (35).

One form of stem cell that produces three differentiated primary germ layers is IPSCs, which are produced from somatic stem cells. Because they are derived from the patient's body cells, they have a lower risk of rejection (36). Sources of MSCs include umbilical cord, bone marrow, and adipose tissue (37). Previous pre-clinical and clinical trials have shown that MSCs are effective in treating a variety of female reproductive disorders and can be used to treat PCOS (38). Other stem cell classifications are based on the ability to differentiate totipotent, pluripotent, and multipotent. The ability to transform into a differentiated cell decreases from the first category to the third category, respectively (25, 26, 28). Based on the above content and recent studies, it can be theorized that stem cells have a high capacity for the treatment of various diseases (Figure 3) (26, 33, 39).

3 Exosome: definition and application in PCOS treatment

Initially, it was seen that extracellular vesicles (EVs) bud straight from the plasma membrane; however, EVs secreted by adult reticulocytes have a more intricate secretion pattern, as found in the 1980s by Pan and Harding. Cellular communication is mostly mediated by EVs, once known as cellular trash. EXOs (40 to 120 nm) are the smallest of the three EV subtypes. Johnston, a pioneer in this field, gave this EV the term "exosome" for the first time in 1987 because of its uniqueness (40, 41). EXOs are produced when multivesicular bodies (MVBs) fuse with the plasma membrane. MVBs are absorbed by the process of endocytosis, which is controlled by many processes that also govern the development of early endosomes and the inner budding of the plasma membrane. While various payloads are sorted into intraluminal vesicles (ILVs) to produce MVBs in early endosomes, a subset of proteins is returned to the plasma membrane. Finally, complete MVBs may also merge with lysosomes for degradation or with the plasma membrane to

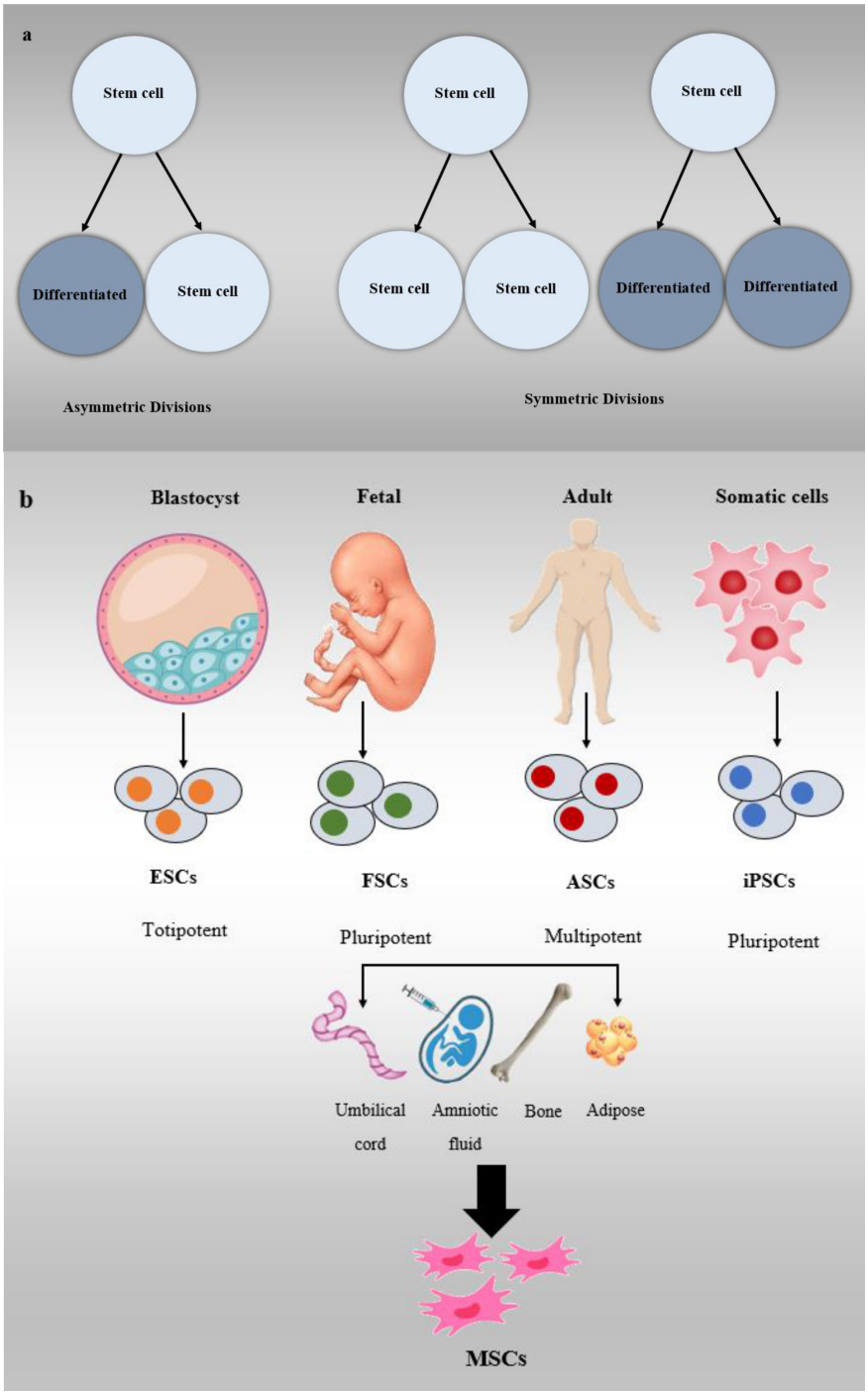

FIGURE 3
Stem cell classifications, **(A)** Symmetric and asymmetric division in stem cells, **(B)** Stem cells are divided into different types including ESCs, FSCs, ASCs, and iPSCs.

discharge ILVs as EXOs (42, 43). In recent decades, EXOs have been shown to have the potential to be used as transport vehicles and to contain several components such as miRNAs, nucleic acid RNAs, and proteins, which play important roles in extracellular communication (Figure 4) (11, 44).

Like MSCs, MSC-EXOs have the innate ability to suppress immune response and inflammation (45). EXOs produced by adipose MSCs have been demonstrated to impact the immune system by increasing the production of immunomodulatory cytokines, decreasing γ interferon expression and transcription factors, and limiting the local inflammatory response (46). Furthermore, MSC-derived EXOs (MSC-EXOs) exhibit an angiogenic effect by stimulating the proliferation of vascular endothelial cells. Many biological processes, including reproduction, tissue renewal and repair, and embryonic development, depend on these EXOs. Their ability to modulate

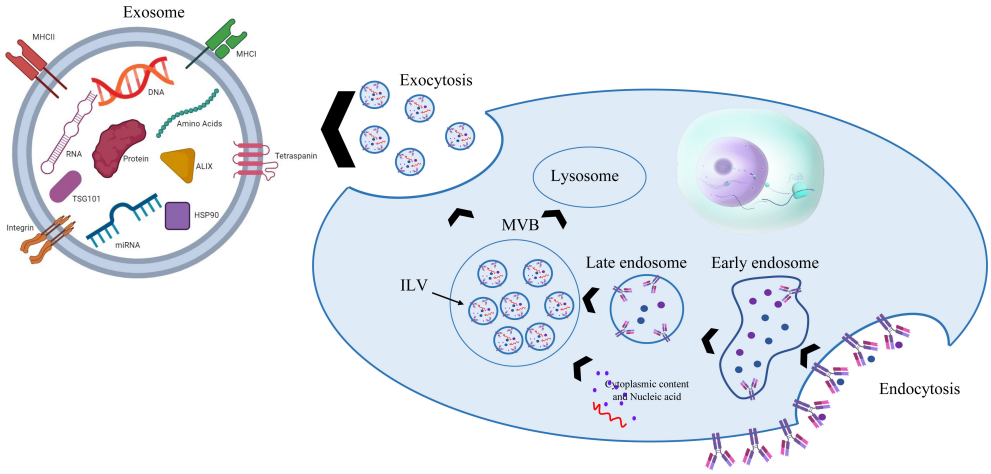

FIGURE 4
Biogenesis and structure of EXOs.

these processes makes them promising candidates for various therapeutic applications, particularly in promoting tissue repair and improving reproductive outcomes (47). MSC-derived exosomes (MSC-EXOs) have the ability to transfer and regulate miRNAs and proteins, leading to the increased expression of Bcl-2 and decreased expression of Bax. Epithelial cells, cardiomyocytes, and bone cells all experience less apoptosis because of this adjustment in Bcl-2 and Bax expression. Furthermore, MSC-EXOs have a significant task in promoting tissue regeneration, aiding in the regeneration of damaged tissues (48–50).

In a rat model, AMSC-EXOs demonstrated the ability to improve fertility, treat PCOS, and protect against metabolic issues. In PCOS rats, miR-21-5p was transferred by AMSC-EXOs, which activate the IRS1/AKT pathway and improve hepatic metabolism. Additionally, they suppress the expression of the B-cell translocation gene. As a result, AMSC-EXOs ameliorate metabolic dysregulation in rats by delivering miRNAs to the liver, presenting a potential therapeutic approach for treating PCOS-related metabolic disturbances (51). AMSC-EXOs edited with miR-323-3p reduce PCOS symptoms by inhibiting cumulus cell apoptosis and increasing cell proliferation by engaging programmed cell death protein 4 (52).

According to research results, EXOs obtained from hUC-MSCs suppress chronic inflammation by decreasing the generation of inflammatory mediators such as TNF-α, and IFN-γ, elevating IL-10 levels and anti-inflammatory cytokines and reducing ovarian granulosa cell apoptosis. This research provides more proof that blocking the NF-κB signaling is the method through which anti-inflammation is accomplished (53).

According to research findings, MSC-derived exosomes are essential molecules that control androgen synthesis in an *in vitro* model and restore fertility in a mouse model of PCOS. In the context of PCOS, intravenous and intraovarian injection have both demonstrated novel therapeutic potential. In systemic regulation, such as blood sugar control, intravenous administration produces a more favorable outcome. Contrarily, intraovarian injection exhibits greater efficacy in regaining ovarian function. Although more clinical trials are required in our future research, MSC-derived exosomes may offer PCOS patients a promising therapeutic option (54).

Female rats were treated cell-free (Condition media (CM) of stem cell and exosome) after letrozole-induced PCOS. According to the study's findings, the treatment of PCOS with both EXO and CM produced from BM-MSCs appears promising. However BM-MSCs-derived CM are superior to BM-MSCs-derived exosomes in biochemical, morphological, and functional aspects. This can be due to the fact that CM also includes numerous other biological components besides EXO (Table 1) (55).

4 Stem cell application in PCOS treatment

MSC-based therapy has arisen as a potentially beneficial alternative for PCOS because of its immunomodulatory activities, particularly in inflammatory-related disorders, as well as its differentiation potentials and self-renewal capabilities. Extensive research has revealed the capacity of MSCs to improve and repair ovarian fertility, primarily through paracrine signaling pathways. The RAP1/NFkb signaling path acts as a crucial character in controlling MSC activity. The function of MSCs is significantly impacted by their paracrine activities, regulating inflammatory and immune responses, facilitating tissue healing, and encouraging the differentiation of progenitor cells into specialized tissue cells. According to research, women with PCOS often experience chronic inflammation; thus, they are more likely to develop type 2 diabetes (DMT2), metabolic syndrome, obesity, and insulin resistance. MSC-based therapy holds promise in addressing the underlying inflammation and restoring ovarian function, making it

TABLE 1 EXOs application in PCOS treatment.

EXOs sources	Bioactive compounds	Modeling	Finding	Ref.
UC-hMSCs	IL-10 IFN-γ TNF-α	Human	Anti-inflammatory Suppressed of NF-κB Activated signaling	(53)
AMSCs	miR-21-5p	Mice	Protect against metabolic problems, Decreased PCOS Increased fertility	(51)
AMSCs	miR-323-3p	Mice	Proliferation of cells Inhibited apoptosis in CCs through targeting PDCD4	(52)
UC-hMSCs	IL-10	*In vitro* Mice	Restored ovarian function Blood glucose control Restored fertility	(54)
BM-MSCs	IL-10 TNF-α	Rat	Restored normal histological structure of the ovaries Restored fertility	(55)

a potential therapeutic approach for managing PCOS and its associated complications (56).

Research findings indicate that patients with PCOS show poor results in in vitro maturation (IVM) of oocytes. However, MSCs secrete a wide variation of growth factors and cytokines that can aid in the maturation of oocytes. One study showed that the addition of 50% human bone marrow mesenchymal stromal cell-conditioned medium (hBM-MSC-CM) to the IVM medium significantly improved the fertilization rate as well as the nuclear and cytoplasmic maturation of germinal vesicle (GV) oocytes. It also led to an increase in blastocyst formation and the two-cell rate of developed oocytes from PCOS-affected mice. These findings suggest that MSC-secreted factors can positively influence the oocytes maturation in PCOS patients during in vitro procedures (57).

In animal models of PCOS, treatment with BM-MSC has shown meaningful reductions in the expression of steroidogenic genes, leading to the restoration of fertility and a decrease in inflammation. Interleukin-10 (IL-10) was discovered to be a key mediator of the therapeutic benefits of BM-MSC treatment in PCOS animals. The reported results indicate that BM-hMSC therapy effectively improves fertility and positively impacts metabolic and reproductive parameters in PCOS animal models (4).

According to studies on the causes of PCOS, inflammation and oxidative stress may contribute to the pathophysiology of the condition (58). Thus, targeting the previously mentioned systems can be an effective treatment method. As a result of their immunomodulatory, anti-oxidative, and anti-apoptotic properties, BM-MSCs are being employed in the treatment of many inflammatory illnesses (58, 59). Furthermore, it has been shown that BM-MSC transplantation is effective in the treatment of ovarian dysfunction diseases and damage (58).

PCOS patients treated with BM-MSCs showed a significant increase in the total ovary, cortex, and oocyte volumes; zona pellucida thickness; and the number of antral follicles as well as a significant decrease in the number of preantral and primary follicles compared to the PCOS ills. Moreover, the blood levels of FSH and TAC were considerably elevated, whereas the levels of LH, testosterone, and MDA and the proportion of apoptotic cells that were TUNEL-positive substantially dropped in PCOS patients treated with BM-MSCs (Table 2) (58).

5 EXO as a biomarker in PCOS

Various studies around the world have shown that different types of EXO contents, especially miRNAs, may play a role in PCOS etiology by influencing the production process and the transfer of various substances, providing the potential that they may be utilized as diagnostic and therapeutic biomarkers (61, 62).

Several experiments have investigated the function of EXOs in the context of metabolic disorders in PCOS. For instance, a study conducted in 2020 analyzed human follicular fluid (HFF) from

TABLE 2 Stem cells application in PCOS treatment.

Stem cell type	Modeling	Finding	Ref.
UC-MSC	Mice	Suppression of ovarian systemic and local inflammatory responses	(38)
BM-hMSC	Mice	Decreased expression of steroidogenic gene Decreased inflammation Increased fertility	(60)
hBM-MSC-CM	Mice	Improved the oocyte-IVM, cytoplasmic development and fertilization	(57)
BM-MSCs	Mice	Increased the volume of the ovary, cortex, the number of corpuses luteum and antral follicles Decreased the number of primary and preantral follicles Decreased the serum level of testosterone, LH, MDA, and percentage of TUNEL-positive apoptotic cells	(58)
Conditioned media of BM-MSCs	Rat	Restored normal histological structure of the ovaries Restored fertility	(55)

people with and without PCOS focusing particularly on EXOs. The study revealed ten miRNAs whose expression was noticeably increased in PCOS patients (miR-6087, miR-193b-3p, miR-4745-3p, miR-199a-5p, miR-199a-3p, miR-4532, miR-629-5p, miR-199b-3p, miR-25-3p, and miR-143-3p). Conversely, these miRNAs (miR-200c-3p, miR-483-5p, miR-382-5p, miR-98-5p, miR-23b-3p, miR-200a-3p, miR-10a-5p, miR-141-3p, miR-3911, and miR-483-3p) showed a substantial reduction in expression in PCOS patients. These results present valuable insight into the potential function of EXOs and miRNAs in the metabolic alterations associated with PCOS (63).

In their study, Hu et al. focused on transfer RNAs (tRNAs) and piwi-interacting RNAs (piRNAs) in relation to PCOS pathogenesis. piRNAs are little RNA molecules that range in size from 24 to 32 nucleotides and are prevalent in the germline of all animal species. The study found different expression patterns of piRNAs when compared to controls in PCOS patients. Specifically, ten piRNAs exhibited markedly increased expression (pir-57942, pir-36441, pir-34896, pir-54998, pir-51671, pir-33221, pir-36040, pir-33226, pir-43997, and pir-33405), while ten others (pir-35414, pir-43772, pir-35413, pir-43771, pir-35469, pir-35463, pir-35468, pir-33065, pir-33387 and pir-35467) showed a substantial reduction in expression. Additionally, the authors of the study investigated tRNAs in EXOs HFF in relation to PCOS. They discovered ten tRNAs with markedly increased expression (tsrna-12363, tsrna-12365, tsrna-12364, tsrna-12362, tsrna-12360, tsrna-12361, tsrna-17099, tsrna-12359, tsrna-12395, and tsrna-17100) and ten others with substantially reduced expression (tsrna-06176, tsrna-06177, tsrna-14937, tsrna-14935, tsrna-15209, tsrna-15199, tsrna-14934, tsrna-03939, tsrna-03940 and tsrna-15198). These findings suggest that altered expression of piRNAs and tRNAs may play important mechanistic roles in PCOS pathogenesis. Moreover, previous research has associated mitochondrial tRNA mutations with PCOS, further supporting the potential significance of these findings in understanding the underlying mechanisms of PCOS (63, 64).

In an attempt to better understand how highly expressed miRNAs in follicular fluid affect steroidogenesis, particularly estradiol and progesterone secretion, Sang et al. found that 8 out of 12 miRNAs significantly influenced steroidogenesis. Estradiol levels were specifically controlled by miR-320, miR-132, miR-24, miR-520c-3p, and miR-222, whereas progesterone levels were regulated by miR-193b, miR-24, and miR-483-5p. Additionally, the target genes associated with these miRNAs were involved in cell growth and development of the immune system (65).

A 2022 study identified 157 differentially expressed miRNAs in PCOS, 33 of which were decreased, while 124 of them were substantially increased. The metabolic pathway was most affected by the differentially expressed miRNAs, showing their relevance in the pathophysiology of PCOS. The research also identified a network of miRNAs and lncRNAs connected with metabolic pathways, which shed light on the main processes driving PCOS. The study also discovered 5 miRNAs as possible indicators for PCOS diagnosis: hsa-miR-196a-3p, hsa-miR-106a-3p, hsa-miR-143-5p, hsa-miR-20a-5p, and hsa-miR-34a-5p. These miRNAs may offer interesting options for enhancing the early and accurate identification of PCOS in clinical settings (66).

In studies conducted by Wang et al. (66, 67) miR-4632, miR-146a-5p, and miR-92-5p were found to be closely associated with the incidence of PCOS. According to these studies, significant roles in the progression of PCOS may be played by long non-coding RNAs (lncRNAs), which contribute to a number of cellular functions and pathways of signaling such as the Hippo signaling pathway, endocytosis, infection with human T-lymphotropic virus type 1 (HTLV-1) and mitogen-activated protein kinases (MAPK). These results emphasize the possible uses of miRNAs and lncRNAs as treatment and diagnostic targets for PCOS and offer insightful information on the molecular processes underlying the illness (67). Researchers at Northern Jiangsu People's Hospital examined follicular fluids produced after fertility treatment with IVF cycles and found that PCOS patients exhibited clear upregulation of certain hormone levels compared to non-PCOS patients. Particularly, blood levels of luteinizing hormone (LH), testosterone (T), estradiol (E2), anti-Mullerian hormone (AMH), and serum prolactin (PRL) were substantially greater in PCOS individuals. These results may help us learn more about PCOS and its treatment, as they provide light on the hormonal abnormalities connected to the disorder (67).

Research on the follicular fluid of women having PCOS found that out of 235 miRNAs, 29 had differing expression levels in the PCOS and control groups. In women with PCOS, the expression levels of five of these miRNAs—has-miR-9, 18b, 32, 34c, and 135a—significantly increased, suggesting that they may be involved in controlling the levels of inflammation and insulin. Additionally, three target genes (synaptotagmin1 (SYT1), insulin receptor substrate 2 (IRS2), and interleukin 8 (IL8)) were identified with significantly decreased expression in women with PCOS, which are associated to the PCOS phenotype (68).

EXOs exist in other body fluids in addition to follicular fluid; some studies have used serum samples to research the function of EXOs as PCOS biomarkers (63, 69–72). Long et al. determined that three miRNAs (miR-30c, miR-222, and miR-146a) have considerably greater production levels in patients with PCOS. Furthermore, serum insulin and miR-222 were favorably correlated, but miR-146a was inversely correlated with serum testosterone levels. Genes targeted by these miRNAs were discovered to be important in endocrine processes, apoptosis, cell cycles, metastasis, and the Jak-STAT, Wnt, and MAPK signaling pathways (73).

Another study on the serum EXOs of PCOS patients identified a relationship between PCOS and individual miRNAs, miR-126–3p, and miR-146a-5p. IL6 and tumor necrosis factor levels in the blood were discovered to be correlated with miR-146a, and these two variables are thought to be crucial in the development of PCOS (10). In another research on the microRNA profile of serum EXOs' sequencing in women with and without PCOS, four miRNAs, namely hsa-miR-192-5p, has-miR-1299, hsa-miR-145-5p, and hsa-miR-6818-5p, were identified as potential biomarkers for PCOS. These miRNAs may hold promise for improving the diagnosis and management of PCOS because of their differential expression patterns in women with the condition compared to those without it (74).

Independent of age and body mass index (BMI), the full profiling of miRNAs in the blood of PCOS women indicated

raised amounts of miR-485-3p and miR-1290 and lowered levels of miR-139-3p, miR-21-3p, miR-572, miR-361-5p, and miR-143-3p. Five miRNAs (miR-1290, -20a-5p, -139, -433, and -361-5p) were significantly related to high testosterone levels after adjusting for age and BMI. Five miRNAs were shown to be related to both abnormal glucose homeostasis and dyslipidemia particularly (miR-20a-5p, -433-3p, 1225-3p, -1290, and -361-5p) (75).

Some studies have emphasized the significance of other exosomal indications in the advancement of PCOS. For example, He et al. reported elevated miR-200c and miR-141 expressions in the granulosa lutein cells (GLCs) of PCOS patients. These two miRNAs may have crucial functions in the control of the PI3K and Wingless type protein (Wnt) signaling pathways, which may have an impact on the occurrence and development of PCOS (69, 76, 77).

In addition to the involvement of nucleic acids in PCOS, recent studies have also explored the impact of other EXO contents on the disease. Han et al. discovered that the S100-A9 protein present in EXOs, secreted by ovarian cells, granulosa cells, and leukocytes, could activate NF-κB signaling pathways within granulosa cells. This activation, in turn, led to an increase in inflammatory cytokine production and steroidogenesis disruption. These results imply that S100-A9 in EXOs may be essential for controlling granulosa cell activity and have an impact on the pathophysiology of PCOS (61, 78). Furthermore, by inhibiting DAPK1, miR-141-3p may prevent ovarian granulosa cell death and contribute to the development of PCOS (79).

Exosomal DENND1A.V2 RNA was detected in greater concentrations in PCOS patients' urine, according to research. Additionally, PCOS theca cells expressed DENND1A.V2 protein at a greater level (80). DENND1A.V2 may impact insulin or luteinizing hormone (LH)-receptor turnover, influencing ovarian function in PCOS patients, according to another study. A component of the insulin and MAPK signaling networks, DENND1A.V2 functions as a guanine nucleotide-exchange factor for Rab, and Rab-5B, a protein associated with Ras, binds with the DENN domain. These conclusions suggest a potential role for DENND1A.V2 in the pathophysiology of PCOS through its involvement in the regulation of insulin and signaling pathways (61). According to one research, PCOS patients had substantially greater levels of mRNA expression for the proteins CYP11A, CYP19A, and HSD17b1 in their follicular fluid relative to study controls (81, 82).

Endometrial cancer, a significant concern and consequence of PCOS, has been the focus of research efforts aiming to reduce its incidence in individuals with PCOS. A recent study conducted in China investigated the levels of 55 mature miRNAs in serum EXOs of PCOS patients and found that miR-27a-5p exhibited the most significant elevation in the serum EXOs of PCOS patients. The study identified the SMAD4 gene as the target of miR-27a-5p, and its effect was shown to stimulate endometrial cancer cells to move around and invade other tissues. These findings highlight the potential role of miR-27a-5p in promoting endometrial cancer in the context of PCOS, providing valuable insights for potential therapeutic strategies to mitigate the risk of endometrial cancer in individuals with PCOS (Table 3) (83).

TABLE 3 Application of exosome as a biomarker in PCOs.

Model study in PCOS	EXOs source	RNA type	Ref.
HFF samples from IVF undergoing female (*in vitro*)	HFF	miRNAs/ piRNAs/ tRNAs	(63)
Human Serum samples (*in vitro*)	Human serum samples	miRNA	(74)
Human serum samples	Human serum samples	miRNA	(83)
HFF samples from IVF and ICSI undergoing female (*in vitro*)	HFF	miRNA	(78)
HFF (*in vitro*)	HFF	miRNA/ lncRNA	(66)
HFF samples from ICSI (*in vitro*)	HFF	miRNA	(65)
HFF samples from IVF and ICSI (*in vitro*)	HFF	miRNA	(82)
HFF samples from IVF (*in vitro*)	HFF	lncRNAs	(67)
Human serum (*in vitro*)	Human serum	miRNA	(73)
Human serum (*in vitro*)	Human serum	miRNA	(10)
Granulosa cells undergoing IVF and ICSI from FF (*in vitro*)	HFF granulosa cells	miRNA	(76)
Rat ovarian granulosa cells (*in vivo*)	Rat granulosa cells	miRNA	(79)
Human Theca cells	Human Theca cells	miRNA	(80)

Conclusion

We offer here a potentially successful avenue for the development of novel cell-based (stem cells) and cell-free (EXOs) diagnostic and therapeutic approaches for PCOS-related fertility therapy. Recent research indicates that stem cells and EXOs suppress inflammation and apoptosis, control steroidogenesis, and prevent the synthesis of androgens both *in vivo* and *in vitro*. Our knowledge of exosomal miRNA expression profiles in PCOS patients is also improved by this study. Consequently, it is worthwhile to challenge the effectiveness and efficiency of these compounds in the treatment of PCOS. We anticipate that with further developments in the study of EXOs and stem cells, these two topics will be able to significantly contribute to the early and more precise diagnosis of PCOS as well as be useful treatment and interventional tools for patients with this condition. Even though the research directions for EXOs and stem cells still need to be explored, with the persistent efforts of researchers and medical professionals, these technologies might someday become useful aids and significant medical advancements, improving the health of women with PCOS.

Author contributions

MH: Conceptualization, Writing – original draft, Investigation. KK: Conceptualization, Investigation, Writing – original draft. EG: Conceptualization, Investigation, Writing – original draft. LR: Writing – original draft, Conceptualization, Project administration, Supervision. MK: Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Glossary

EXO	Exosome
PCOS	Polycystic ovary syndrome
NIH	National Institutes of Health
MSCs	Mesenchymal stem cells
ASCs	Adipose stem cells
ASCs-EXOs	Adipose stem cells exosomes
ESCs	Embryonic stem cells
EDSCs	Epidermal stem cells
NSCs	Neural stem cells
HSCs	Hematopoietic stem cells
IPSCs	Induced pluripotent stem cells
EVs	Extracellular vesicles
NFkb	Nuclear factor kappa light
RAP1	Repressor activator protein 1
CM	Conditioned media
UC	Umbilical cord
PDCD4	Programmed Cell Death 4
CCs	Cumulus cells
IVM	In vitro maturation
GVs	Germinal vesicles
UC-MSCs	Umbilical cord Mesenchymal stem cells
DMT2	Type 2 diabetes mellitus
BM-hMSCs	Human bone marrow Mesenchymal stem cells
IL-10	Interleukin-10
BM-MSCs	Bone marrow Mesenchymal stem cells
BM-hMSC-CM	Human bone marrow Mesenchymal stem cell Conditioned media
TAC	Total antioxidant capacity
FSH	Follicle-stimulating hormone
LH	Luteinizing hormone
MDA	Malondialdehyde
TUNEL	Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling
MSC-EXOs	Mesenchymal stem cell exosomes
AMSCs	Adipose mesenchymal stem cells
TNF-α	Tumor necrosis factor alpha
IFN-γ	Interferon-gamma
UC-hMSCs	Human umbilical cord Mesenchymal stem cells
PDCD4	Programmed Cell Death 4

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miRNAs	microRNAs
HFF	Human follicular fluid
piRNAs	Piwi-interacting RNA
TRNAs	Transfer RNA
LncRNAs	Long noncoding RNAs
MAPK	Mitogen-activated protein kinases
HTLV-1	Human T-lymphotropic virus type 1
T	Testosterone
PRL	Prolactin
AMH	Anti-Mullerian hormone
IL-8	Interleukin 8
IRS2	Insulin receptor substrate 2
SYT1	Synaptogamin 1
JAK-STAT	Janus kinases, signal transducer, and activator of transcription proteins STATs
AFC	Antral follicle count
GLCs	Granulosa-lutein cells
Wnt	Wingless type protein
PI3K	Phosphatidylinositol-3-kinase
DAPK1	Death-associated protein kinase 1
DENND1A.V2 RNA	DENN domain-containing 1A variant 2
CYP11A	Proteins Cytochrome P450 Family 11 Subfamily A Member 1
CYP19A	Proteins Cytochrome P450 Family 19 Subfamily A Member 1
HSD17b1	17β-Hydroxysteroid dehydrogenase 1
NIH	National Institutes of Health
LH	Luteinizing hormone
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
HA	Hyperandrogenism
IR	Insulin resistance
CTGF	Connective tissue growth factor
E2	Estradiol
RAB5B	Ras-related protein Rab-5B

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Effect of hyperlipidemia on the outcome of *in vitro* fertilization in non-obese patients with polycystic ovary syndrome

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Introduction: It is little known whether hyperlipidemia alone has adverse effects on the outcome of *in vitro* fertilization (IVF) in patients with polycystic ovarian syndrome (PCOS).

Methods: The PCOS patients with body mass index (BMI) < 30 kg/m^2 were performed IVF or intracytoplasmic sperm injection treatment, including 208 fresh cycles and 127 frozen embryo transfer (FET) cycles. All the patients were divided into hyperlipidemia and control groups, and embryo quality and pregnancy outcomes between the two groups were compared.

Results: In the fresh cycles, total gonadotropin dosage in the control group was significantly lower than that in the hyperlipidemia group, and serum estradiol levels on trigger day were reversed ($P < 0.05$). The embryo fragment score was positively correlated with serum low-density lipoprotein level ($r = 0.06$, $P < 0.05$) and negatively with serum high-density lipoprotein (HDL) and lipoprotein A levels ($r = -0.489$ and -0.085, $P < 0.01$). Logistic regression analysis found that HDL was beneficial for clinical pregnancy (OR = 0.355, 95% *CI*: 0.135-0.938, $P < 0.05$). In the FET cycles, there were no differences in pulse index, systolic/diastolic ratio and serum estradiol and progesterone levels between the two groups, but resistance index in the hyperlipidemia group was significantly higher than that in the control group ($P < 0.05$).

Conclusion: Hyperlipidemia may increase the dosage of gonadotropin and have adverse effect on the embryo quality, endometrial receptivity, and clinical outcomes of lean PCOS patients. It is recommended that the non-obese patients with hyperlipidemia and PCOS perform lipid-lowering treatment before undergoing embryo transfer.

KEYWORDS

polycystic ovary syndrome, hyperlipidemia, *in vitro* fertilization, pregnancy outcome, non-obese

1 Introduction

In women of reproductive age, polycystic ovarian syndrome (PCOS) is the most prevalent endocrine condition and is characterized by hyperandrogenism, recurrent anovulation, and polycystic ovaries. A portion of PCOS is frequently linked to metabolic abnormalities such as obesity, insulin resistance (IR), poor glucose tolerance, lipid metabolic diseases, and others. Among these features obesity associated with IR is a major trigger of these disorders. Numerous researches in recent years have revealed that IR and obesity are associated with particular reproductive health issues, such as reduced clinical pregnancy rates in assisted reproductive technology (ART) cycles (1). However, there are few reports on the effects of dyslipidemia, especially in non-obese patients with PCOS, on the outcomes of assisted reproduction. It has been demonstrated that dyslipidemia in PCOS patients may exist without obesity (2), but it can occasionally exacerbate metabolic abnormalities such as obesity, IR, and others. About 70% of obese patients with PCOS have lipid abnormalities, and about 20% of lean PCOS individuals have impaired lipid metabolic disorders (3, 4). Some studies have proved that hyperlipidemia affected pregnancy outcomes of ART, such as implantation rate, clinical pregnancy rate, and miscarriage rate (5), which might be a result of alterations in the microenvironment of oocytes and endometrium brought on by lipids. Our study aimed to determine whether dyslipidemia affected the embryo quality and pregnancy outcome of non-obese PCOS patients undergoing *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI), which might indirectly reflect the oocyte quality and endometrial receptivity.

The ART cycle includes fresh embryo transfer and frozen embryo transfer (FET) cycles. Considering the many differences between fresh and FET cycles, such as the higher dosage of gonadotropin (Gn), different ovarian stimulation protocols, higher estrogen levels on the human chorionic gonadotropin (HCG) trigger day, and unfrozen embryos in fresh cycles, this study investigated the fresh and FET cycles separately and compared the effects of dyslipidemia on their pregnancy outcomes. Meanwhile, considering that lipids are the substrate of steroid hormones and that abnormal lipid metabolism may have an impact on endogenous hormones, this study only selected the hormone replacement therapy (HRT) protocol for the FET cycle, which may minimize the impact of confounding factors on the results by supplementing exogenous hormones as the endometrial preparation protocol.

2 Materials and methods

2.1 Patients

The clinical data of patients undergoing conventional IVF or ICSI treatment in Center for Reproductive Medicine of Zhongda Hospital during October 2017 and June 2021 were analyzed retrospectively. The implementation of IVF or ICSI was approved by the Reproductive Medicine Ethics Committee of Zhongda Hospital affiliated to Southeast University (Reproduction No. 2015-1), and all patients signed the informed consent. The inclusion criteria of the patients included: (1) females aged < 35 years old, (2) body mass index (BMI) < 30 kg/m^2, and (3) patients diagnosed with PCOS. The diagnostic criteria for PCOS were based on the unified standards formulated by the Rotterdam International Conference in 2003 (6). The patients with any two of the following three conditions were diagnosed with PCOS: (1) infrequent ovulation or anovulation, (2) hyperandrogenism or clinical manifestations of high blood androgen, and (3) ultrasound findings of polycystic ovaries in one or two ovaries, 12 or more follicles with a diameter of 2-9 mm, and/or ovarian volume ≥ 10 ml after exclusion of other etiologies such as congenital adrenal hyperplasia, androgen-secreting tumors, Cushing syndrome, 21-hydroxylase-deficient nonclassic adrenal hyperplasia, androgenic/anabolic drug use or abuse. Exclusion criteria included: (1) women with endocrine or metabolic diseases such as thyroid dysfunction, hyperprolactinemia, type 2 diabetes mellitus, and cardiovascular disease, (2) oocyte donation cycles, and (3) chromosome abnormality or other genetic mutations. All the patients undergoing IVF or ICSI treatments were divided into dyslipidemia (hyperlipidemia) and normal (control) groups. The diagnostic criteria of dyslipidemia were based on the 2016 guidelines for the prevention and treatment of dyslipidemia in Chinese adults (7). Dyslipidemia was defined as having at least one of the following indexes: total cholesterol (TC) ≥ 5.18 mmol/L or ≥ 200 mg/dL, low-density lipoprotein cholesterol (LDL-C) ≥ 3.37 mmol/L or ≥ 130 mg/dL, high-density lipoprotein cholesterol (HDL-C) < 1.04 mmol/L or < 40 mg/dL, and triglyceride (TG) ≥ 1.7 mmol/L or ≥ 150.62 mg/dL. The patients underwent fresh embryo transfer or frozen embryo transfer (FET) for the first time were recruited for the study. A total of 208 fresh cycles and 127 FET cycles were included. The study conforms to the WMA Declaration of Helsinki.

2.2 Stimulation protocols for fresh cycles

Gonadotropin-releasing hormone (GnRH) antagonist and long GnRH agonist (GnRH-a) protocols were applied for controlled ovarian stimulation (COS). The initial gonadotropin dose was determined according to the patient's age, body weight, number of antral follicles, etc. Stimulation was monitored using estradiol concentrations, together with ultrasound measurements of follicle numbers and diameters. When at least 2 follicles were 18 mm or 3 follicles were 17 mm in diameter, 0.2 mg GnRH-a or 5000-10000 U human chorionic gonadotropin (HCG) were injected for triggering. Oocyte retrieval was performed 36 hours after HCG was injected through the transvaginal route with an ultrasound guidance. Embryo transplantation was performed 3 days after ovum pick-up (OPU) on the basis of exact situations such as estradiol concentration and endometrial thickness. One to two embryos

with best quality were transferred into the uterus, and then the patients were given routine corpus luteum support. The serum HCG levels of the patients were detected 14 days after embryo transfer, and serum HCG level > 50 U/L was regarded as biochemical pregnancy. B ultrasound examination was done 4 weeks after embryo transfer, and clinical pregnancy was considered if the gestational sac was found.

2.3 Endometrial preparation protocols for the FET cycle

All patients in the FET cycle used HRT for endometrial preparation. First, 4 mg of oral estradiol valerate (Progynova; Bayer, Germany) was administered per day from day 2–4 of the menstrual cycle. The patients were evaluated by transvaginal ultrasound 7 days later to adjust the dosage of estradiol based on the endometrial thickness. A supplementation of vaginal estradiol was added if the endometrial thickness continued to be unsatisfactory. When the endometrial thickness reached 8 mm, and serum progesterone level was below 1.5 ng/mL, intramuscular administration of 40 mg progesterone (Zhejiang Xianju Pharmaceutical Co., Ltd, Taizhou, Zhejiang, China) or vaginal supplementation with 90 mg progesterone (8% Crinone, Merck-Serono, Germany) was added. The parameters of resistance index (RI), pulse index (PI) and systolic/diastolic ratio (S/D) were measured on the day of progesterone administration using Voluson S8 color doppler ultrasonic diagnostic apparatus (GE Ultrasound Korea, Ltd.). One to two embryos with good quality were transferred into the uterus, and then the patients were given routine corpus luteum support. The serum HCG levels of the patients were detected 14 days after embryo transfer. If the serum HCG level was higher than 50 U/L, the patient was regarded as a biochemical pregnancy. Clinical pregnancy was confirmed by ultrasound, and one or two gestational sacs were visible approximately 4 weeks after embryo transfer.

2.4 Assessment of embryo quality

Embryo quality was evaluated on day 3 by assessing the embryo cell number and embryo fragment score (EFS). EFS was graded on the basis of the percentage of fragmentation as follows: < 5% of fragmentation, score 4; 5%–10% of fragmentation, score 3; 11%–25% of fragmentation, score 2; 26%–50% of fragmentation, score 1; and ≥ 51% of fragmentation, score 0 (8). Good quality embryos were defined as having over 6 cells with relatively equal sized blastomeres and less than 25% of fragmentation (EFS 2-4).

2.5 Laboratory analysis

Commercially available kits for the determinations of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), estradiol (E2), progesterone, anti-Müllerian hormone (AMH) and insulin were purchased from Abbott Laboratories, Inc. USA, and serum FSH, LH, T, E2, progesterone, AMH and insulin levels were determined by chemiluminescence assay using an automated Abbott Architect i1000 system (Abbott Laboratories, Inc., USA). Commercially available kits for the determinations of TG, TC, LDL-C, HDL-C, and lipoprotein A were purchased from Shanghai Zhicheng Biotechnology Co., Ltd., China. Calibration and quality control products were purchased from Randox Laboratories Ltd., Northern Ireland, United Kingdom. The determinations of serum lipids were carried out using a Beckman Coulter AU5800 automatic biochemistry analyzer (Beckman Coulter, Inc., USA).

2.6 Statistical analysis

Statistical analysis was performed with SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The measurement data were first performed by one-sample nonparametric tests (Kolmogorov-Smirnov test) to determine whether they were normal distribution. The data conforming to the normal distribution were expressed as mean ± standard deviation (x ± s), and those conforming to the non-normal distribution were expressed as median [P_{25}, P_{75}]. The comparisons between the control and hyperlipidemia groups were analyzed by independent samples *t*-test for normal distribution data or Mann-Whitney *U* test for non-normal distribution data. The count data were presented as percentages, and the comparisons between the control and hyperlipidemia groups were analyzed by the $\chi 2$ test. The standard errors (*SE*) for percentages in two samples were calculated using the following formulas (9): the observed percentage (p) in the combined samples = $(n1p1 + n2p2)/(n1 + n2)$, and $SE = [p(100\text{-p})(1/n1 + 1/n2)]^{1/2}$. Among them, $n1$ and $n2$ were the sample size of two groups, respectively, and $p1$ and $p2$ were the percentage of two groups, respectively. The Spearman rank correlation coefficients between EFS and lipids levels were calculated to evaluate the correlations of lipids levels with embryo quality. The multiple logistic regression analysis was used to analyze the impacts of blood lipids on the clinical outcomes of non-obese PCOS patients. They all performed the 2-sided test. $P \leq 0.05$ was considered to be statistically significant.

3 Results

3.1 Comparisons of basic clinical information between the control and hyperlipidemia groups in the non-obese PCOS patients undergoing fresh cycle transplantation

The basic clinical information of the PCOS patients undergoing fresh cycle transplantation was presented in Table 1. There were no statistical differences in the age, body mass index (BMI), number of

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TABLE 1 Comparisons of basic clinical information between the control and hyperlipidemia groups in the non-obese PCOS patients undergoing fresh cycle transplantation.

Indexes	Control (*n* = 113)	Hyperlipidemia (*n* = 95)	*P*-value
Age (year)	28.7 ± 3.1	29.4 ± 3.1	0.08
BMI (kg/m^2)	23.0 ± 2.1	23.4 ± 2.1	0.22
Basal FSH (mIU/ml)	6.2 ± 1.4	6.0 ± 1.6	0.42
Basal LH (mIU/ml)	7.9 ± 3.7	8.2 ± 3.7	0.55
T (ng/ml)	0.66 ± 0.38	0.59 ± 0.59	0.42
AMH (ng/ml)	10.7 ± 4.1	9.6 ± 4.7	0.07
AFC (*n*)	25.4 ± 6.9	25.5 ± 6.0	0.89
FBG (mmol/L)	5.2 ± 0.5	5.3 ± 0.6	0.13
Insulin (pmol/L)	97.9 ± 40	118.4 ± 65.9	0.18
TG (mmol/L)	1.1 [0.83, 1.39]	2.43 [1.47, 3.44]	<0.01
TC (mmol/L)	4.4 ± 1.0	5.2 ± 1.3	<0.01
HDL (mmol/L)	1.54 [1.26, 2.65]	1.18 [1.00, 1.53]	<0.01
LDL (mmol/L)	2.6 ± 0.6	3.0 ± 0.8	<0.01
Lipoprotein A (mmol/L)	61.0 [40.0, 127.0]	112.0 [57.0, 255.0]	<0.01

PCOS, polycystic ovarian syndrome; BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; T, testosterone; AMH, anti-Müllerian hormone; AFC, antral follicle count; FBG, fasting blood glucose; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. The measurement data were first performed by one-sample nonparametric tests (Kolmogorov-Smirnov test) to determine whether they were normal distribution. The data conforming to the normal distribution were expressed as mean ± standard deviation (x ± s), and those conforming to the non-normal distribution were expressed as median [P_{25}, P_{75}]. The comparisons between the control and hyperlipidemia groups were analyzed by independent samples t-test for normal distribution data or Mann-Whitney U test for non-normal distribution data. A P-value ≤ 0.05 was considered statistically significant.

antral follicles (AFC), fasting blood-glucose (FBG), insulin, FSH, LH, testosterone, and AMH ($P > 0.05$) except serum lipid levels such as TG, TC, HDL, LDL, and lipoprotein A ($P < 0.05$) between hyperlipidemia and control groups.

3.2 Comparisons of COS protocols and clinical outcomes of assisted pregnancy between the control and hyperlipidemia groups in the non-obese PCOS patients undergoing fresh cycle transplantation

The COS protocols and clinical outcomes of the non-obese PCOS patients undergoing fresh cycle transplantation were presented in Table 2. There were no significant differences in the Gn usage time, endometrial thickness, progesterone level on trigger day, oocytes retrieved, normal fertilization rate, good quality embryos rate, implantation rate, clinical pregnancy rate, and miscarriage rate between hyperlipidemia and control groups. However, the total Gn dosage in the hyperlipidemia group was significantly higher than that in the control group, while the estradiol level on trigger day in the hyperlipidemia group was significantly lower than that in the control group.

TABLE 2 Comparisons of COS protocols and clinical outcomes of assisted pregnancy between the control and hyperlipidemia groups in the non-obese PCOS patients undergoing fresh cycle transplantation.

Indexes	Control (*n* = 113)	Hyperlipidemia (*n* = 95)	*P*-value
Gn usage time (days)	8.8 ± 1.2	9.1 ± 1.4	0.13
Gn dosage (IU)	1740.9 ± 449	2125.6 ± 407	<0.01
Initial dose of Gn (IU)	207.5 ± 49.6	213.2 ± 56.9	0.43
COS protocols (*n*, %)			0.53
GnRH agonist	37 (32.7)	35 (36.8)	
GnRH antagonist	76 (67.3)	60 (63.2)	
Endometrial thickness (mm)	11.1 ± 2.2	11.5 ± 2.8	0.25
E2 on trigger day (pg/ml)	5365.0 [4113.0, 8179.5]	4532.0 [3470.0, 6584.5]	<0.01
P on trigger day (ng/ml)	1.2 ± 1.0	1.3 ± 0.8	0.32
Oocytes retrieved (*n*)	14.1 ± 3.9	13.4 ± 4.5	0.25
Normal fertilization rate	0.63 ± 0.17	0.66 ± 0.19	0.28
Good quality embryos rate	0.58 ± 0.19	0.59 ± 0.18	0.97
Clinical pregnancy rate (*n*, %)	17/36 (47.6)	13/28 (46.4)	0.95
Implantation rate (*n*, %)	21/44 (47.7)	15/40 (37.5)	0.34
Miscarriage rate (*n*, %)	2/17 (11.8)	2/13 (15.4)	0.77

COS, controlled ovarian stimulation; PCOS, polycystic ovarian syndrome; Gn, gonadotropin; GnRH, gonadotropin-releasing hormone; E2, estradiol; P, progesterone. Clinical pregnancy rate refers to the number of cycles achieving clinical pregnancy/the number of embryo transfer cycles; Implantation rate refers to the number of gestational sacs/the number of transplanted embryos; Miscarriage rate refers to the number of miscarriage cycles/the number of clinical pregnancy cycles. The measurement data were first performed by one-sample nonparametric tests (Kolmogorov-Smirnov test) to determine whether they were normal distribution. The data conforming to the normal distribution were expressed as mean ± standard deviation (x ± s), and those conforming to the non-normal distribution were expressed as median [P_{25}, P_{75}]. The comparisons between the control and hyperlipidemia groups were analyzed by independent samples t-test for normal distribution data or Mann-Whitney U test for non-normal distribution data. The count data were presented as percentages, and the comparisons between the control and hyperlipidemia groups were analyzed by the $\chi 2$ test. The standard errors (SEs) of the clinical pregnancy rate, implantation rate and miscarriage rate between the two groups were 1.73, 1.43, and 1.34, respectively. A P-value ≤ 0.05 was considered statistically significant.

3.3 Correlations of lipid levels with embryo quality in the non-obese PCOS patients undergoing fresh cycle transplantation

To further investigate the effects of lipid levels on embryo quality, we analyzed the correlations of lipid levels with EFS, and

found that there were no significant correlations between EFS and TG or TC, but there were a positive correlation between EFS and LDL (r = 0.06, P = 0.015) and negative correlations between EFS and HDL or lipoprotein A (r = -0.489, P < 0.01; r = -0.085, P < 0.01) (Table 3).

3.4 Multiple logistic regression analysis between blood lipids and the clinical outcomes of non-obese PCOS patients undergoing fresh cycle transplantation

The multiple logistic regression analysis between blood lipids and the clinical outcomes of non-obese PCOS patients undergoing fresh cycle transplantation was shown in Table 4. The results showed no any correlation between blood lipids and clinical outcomes, as shown by the crude odd ratios (ORs). However, after correcting the Gn dosage and estradiol on trigger day, HDL was shown to be a protective factor for clinical pregnancy (adjusted OR [AOR] = 0.355, 95% *CI*: 0.135-0.938, P = 0.037) and TC might be a potential risk factor (AOR = 4.072, 95% *CI*: 0.989-16.761, P = 0.052). It is indicated that HDL is beneficial for clinical pregnancy and TC may be harmful to clinical pregnancy.

TABLE 3 Correlations of lipid levels with EFS in the non-obese PCOS patients undergoing fresh cycle transplantation (n = 208).

Variable	EFS (r)	*P-value*
TG	−0.013	0.597
TC	0.042	0.088
HDL	−0.489	<0.01
LDL	0.06	0.015
Lipoprotein A	−0.085	<0.01

PCOS, polycystic ovarian syndrome; EFS, embryo fragment score; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. The correlations of lipid levels with EFS were analyzed by the Spearman rank correlation. A P-value ≤ 0.05 was considered statistically significant.

3.5 Comparisons of basic clinical information and pregnancy outcomes between the control and hyperlipidemia groups in the non-obese PCOS patients undergoing FET

The basic clinical information and pregnancy outcomes of non-obese PCOS patients undergoing FET were shown in Table 5. Serum lipid levels, including TC, TG, LDL, and HDL, in the hyperlipidemia group were significantly higher than those in the control group (P < 0.01). However, there were no significant differences in the age, BMI, FBG, serum insulin level, serum P and E2 levels on the day of progesterone administration, PI, S/D, endometrial thickness, transferred embryos, implantation rate, clinical pregnancy rate, and miscarriage rate (all P > 0.05) except RI (P = 0.002) between the hyperlipidemia and control groups.

3.6 Multiple logistic regression analysis between blood lipids and the clinical outcomes of non-obese PCOS patients undergoing FET

The multiple logistic regression analysis between blood lipids and the clinical outcomes of non-obese PCOS patients undergoing FET was shown in Table 6. The results showed no any correlation between blood lipids and clinical outcomes, whether corrected or uncorrected for RI.

TABLE 4 Multiple logistic regression analysis between blood lipids and the clinical outcomes of non-obese PCOS patients undergoing fresh cycle transplantation.

Variables	Pregnancy rate		Implantation rate		Miscarriage rate	
	Crude OR (95% *CI*)	Adjusted OR (95% *CI*)	Crude OR (95% *CI*)	Adjusted OR (95% *CI*)	Crude OR (95% *CI*)	Adjusted OR (95% *CI*)
TG	0.823 (0.529-1.280) P = 0.387	0.742 (0.430-1.282) P = 0.284	0.944 (0.653-1.364) P = 0.757	0.807 (0.518-1.259) P = 0.345	1.046 (0.418-2.617) P = 0.923	1.383 (0.420-4.466) P = 0.588
TC	2.537 (0.700-8.246) P = 0.122	4.072 (0.989-16.761) P = 0.052	1.435 (0.733-2.811) P = 0.292	2.544 (0.873-7.410) P = 0.087	0.988 (0.212-4.597) P = 0.988	0.973 (0.117-8.069) P = 0.980
HDL	0.540 (0.246-1.185) P = 0.125	0.355 (0.135-0.938) P = 0.037	0.646 (0.341-1.222) P = 0.179	0.541 (0.257-1.138) P = 0.105	1.207 (0.173-8.443) P = 0.850	1.380 (0.158-12.072) P = 0.771
LDL	0.821 (0.180-3.737) P = 0.799	0.575 (0.102-3.244) P = 0.531	1.186 (0.404-3.483) P = 0.756	0.731 (0.175-3.065) P = 0.669	0.497 (0.038-6.456) P = 0.593	0.402 (0.022-7.369) P = 0.539
Lipoprotein A	0.999 (0.997-1.002) P = 0.693	1.000 (0.997-1.002) P = 0.730	1.000 (0.997-1.002) P = 0.790	1.000 (0.997-1.002) P = 0.826	1.006 (0.991-1.020) P = 0.427	1.006 (0.991-1.021) P = 0.432

TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. The multiple logistic regression analysis was conducted using pregnancy rate, implantation rate, or miscarriage rate as dependent variables and TG, TC, HDL, LDL, and lipoprotein A as covariates. The results were expressed in odd ratio (OR) and 95% confidence interval (CI). Crude OR and adjusted OR referred to uncorrected and corrected Gn dosage and estradiol on trigger day, respectively. A P-value ≤ 0.05 was considered statistically significant.

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TABLE 5 Comparisons of basic clinical information and pregnancy outcomes between control and hyperlipidemia groups in the PCOS patients undergoing FET.

Indexes	Control (*n* = 76)	Hyperlipidemia (*n* = 51)	*P-value*
Age (years old)	29.2 ± 2.9	29.8 ± 2.1	0.23
BMI (kg/m^2)	23.6 ± 3.0	24.3 ± 1.8	0.12
FBG (mmol/L)	5.06 ± 0.45	5.05 ± 0.6	0.98
Insulin (pmol/L)	87.4 ± 36.4	94.2 ± 33.4	0.29
TG (mmol/L)	1.22 [0.91, 1.56]	2.36 [1.74, 2.80]	<0.01
TC (mmol/L)	4.47 [4.07, 5.44]	5.04 [4.73, 5.81]	<0.01
HDL (mmol/L)	1.55 [1.36, 1.69]	1.40 [1.01, 1.49]	<0.01
LDL (mmol/L)	2.58 ± 0.64	3.13 ± 0.68	<0.01
Lipoprotein A (mmol/L)	86.5 [51.5, 152.2]	112.0 [82.0, 143.0]	0.04
Endometrial thickness (mm)	10.86 ± 1.61	11.36 ± 1.56	0.087
RI	0.82 ± 0.05	0.84 ± 0.05	0.002
PI	2.30 ± 0.47	2.35 ± 0.41	0.49
S/D	6.21 ± 1.62	6.42 ± 1.65	0.46
Serum E2 on the day of progesterone administration (pg/ml)	273.2 ± 93.9	276.4 ± 104.2	0.86
Serum P on the day of progesterone administration (ng/ml)	0.58 ± 0.24	0.63 ± 0.24	0.19
Transferred embryos (*n*)	1.6 ± 0.49	1.5 ± 0.50	0.54
Clinical pregnancy rate (*n*, %)	44/76 (57.9)	27/51 (52.9)	0.58
Implantation rate (*n*, %)	57/119 (47.9)	31/77 (40.3)	0.29
Miscarriage rate (*n*, %)	5/44 (11.4)	3/27 (11.1)	0.97

PCOS, polycystic ovarian syndrome; FET, frozen embryo transfer; BMI, body mass index; FBG, fasting blood glucose; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; RI, resistance index; PI, pulse index; S/D, systolic/diastolic ratio; E2, estradiol; P, progesterone. Clinical pregnancy rate refers to the number of cycles achieving clinical pregnancy/the number of embryo transfer cycles; Implantation rate refers to the number of gestational sacs/the number of transplanted embryos; Miscarriage rate refers to the number of miscarriage cycles/the number of clinical pregnancy cycles. The measurement data were first performed by one-sample nonparametric tests (Kolmogorov-Smirnov test) to determine whether they were normal distribution. The data conforming to the normal distribution were expressed as mean ± standard deviation (x ± s), and those conforming to the non-normal distribution were expressed as median [P_{25}, P_{75}]. The comparisons between the control and hyperlipidemia groups were analyzed by independent samples t-test for normal distribution data or Mann-Whitney U test for non-normal distribution data. The count data were presented as percentages, and the comparisons between the control and hyperlipidemia groups were analyzed by the $\chi 2$ test. The standard errors (SEs) of the clinical pregnancy rate, implantation rate and miscarriage rate between the two groups were 1.35, 0.97, and 0.82, respectively. A P-value ≤ 0.05 was considered statistically significant.

4 Discussion

PCOS is the most common endocrine disorder in reproductive-aged women. Women with PCOS have increased risk of metabolic disorders such as IR, hyperandrogenism, obesity and hyperlipidemia. Researchers have confirmed the effects of obesity and IR on fertility. A compensatory hyperinsulinemic state caused by obesity and PCOS may disrupt endometrial homeostasis and result in insulin receptors decrease and defective decidualization (10). Hyperandrogenism could affect the window of implantation by decreasing the expression levels of *HOXA10* and *WT1* genes and influencing endometrial decidualization. Cui et al. (11) verified that the obese PCOS patients undergoing IVF/ICSI treatment had lower clinical pregnancy rate. Dyslipidemia often coexists with obesity and IR, and some studies have found that the incidence of hyperlipidemia in PCOS patients increased by 16.1% when compared with the general population (12). However, hyperlipidemia may exist without obesity in PCOS patients, and sometimes it can aggravate obesity, IR and a variety of metabolic abnormalities, which have serious effects on cardiovascular system and fertility (2). Therefore, we speculate that hyperlipidemia may affect the pregnancy outcome of ART. However, the relationship between serum lipids and the pregnancy outcomes of IVF/ICSI in PCOS patients is rarely reported.

The present study was to elucidate the characteristics of serum lipids and their effects on oocyte and endometrium in non-obese women with PCOS undergoing IVF/ICSI. The women chosen for the retrospective analysis had BMI below 30 kg/m^2, and their hyperandrogenism were pretreated.

Our investigation found that the non-obese PCOS patients with hyperlipidemia required higher Gn dosage during ovulation induction, which was consistent with previous research results (13). However, the specific reasons are still unclear. Some researchers found that the serum level of sex hormone binding globulin in the patients with hyperlipidemia decreased, and that free testosterone levels increased, which would reduce the sensitivity of ovary to Gn (14). It was also found that tumor necrosis factor (TNF) was involved in the regulation of oocyte maturation and apoptosis, and that high TNFα levels might lead to the arrest of oocyte maturation and chromosome abnormality, and reduce the sensitivity of ovary to Gn (15). While, the increase of blood lipids especially triglyceride was positively correlated with TNFα (16).

An analysis of 1394 treatment cycles of 943 patients found that daily dose of Gn and total Gn dosage were negatively correlated with the number of oocytes, implantation rate, clinical pregnancy rate, and live-birth rate, which recommended that daily Gn dose is preferably less than 450 IU or total Gn dosage less than 3000 IU/cycle (17). Another study found that daily Gn dose exceeding 300 IU was significantly associated with a lower live birth rate (18). Excessive Gn exposure may lead to an unfavorable endometrium or a detrimental metabolic environment (19). It is speculated that

TABLE 6 Multiple logistic regression analysis between blood lipids and the clinical outcomes of non-obese PCOS patients undergoing FET.

Variables	Pregnancy rate		Implantation rate		Miscarriage rate	
	Crude OR (95% *CI*)	Adjusted OR (95% *CI*)	Crude OR (95% *CI*)	Adjusted OR (95% *CI*)	Crude OR (95% *CI*)	Adjusted OR (95% *CI*)
TG	1.048 (0.707-1.554) *P* = 0.815	1.058 (0.712-1.572) *P* = 0.700	0.903 (0.651-1.252) *P* = 0.541	0.899 (0.646-1.249) *P* = 0.524	0.709 (0.340-1.476) *P* = 0.358	0.725 (0.344-1.529) *P* = 0.399
TC	1.054 (0.561-1.979) *P* = 0.870	1.043 (0.554-1.961) *P* = 0.897	1.163 (0.710-1.906) *P* = 0.549	1.166 (0.711-1.913) *P* = 0.542	0.978 (0.252-3.796) *P* = 0.974	0.975 (0.241-3.935) *P* = 0.972
HDL	1.185 (0.533-2.635) *P* = 0.677	1.141 (0.506-2.570) *P* = 0.750	0.960 (0.527-1.749) *P* = 0.895	0.973 (0.530-1.786) *P* = 0.930	1.134 (0.083-15.411) *P* = 0.925	0.964 (0.061-15.188) *P* = 0.979
LDL	1.188 (0.518-2.724) *P* = 0.685	1.250 (0.533-2.932) *P* = 0.608	0.962 (0.502-1.843) *P* = 0.908	0.945 (0.486-1.837) *P* = 0.866	0.598 (0.100-3.568) *P* = 0.573	0.671 (0.105-4.311) *P* = 0.675
Lipoprotein A	0.997 (0.992-1.002) *P* = 0.210	0.997 (0.992-1.002) *P* = 0.202	0.997 (0.993-1.000) *P* = 0.073	0.997 (0.993-1.000) *P* = 0.074	1.005 (0.993-1.018) *P* = 0.434	1.005 (0.992-1.018) *P* = 0.427

TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. The multiple logistic regression analysis was conducted using pregnancy rate, implantation rate, or miscarriage rate as dependent variables and TG, TC, HDL, LDL, and lipoprotein A as covariates. The results were expressed in odd ratio (OR) and 95% confidence interval (CI). Crude OR and adjusted OR referred to uncorrected and corrected resistance index (RI), respectively. A P-value ≤ 0.05 was considered statistically significant.

increasing Gn dosage may cause high circulating progesterone level and a premature luteinization of the endometrium (20). It is also well established that the use of exogenous Gn affects the endometrium and can result in functional genomic disorder of the endometrium (21). Therefore, high Gn dosage in non-obese PCOS patients with hyperlipidemia may also affect endometrial receptivity.

In addition, numerous studies have demonstrated that the metabolic problems related to hyperlipidemia, such as hyperinsulinemia and aberrant adipokines, may affect embryonic development and endometrial receptivity (22). Some studies have demonstrated that hyperlipidemia has an adverse effect on embryo quality, and that the exact mechanisms need to be further verified. Some researches showed that hyperlipidemia could produce a large number of reactive oxygen species (ROS), and then destroy the morphology and integrity of endoplasmic reticulum of granulosa cells. All these changes would disturb the maturation of oocytes and reduce the blastocyst formation rate (8, 23, 24). Some animal experiments found that high fat diets might cause important defects and chromosome dislocations during meiosis of oocytes (25).

In our study, although there were no significant differences in the normal fertilization rate and good quality embryos rate between the control and hyperlipidemia groups, the embryo fragmentation score (EFS) was positively correlated with LDL and negatively with HDL and lipoprotein A ($P < 0.05$). Moreover, the multiple logistic regression analysis showed that HDL was beneficial for clinical pregnancy (AOR = 0.355, 95% *CI*: 0.135-0.938, $P = 0.037$) and TC may be a potential risk factor for clinical pregnancy (AOR = 4.072, 95% *CI*: 0.989-16.761, $P = 0.052$). Some studies also demonstrated that the levels of follicular fluid and plasma HDL were negatively correlated with embryo fragmentation (26). It was reported that transferring embryos with more fragments had lower implantation and pregnancy rates than transferring embryos with minimal fragments (27). Browne et al. (28) confirmed that HDL exhibited important cytoprotective effect on oocytes and granulosa cells around them. The most compelling evidence for the importance of HDL in mammalian oocyte development and competence was exemplified by studies of SR-BI KO mice (29). The SR-BI known as HDL receptor could facilitate the uptake of cholesterol for steroidogenesis. Oocyte and embryo morphology were disrupted in SR-BI KO female mice. However, it is unclear how HDL works to safeguard embryonic growth. Some researchers thought that it might be associated with antioxidation. The HDL particles include phospholipids, triglycerides and proteins such as PON1, which play an antioxidant role and would protect embryos from the damage of ROS (30). Theoretically, follicular fluid HDL is a filtration byproduct of human plasma. The plasma HDL level might partly reflect the HDL level in follicular fluid. However, the HDL particles in follicles contain less cholesterol and rich phospholipids relative to those in serum. Moreover, the HDL particles in follicles are heterogeneous in structure, and it is unclear which type of HDL is primarily related to embryo fragmentation. Kim et al. (31) found that higher concentrations of follicular fluid HDL subfractions in the large and medium-sized particles were associated with poorer embryo quality. Therefore, we speculate that HDL plays a protective role in oocyte and embryo development, but which kind of HDL is necessary still needs to be further explored.

Although we found a harmful effect of hyperlipidemia on embryos in fresh cycles, there were no differences in clinical pregnancy rate and miscarriage rate between hyperlipidemia and control groups. This may be attributed to the fact that the best embryos with few fragments in the first cycle were transplanted. Considering that serum E2 levels on trigger day in the control group were significantly higher than that in the hyperlipidemia group, and that serum E2 level was crucial for embryo implantation, we further investigated the FET outcomes of the two groups. All the patients undergone the FET for the first time received HRT. There were no significant differences in serum E2 levels and endometrial thickness

on the day of progesterone administration. Meanwhile, all the transferred embryos were of good quality, similar to the fresh transfer cycle. The results showed that there were no significant differences in the implantation rate, pregnancy rate, and miscarriage rate between hyperlipidemia and control groups, but the resistance index (RI) was significantly different.

Uterine blood supply mainly comes from the uterine artery and its branches, including the arcuate artery supplying the myometrium and the spiral artery supplying the endometrium. During the embryo implantation stage, the blood supply to the uterus alters to ensure adequate blood supplementation. Kim et al. (32) used Doppler ultrasonography to measure endometrial and subendometrial vascularity on the day of HCG administration during IVF-ET, and found that endometrial and subendometrial vascularity in the pregnancy group was significantly higher than that in the control group. It was reported that a successful FET cycle was related to increased blood flow to the endometrium, which boosted endometrial receptivity (33). However, the results of this study showed that the difference of RI was not enough to lead to the difference of implantation rate. Subendometrial vascularity indexes such as vascularity index (VI) and vascularity flow index (VFI) are more accurate than uterus artery indexes including RI, PI and S/D. Some studies found that there were no differences in uterus artery indexes such as PI, RI and S/D scores between pregnancy and non-pregnancy groups (32, 34). In recent studies subendometrial vascularity indexes were more prone to be selected to detect endometrial receptivity. However, due to the significant technical requirements for measuring subendometrial blood vessels, we only measured the uterus artery indexes. This is one of the limitations of our study. This study is only conducted in one institute, which requires more data to confirm. Another limitation of this study is the limited sample size. Analyzing the FET cycle and fresh cycle separately may further reduce the testing power of the used samples. In the future, we will increase the sample size to further confirm the impact of hyperlipidemia on the clinical outcomes of ART in PCOS patients and explore its possible mechanisms.

5 Conclusions

In conclusion, our study found that the total Gn dosage in the hyperlipidemia group was significantly higher than that in the control group, and that the embryo fragment score was positively correlated with LDL and negatively with HDL and lipoprotein A. Moreover, HDL was beneficial for clinical pregnancy. In addition, in FET cycles, the resistance index in the hyperlipidemia group was significantly higher than that in the control group. It is indicated that hyperlipidemia may increase the dosage of gonadotropin and have adverse effect on the embryo quality, endometrial receptivity, and clinical outcomes of lean PCOS patients. It is recommended that the non-obese patients with hyperlipidemia and PCOS perform lipid-lowering treatment before undergoing embryo transfer.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by The clinical data of patients undergoing conventional IVF or ICSI treatment in our hospital during October 2017 and June 2021 were analyzed retrospectively. The implenmentation of IVF or ICSI was approved by the Reproductive Medicine Ethics Committee of Zhongda Hospital affiliated to Southeast University (Reproduction No. 2015-1), and all patients signed the informed consent. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

FY: Data curation, Formal Analysis, Writing – original draft. J-CL: Conceptualization, Data curation, Writing – review & editing. TS: Data curation, Writing – original draft. Y-HJ: Data curation, Writing – original draft. Y-JL: Conceptualization, Resources, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

PCOS	polycystic ovarian syndrome
BMI	body mass index
IVF	*in vitro* fertilization
ICSI	intracytoplasmic sperm injection
FET	frozen embryo transfer
IR	insulin resistance
ART	assisted reproductive technology
TC	total cholesterol
LDL-C	low-density lipoprotein cholesterol
HDL-C	high-density lipoprotein cholesterol
TG	triglyceride
RI	resistance index
PI	pulse index
S/D	systolic/diastolic ratio
GnRH	Gonadotropin-releasing hormone
Gn	gonadotropin
GnRH-a	GnRH agonist
COS	controlled ovarian stimulation
HCG	human chorionic gonadotropin
OPU	ovum pick-up
EFS	embryo fragment score
FSH	follicle-stimulating hormone
LH	luteinizing hormone
T	testosterone
E2	estradiol
P	progesterone
AMH	anti-Müllerian hormone
AFC	antral follicles
FBG	fasting blood-glucose
TNF	tumor necrosis factor

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Causal association of immune cells and polycystic ovarian syndrome: a Mendelian randomization study

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Background: Polycystic ovarian syndrome (PCOS) is a common reproductive disorder that affects a considerable number of women worldwide. It is accompanied by irregular menstruation, hyperandrogenism, metabolic abnormalities, reproductive disorders and other clinical symptoms, which seriously endangers women's physical and mental health. The etiology and pathogenesis of PCOS are not completely clear, but it is hypothesized that immune system may play a key role in it. However, previous studies investigating the connection between immune cells and PCOS have produced conflicting results.

Methods: Mendelian randomization (MR) is a powerful study design that uses genetic variants as instrumental variables to enable examination of the causal effect of an exposure on an outcome in observational data. In this study, we utilized a comprehensive two-sample MR analysis to examine the causal link between 731 immune cells and PCOS. We employed complementary MR methods, such as the inverse-variance weighted (IVW) method, and conducted sensitivity analyses to evaluate the reliability of the outcomes.

Results: Four immunophenotypes were identified to be significantly associated with PCOS risk: Memory B cell AC (IVW: OR [95%]: 1.123[1.040 to 1.213], p = 0.003), CD39+ CD4+ %CD4+ (IVW: OR [95%]: 0.869[0.784 to 0.963], p = 0.008), CD20 on CD20- CD38-(IVW: OR [95%]:1.297[1.088 to 1.546], p = 0.004), and HLA DR on CD14- CD16+ monocyte (IVW: OR [95%]:1.225[1.074 to 1.397], p = 0.003). The results of the sensitivity analyses were consistent with the main findings.

Conclusions: Our MR analysis provides strong evidence supporting a causal association between immune cells and the susceptibility of PCOS. This discovery can assist in clinical decision-making regarding disease prognosis and treatment options, and also provides a new direction for drug development.

KEYWORDS

immune cells, causality, pcos, MR analysis, SNP

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent reproductive endocrine and metabolic disorder that commonly affects women of childbearing age. It is characterized by chronic anovulation and hyperandrogenism. Clinical symptoms of PCOS include menstrual irregularities, hirsutism, acne, and polycystic ovarian morphology. Additionally, individuals with PCOS may also experience metabolic conditions such as obesity, insulin resistance, and dyslipidemia (1). The pathogenic mechanisms of PCOS remain unclear; in addition to the ovarian-pituitary-hypothalamic-gonadal axis, pathogenesis of PCOS must also consider ovarian local cytokines, immunology, and genetics.

Recent research has highlighted the importance of the inflammatory immune mechanism in the occurrence and development of PCOS. Numerous studies have reported that chronic low-grade inflammation is closely associated with and interacts with PCOS (2–4). The identification of leukocytosis in polycystic ovaries may indicate that polycystic ovaries are associated with a pro-inflammatory state (5, 6). The expression of IFN-c, a cytokine produced by Th1, was significantly increased in PCOS patients compared to the control group (3). The regulation of granulosa cells and immune cells is impaired in patients with PCOS, which may contribute to accelerated anovulation (7). Systemic and ovarian cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-18, can alter the local microenvironment in the ovary, disrupt ovarian function, increase androgen production, and contribute to insulin resistance through various mechanisms (2). Immune factors such as vascular endothelial growth factor (VEGF) and transforming growth factor-β1, along with inflammation in the follicular microenvironment, may play a role in the dysfunction of the hypothalamic-pituitary-gonadal axis and the development of follicular dysplasia. Patients with PCOS had higher levels of antinuclear antibody, histone antibody resistance, and ds-DNA antibody levels than the control group. In addition, an increase in thyroperoxidase or thyroglobulin antibodies in patients with PCOS was found to be associated with the development of autoimmune thyroiditis (8).

Wu et al. found that T lymphocytes play a significant role in the local pathological mechanisms of PCOS (9). T lymphocytes secrete inflammatory and immunomodulatory molecules that regulate ovarian function. Additionally, dysregulation of T-cell subsets has been observed in the peripheral blood and ovaries of patients with PCOS due to disrupted sex hormone levels (10). Animal models with elevated androgens have been linked to reproductive dysfunction, including oligo-anovulation, menstrual disturbances, and subfertility, which are commonly observed in PCOS (11–15). Androgens have immunomodulatory effects, and the presence of elevated androgens is associated with altered immune function, which can have an impact on reproductive function (16, 17). Medawar identified the importance of the immune system in reproduction, and subsequent studies have highlighted the significance of regulatory T (Treg) cells frequencies in maintaining normal ovarian function and menstrual cycles (18–20). Androgens appear to modulate the differentiation of T cells and the ratios of Treg cells (3, 17, 21, 22). Furthermore, the differentiation of T cells is also modulated by epigenetic mechanisms (23–25), which may be the case in PCOS (17).

PCOS has been shown to be significantly associated with B lymphocytes. In addition to their role in humoral immunity, B lymphocytes are involved in antigen detection and regulation of antigen processing and presentation. This group of lymphocytes plays a crucial role in the development of insulin resistance associated with obesity and glucose intolerance. They contribute to insulin resistance by activating pro-inflammatory T cells and producing pathological antibodies (26). The pathogenic role of B cells was identified in PCOS, as the activity of these cells increased in women with PCOS compared to the control group. Moreover, mice with PCOS that were treated with CD19 antibody exhibited a reduction in B cells in their peripheral blood, which led to a reduction in their cystic follicles and an increase in their corpus luteum. Based on these findings, it can be concluded that manipulation of these cells and antibodies could be potential targets for treating insulin resistance and PCOS (27).

Mendelian randomization (MR) is an analytical method used in epidemiological etiology inference, which is based on the Mendelian independent distribution law. It is essential for the causal sequence of MR to be reasonable (28, 29). Previous observational studies have identified multiple associations between immune cell traits and PCOS, supporting the hypothesis of a correlation between them. In this study, a comprehensive two-sample MR analysis was conducted to establish a causal association between immune cell signatures and PCOS.

Materials and methods

Study design

We assessed the causal relationship between 731 immune cell signatures and PCOS based on a two-sample MR analysis. In order to acquire dependable outcomes, three hypotheses must be met during the execution of MR analysis: a robust association between genetic variants and exposure factors, an absence of correlation between genetic variants and confounding variables, and the influence of genetic variants on the outcome solely through exposure factors, excluding other pathways.

Genome-wide association study data sources for PCOS

The GWAS statistics for PCOS were sourced from FinnGen Research's data release in July 2021 (https://gwas.mrcieu.ac.uk/datasets/finn-b-E4_POCS/). The diagnostic criteria of PCOS were based on ICD-9 and ICD-10 standards (presence of two of the three criteria: chronic anovulation, hyperandrogenism, polycystic ovaries on ultrasonography), and the GWAS statistics encompassed 16,379,676 loci variations from 642 cases and 118,228 controls.

Immunity-wide GWAS data sources

The GWAS summary statistics for each immune trait can be accessed from the GWAS Catalog, with accession numbers ranging from GCST90001391 to GCST90002121 (30). A total of 731 immunophenotypes were included in the analysis, which comprised of absolute cell (AC) counts (n = 118), median fluorescence intensities (MFI) representing surface antigen levels (n = 389), morphological parameters (MP) (n = 32), and relative cell (RC) counts (n = 192). The AC, MFI, and RC features encompassed B cells, CDCs, mature stages of T cells, monocytes, myeloid cells, TBNK (T cells, B cells, natural killer cells), and Treg panels. The MP feature consisted of CDC and TBNK panels. The original GWAS on immune traits utilized data from 3,757 European individuals, and there were no overlapping cohorts. Approximately 22 million SNPs were genotyped with high-density arrays and imputed using the Sardinian sequence-based reference panel (31). Associations were tested while adjusting for covariates such as sex, and age.

Selection of instrumental variables

Based on recent research (30), the significance level for instrumental variables (IVs) associated with each immune trait was set to 1×10^{-5}. To ensure reliable results, a threshold for strong linkage disequilibrium (LD) effect was applied ($r^2 < 0.001$) (32), with LD r^2 calculated using the 10000 Genomes Project as a reference panel. The proportion of phenotypic variation explained (PVE) and F statistic were calculated for each IV to assess IV strength and avoid weak instrumental bias. Furthermore, to mitigate bias introduced by weak instruments, IVs with F statistics greater than 10 were deemed strong instruments and retained for subsequent analysis. The exposure and outcome SNPs were harmonized to align effect estimates for the same effect allele. Palindromic SNPs with intermediate effect allele frequencies (EAFs > 0.42) or SNPs with incompatible alleles were excluded (33).

Data analysis

We conducted a range of MR analyses, encompassing MR Egger, weighted median, inverse-variance weighted (IVW), simple mode, weighted mode, and MR-PRESSO approaches. Among these, the IVW method is frequently employed (34).

To evaluate the presence of variance, we performed heterogeneity examinations utilizing both the MR Egger and IVW techniques. The Cochrane's Q value was employed to appraise the variability of genetic instruments, whereby a p-value exceeding 0.05 indicates a lack of noteworthy diversity. To scrutinize the existence of horizontal pleiotropy, we utilized the MR Egger regression equation, where a p-value surpassing 0.05 indicates an absence of indications of horizontal pleiotropy (35).

Additionally, in order to assess the potential impact of directional pleiotropy, we scrutinized each SNP for potential associations with secondary phenotypes using the GWAS Catalog (http://www.phenoscanner.medschl.cam.ac.uk/). Subsequently, we re-performed the MR analyses, excluding SNPs associated with other phenotypes. Moreover, we conducted leave-one-out sensitivity analyses on significant findings to determine if a single SNP was accountable for the observed causal relationship. The overall research design is depicted in Figure 1. The MR analyses were carried out utilizing the 'TwoSampleMR' package (version 0.5.7) within the R software environment (version 4.2.1) (35, 36).

Results

Exploration of the causal effect of immunophenotypes on PCOS

At the significance of 0.001, we detected Memory B cell AC, CD20 on CD20- CD38-, HLA DR on CD14- CD16+ monocyte were significantly associated with an increased risk of PCOS, while CD39+ CD4+ %CD4+ retained a robust association with an decreased risk of PCOS.

The OR of Memory B cell AC (B cell panel) on PCOS risk was estimated to be 1.123 (95% CI = 1.040 to 1.213, $p = 0.003$) by using the IVW method. Similar results were observed by using MR-Egger (OR [95%]: 1.146 [1.045 to 1.258], $p = 0.008$). The genetically predicted CD39+ CD4+ %CD4+ (Treg panel) exhibited a noticeable protective effect against PCOS (IVW: OR [95%]:0.869 [0.784 to 0.963], $p = 0.008$). The genetically predicted CD20 on CD20- CD38- (B cell panel) showed a positive correlation with the risk of PCOS, as evidenced by IVW method (OR [95%]:1.297 [1.088 to 1.546], $p = 0.004$). The OR of HLA DR on CD14- CD16+ monocyte (Monocyte panel) on PCOS risk was estimated to be 1.225 (95% CI = 1.074 to 1.397, $p = 0.003$) by using the IVW method. (Supplementary Table 1, Figure 2).

Sensitivity analysis

The MR-Egger intercept test and MR-PRESSO global test results indicated no evidence of heterogeneity or horizontal pleiotropy in the associations between immunophenotypes and PCOS. These results are presented in Supplementary Table 2. Furthermore, the leave-one-out analysis demonstrated the robustness of the MR results. Excluding any single SNP associated with immunophenotypes and PCOS did not significantly alter the overall findings.

To account for potential directional pleiotropy, we conducted an analysis using the GWAS Catalog to identify SNPs linked to immunophenotypes and PCOS. Two SNPs were discovered to exhibit associations with other traits, as detailed in Supplementary Table 3. After excluding these pleiotropic SNPs, the associations between immunophenotypes and PCOS remained stable, as shown in Supplementary Table 4.

Discussion

Our study integrates large-scale individual and aggregated GWAS datasets to systematically elucidate the role of immune

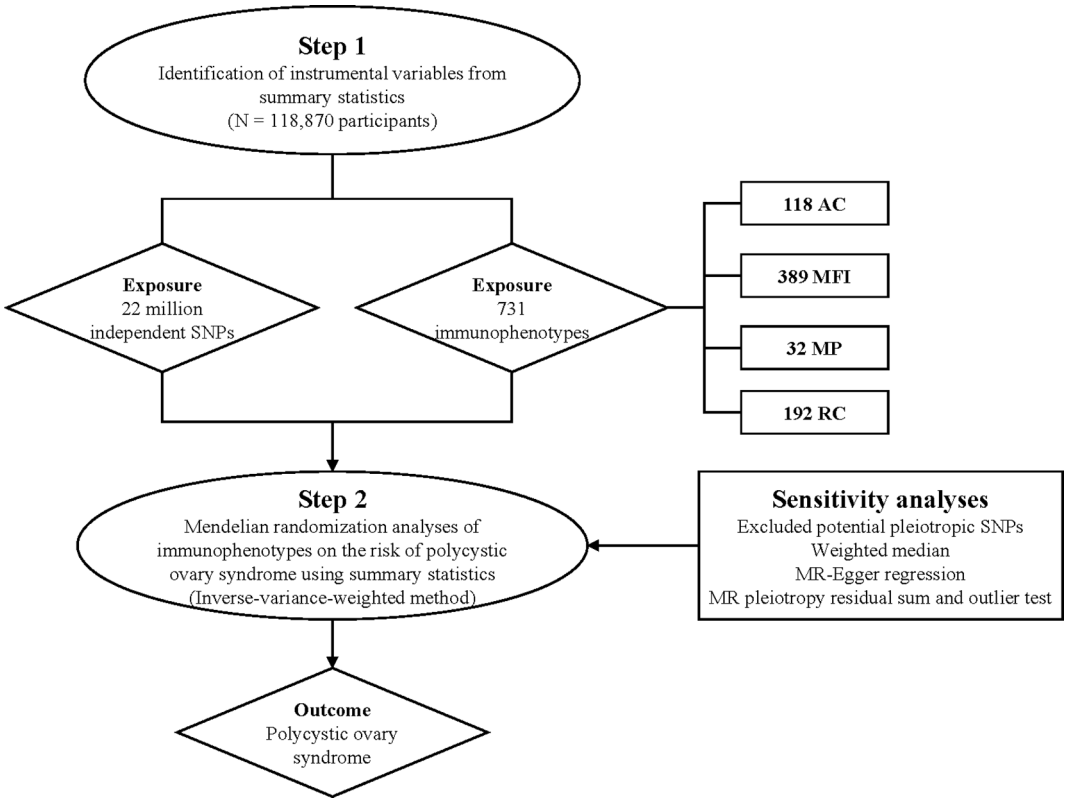

FIGURE 1
Research overview and design of Mendelian randomization analysis.

cells in the pathogenesis of PCOS from a genetic perspective. Our analysis provides suggestive evidence that immune cells can influence the risk of PCOS through a comprehensive genetic approach based on large-scale GWAS summary data. To the best of our knowledge, this is the first MR analysis to investigate the causal relationship between multiple immunophenotypes and PCOS. By utilizing SNPs as instrumental variables and integrating various two-sample MR methods, we confirmed that four immune cells, including Memory B cell AC, CD39+ CD4+ %CD4+, CD20 on CD20- CD38- and HLA DR on CD14- CD16+ monocyte, were significantly associated with the risk of PCOS.

Our findings indicate that two types of B cells, namely CD20 on CD20- CD38- and Memory B cell AC, were significantly associated with an increased risk of PCOS. Recent studies have highlighted the important role of B cells in the development of PCOS. B lymphocytes are known to produce antibodies in response to self-antigens, and the formation of antigen-antibody complexes can contribute to inflammatory responses in the body, thereby potentially increasing the risk of PCOS (37). CD20 is a distinct antigen found on the surface of B lymphocytes, and it is recognized for its significant involvement in regulating B lymphocyte proliferation, differentiation, and signaling processes (38). Previous studies have characterized Memory B cells as being enriched with autoantibodies and primed for plasma cell differentiation. They have also been associated with excessive accumulation in chronic infections, autoimmune disorders, and immunodeficiencies, suggesting their involvement in the regulation of humoral responses. Consistent with existing data demonstrating increased B cell frequencies in PCOS, hyperandrogenic women with PCOS showed a significant reorganization of their B cell repertoire, leading to elevated frequencies of B memory cells (39).

Our study revealed a correlation between elevated levels of CD39+ CD4+ %CD4+ (Treg panel) and a decreased risk of PCOS. T cells can be categorized into three subsets: T helper cells, Cytotoxic T cells, and Treg cells. Treg cells are crucial for immune system regulation, homeostasis, and prevention of autoimmunity. Previous research on Treg cell proliferation in PCOS patients has shown a decrease in these cells (40). Additionally, studies have indicated reduced levels of anti-inflammatory factors like IL-10 in the bodies and ovaries of PCOS patients, attributed to a reduction in peripheral blood Treg cells (41). Treg cells CD39+ CD4+ %CD4+ are particularly important for reproductive function. During a normal pregnancy, there is an increase in the number of Treg cells CD39+ CD4+ % CD4+, while studies suggest that a decrease in these cells among PCOS patients could contribute to miscarriage or infertility (42).

In our study, we discovered a correlation between HLA DR on CD14- CD16+ monocyte levels and an increased risk of PCOS. Androgens can disrupt the ovarian immune balance in PCOS by interacting with immune cells and cytokines. Research revealed that monocytes entering the ovary can provoke a local inflammatory response, leading to increased production of

traits	method	nsnp	pval	OR (95% CI)
Memory B cell AC	MR Egger	25	0.0084	1.1462 (1.0447 – 1.2577)
	Weighted median	25	0.0426	1.1277 (1.0040 – 1.2666)
	Inverse variance weighted	25	0.0030	1.1232 (1.0403 – 1.2128)
	Simple mode	25	0.9508	0.9940 (0.8221 – 1.2018)
	Weighted mode	25	0.0358	1.1171 (1.0132 – 1.2317)
CD39+ CD4+ %CD4+	MR Egger	25	0.2087	0.9139 (0.7973 – 1.0475)
	Weighted median	25	0.1142	0.9011 (0.7918 – 1.0254)
	Inverse variance weighted	25	0.0077	0.8691 (0.7839 – 0.9635)
	Simple mode	25	0.6857	0.9448 (0.7199 – 1.2399)
	Weighted mode	25	0.1259	0.9095 (0.8089 – 1.0226)
CD20 on CD20− CD38−	MR Egger	16	0.0378	1.3606 (1.0458 – 1.7703)
	Weighted median	16	0.0325	1.3396 (1.0246 – 1.7514)
	Inverse variance weighted	16	0.0037	1.2969 (1.0880 – 1.5458)
	Simple mode	16	0.7753	1.0648 (0.6973 – 1.6260)
	Weighted mode	16	0.0320	1.3496 (1.0526 – 1.7304)
CD20 on CD20− CD38−*	MR Egger	15	0.0346	1.3745 (1.0554 – 1.7900)
	Weighted median	15	0.0340	1.3352 (1.0221 – 1.7441)
	Inverse variance weighted	15	0.0054	1.2856 (1.0770 – 1.5347)
	Simple mode	15	0.8265	1.0499 (0.6850 – 1.6092)
	Weighted mode	15	0.0228	1.3878 (1.0796 – 1.7840)
HLA DR on CD14− CD16+ monocyte	MR Egger	19	0.3778	1.1327 (0.8650 – 1.4833)
	Weighted median	19	0.0971	1.1618 (0.9732 – 1.3869)
	Inverse variance weighted	19	0.0025	1.2246 (1.0737 – 1.3966)
	Simple mode	19	0.2154	1.1934 (0.9111 – 1.5630)
	Weighted mode	19	0.1060	1.1787 (0.9754 – 1.4244)
HLA DR on CD14− CD16+ monocyte*	MR Egger	18	0.3409	1.1579 (0.8641 – 1.5516)
	Weighted median	18	0.1252	1.1710 (0.9570 – 1.4329)
	Inverse variance weighted	18	0.0032	1.2441 (1.0758 – 1.4386)
	Simple mode	18	0.2763	1.1910 (0.8783 – 1.6152)
	Weighted mode	18	0.0736	1.2066 (0.9948 – 1.4635)

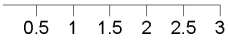

FIGURE 2
The effect of immune cells on polycystic ovarian syndrome. Asterisk (*) represents MR analysis results after excluding SNPs associated with other phenotypes. nsnp, number of single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

ovarian androgens in women with PCOS (43). Tumor necrosis factor-alpha (TNF-α), released by monocytes, has been associated with insulin resistance in PCOS (43). Monocytes and macrophages act as immune sentinels in the innate immune system and can be distinguished by their expression of CD14 and CD16. Monocyte subsets are classified based on phenotypic markers: classic (CD14+CD16-), intermediate (CD14+CD16+), and nonclassical (CD14−CD16+). Our findings have demonstrated the involvement of non-classical monocytes in PCOS. Non-classical monocytes secrete a significant amount of IL-1β in a TLR signaling-dependent manner (44). Compared to classical monocytes, the CD16+ subset exhibits a stronger ability to release pro-inflammatory factors and, as a result, is increased in individuals with PCOS (45, 46).

It is necessary to acknowledge that our study possesses certain inherent limitations that cannot be overlooked. Firstly, it is important to recognize that MR analysis cannot serve as a substitute for clinical trials within the objective realm, as it merely serves as a method for analyzing the causal relationship between exposure and outcome. Therefore, further investigations are required to corroborate the potential association between immune cells and the risk of PCOS. Additionally, our MR analysis was exclusively conducted within the European population due to the limited availability of GWAS data resources. Given the genetic heterogeneity among various ethnic groups, results may vary across different populations. Consequently, forthcoming studies should undertake subgroup analyses encompassing diverse populations in order to arrive at a more comprehensive and encompassing conclusion.

In conclusion, our MR analysis results indicate that Memory B cell AC, CD20 on CD20- CD38-, HLA DR on CD14- CD16+ monocyte increase the risk of PCOS, while CD39+ CD4+ %CD4+ may lead to decreased risk of PCOS. This discovery can assist in clinical decision-making regarding disease prognosis and treatment options, and also provides a new direction for drug development. However, the pathogenesis of PCOS is multifaceted, and the clinical heterogeneity of various types of immune cells involved in PCOS is evident. Therefore, a single treatment may not always yield the desired outcomes. Further research is needed to investigate the interplay between innate immune cells and between innate and adaptive immune cells in PCOS patients.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving humans were approved by The GWAS summary data used in this study were all from the online public platform (https://gwas.mrcieu.ac.uk/). The study protocols were approved by respective local ethics committees, and participants have provided written informed consent. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

NA: Writing – original draft. CY: Investigation, Writing – review & editing. YC: Data curation, Writing – review & editing. JL: Funding acquisition, Supervision, Writing – review & editing.

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The GWAS summary data were acquired from the publicly accessible online platform (https://gwas.mrcieu.ac.uk/). The analyses of the GWAS summary data were conducted using application R version 4.2.1.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1326344/full#supplementary-material

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The role of the autonomic nervous system in polycystic ovary syndrome

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This article reviewed the relationship between the autonomic nervous system and the development of polycystic ovary syndrome (PCOS). PCOS is the most common reproductive endocrine disorder among women of reproductive age. Its primary characteristics include persistent anovulation, hyperandrogenism, and polycystic ovarian morphology, often accompanied by disturbances in glucose and lipid metabolism. The body's functions are regulated by the autonomic nervous system, which consists mainly of the sympathetic and parasympathetic nervous systems. The autonomic nervous system helps maintain homeostasis in the body. Research indicates that ovarian function in mammals is under autonomic neural control. The ovaries receive central nervous system information through the ovarian plexus nerves and the superior ovarian nerves. Neurotransmitters mediate neural function, with acetylcholine and norepinephrine being the predominant autonomic neurotransmitters. They influence the secretion of ovarian steroids and follicular development. In animal experiments, estrogen, androgens, and stress-induced rat models have been used to explore the relationship between PCOS and the autonomic nervous system. Results have shown that the activation of the autonomic nervous system contributes to the development of PCOS in rat. In clinical practice, assessments of autonomic nervous system function in PCOS patients have been gradually employed. These assessments include heart rate variability testing, measurement of muscle sympathetic nerve activity, skin sympathetic response testing, and post-exercise heart rate recovery evaluation. PCOS patients exhibit autonomic nervous system dysfunction, characterized by increased sympathetic nervous system activity and decreased vagal nerve activity. Abnormal metabolic indicators in PCOS women can also impact autonomic nervous system activity. Clinical studies have shown that various effective methods for managing PCOS regulate patients' autonomic nervous system activity during the treatment process. This suggests that improving autonomic nervous system activity may be an effective approach in treating PCOS.

KEYWORDS

polycystic ovary syndrome, autonomic nervous system, sympathetic nervous systems, parasympathetic nervous systems, norepinephrine, heart rate variability

1 Introduction

Polycystic Ovary Syndrome (PCOS) stands as a prevalent multisystem disorder, characterized by disruptions in reproductive, endocrine, and metabolic functions. Due to the heterogeneity of clinical manifestations of PCOS, the diagnostic criteria for PCOS have always been a hotly debated topic. In 1990, the National Institutes of Health (NIH) preliminarily outlined the criteria for diagnosing PCOS, with clinical/biochemical hyperandrogenism and chronic anovulation being considered key for the diagnosis. The diagnosis of PCOS is established when a patient presents two of the above clinical criteria while ruling out other potential causes of hyperandrogenism, such as congenital adrenal hyperplasia, Cushing's syndrome, and androgen-secreting tumors. The NIH criteria for PCOS did not mention polycystic ovary (PCO), although there was controversy, it was widely used before the Rotterdam criteria appeared in 2003 (1). In 2003, the European Society of Human Reproduction and Embryology and the American Society for Reproductive Medicine formulated the Rotterdam diagnostic criteria for PCOS, proposing that at least two of the following three criteria were mandatory: oligo-anovulation, clinical/biochemical hyperandrogenism, and PCO appearance on ultrasonography. This included the ultrasonographic features of polycystic ovarian morphology (PCOM) in the diagnostic criteria (2). The introduction of the Rotterdam criteria significantly increased the number of patients diagnosed with PCOS. In 2006, the Androgen Excess and PCOS Society (AE-PCOS) proposed that in addition to meeting the criteria of oligo-anovulation or PCO, clinical or biochemical hyperandrogenism should be essential for the diagnosis of PCOS (3). The 2018 International Evidence-based Guideline updated the diagnostic criteria for PCOS based on the 2003 Rotterdam Consensus criteria, stating that anti-Müllerian hormone (AMH) can be used instead of ultrasound in the diagnosis of PCOS (4).

Influenced by diagnostic standards, geographical factors, and ethnic/racial factors, the prevalence of PCOS in women varies. Azziz reported a cumulative prevalence of 6.6% in women of reproductive age in Alabama, USA, with no significant statistical difference between black and white women, using the NIH 1990 criteria (5). A prospective study on the prevalence of PCOS in Spain, using the NIH 1990 criteria, reported a prevalence of 6.5% (6). From October 2007 to September 2011, an epidemiological study of 15,924 Han women of childbearing age in 10 provinces and cities of China, referring to the Rotterdam criteria, found a PCOS prevalence of 5.6% (7). According to the NIH 1990 criteria, the prevalence of PCOS in indigenous women aged 15-44 in Darwin and its surrounding areas in the Northern Territory of Australia was 15.3% (8). In South America, a study reported the prevalence of PCOS was 8.5% in women seeking primary health care in Salvador, Brazil, according to the Rotterdam criteria (9). Another study showed that the prevalence of PCOS among the Caucasian population in Ankara, Turkey, as reported according to the NIH, Rotterdam, and AE-PCOS Society criteria, was 6.1%, 19.9%, and 15.3%, respectively (10).

Despite its widespread occurrence and substantial impact on women's health, the precise pathogenesis of PCOS remains incompletely understood, and effective treatment options are currently lacking. Women with PCOS are at increased risk of Obstructive sleep apnea (OSA) (11), hypertension (12), depression (13), obesity (14), type 2 diabetes (15), and the pathogenesis of these diseases is highly associated with the imbalance of the autonomic nervous system (ANS) (16–20).

The ANS encompasses the sympathetic nervous system, parasympathetic nervous system, and enteric nervous system. The enteric nervous system is intrinsic to the gastrointestinal tract and collaborates with the parasympathetic and sympathetic systems to regulate digestion and absorption (21). While the function of the whole body is mainly regulated by the sympathetic nervous system and the parasympathetic nervous system. Nerve function is mediated by neurotransmitters, acetylcholine (ACh) and norepinephrine (NE) being the dominant autonomic neurotransmitters. In the parasympathetic system, both pre- and postganglionic neurons release ACh, with presynaptic ACh primarily acting on nicotinic receptors within autonomic ganglia and postsynaptic ACh primarily affecting muscarinic receptors in effector organs. In the sympathetic system, preganglionic neurons also release ACh, while postganglionic neurons release NE, which serves as the primary sympathetic neurotransmitter acting on α and β adrenergic receptors in target organs (22). The sympathetic and parasympathetic systems interact antagonistically, cooperatively, or independently to provide neural control over nearly all body tissues except skeletal muscle. In response to external and internal perturbations, the ANS regulates processes such as vascular tone, body temperature, heart rate, and glandular secretion to maintain homeostasis. In a healthy state, the autonomic nervous tone maintains an active equilibrium (23).

Apart from its role in maintaining the physiological activities of normal tissues and organs, the ANS is also implicated in the pathogenesis of various diseases. Imbalance of ANS, that is, abnormal increase or decrease of sympathetic or parasympathetic nervous system tension, may be related to the occurrence and development of PCOS. This review article aims to consolidate the latest developments in research pertaining to the relationship between the ANS and PCOS. It explores the role of these neural systems in the onset and progression of PCOS and discusses potential strategies for rectifying ANS balance as a means of treating PCOS. This research provides novel insights into disease prevention and management.

2 Autonomic nervous participate in the regulation of reproductive function

Mammalian ovarian function is under the control of the ANS, which operates in conjunction with the hypothalamic-pituitary-ovarian axis (HPOA), collectively influencing the secretion of ovarian steroid hormones and the process of ovulation (24, 25). In rat, the ovaries receive sympathetic innervation through the ovarian plexus nerves (OPN) and superior ovarian nerve (SON). The left OPN originates from the lumbar ganglion of the

sympathetic trunk (LGST), while the right OPN arises from both LGST and the superior mesenteric ganglion (SMG). After entering the ovary, these OPN nerves travel along the ovarian artery, innervating both the ovarian medulla and cortex regions (26). They may play a role in corpus luteum development and maturation (27). SON originates from the suprarenal ganglion (SG) and communicates with the ovaries through SG, Celiac ganglion (CG), and SMG. SON projects into the ovarian suspensory ligament, running parallel to its long axis, and enters the ovary through the ovarian hilum, distributing within the ovarian stroma and around the follicles, it is distributed in the ovarian stroma and around the follicle, and innervates blood vessels, interstitial glands and ovarian endometrial cells (28). SON contributes to ovarian function by influencing follicular development and steroidogenesis.Approximately 90% of NE in the ovaries is derived from the sympathetic nervous system. NE acts on β2-adrenergic receptors present in ovarian theca cells and granulosa cells, stimulating androgen production, enhancing follicle recruitment, and thereby increasing the likelihood of cystic follicle formation, which can trigger PCOS (29–31). Studies have shown that compared to non-PCOS women, PCOS patients have an increased density of adrenergic nerve fibers, significantly elevated levels of NE and dopamine (DA) in follicular fluid. DA is a precursor to NE and can be converted to NE after absorption by oocyte cells (32). NE may lead to elevated local oxidative stress levels in the ovaries, potentially impairing follicular development (33).

Nerve Growth Factor (NGF) is a marker of sympathetic neural activity. As a neurotrophic factor regulating the adrenergic neurons of the ovary, NGF provides nutritional support for the development of peripheral sympathetic nerve fibers (34). The follicular membrane is a critical area for the distribution and regulation of sympathetic neurons in the ovary, where NGF and its two types of receptors can be synthesized in follicular membrane cells (35). NGF is involved in the development and maintenance of follicles. However, excessive NGF can damage the bidirectional communication between oocytes and cumulus cells, significantly inhibiting the meiosis and maturation of oocytes (36). Dissen reported that NGF levels in the follicular fluid and granulosa cell culture medium are elevated in PCOS patients compared to non-PCOS populations (37). Studies have shown that transgenic mice overexpressing NGF in the follicular membrane (17NF mice) exhibit a higher density of tyrosine hydroxylase-positive nerve fibers in the ovaries, indicating an overdominance of sympathetic nerves. These mice display pathological characteristics of PCOS, such as arrested development of antral follicles, hyperandrogenemia, polycystic ovarian changes, accompanied by increased granulosa cell apoptosis and persistent elevation of serum luteinizing hormone (LH) levels. They also exhibit reproductive dysfunction along with metabolic abnormalities such as hyperinsulinemia, increased body fat, and insulin resistance (IR). Ovarian blockade of NGF can reduce the formation of cystic follicles induced by EV in PCOS rats, restoring their estrous cyclicity and ovulation (38). Furthermore, Manti using 17NF mice found that ovarian overexpression of NGF leads to placental dysfunction, impaired embryonic development, and offspring exhibiting increased sympathetic output until adulthood, with irregular estrous cycles, abnormal morphology and function of adipose tissue, and impaired glucose metabolism, reflecting reproductive-metabolic complications characteristic of PCOS (39). Kisspeptin is a class of neuropeptides widely distributed in the human body, participating in the regulation of the dynamic balance of female reproductive functions. Research indicates that Kisspeptin can stimulate the release of gonadotropin-releasing hormone (GnRH), playing a significant role in the hypothalamic-pituitary-gonadal axis (40). In addition to regulating the release of gonadotropins, Kisspeptin may also directly regulate follicular development through paracrine or autocrine mechanisms (41). A meta-analysis based on 699 patients and 583 controls pointed out that, compared to non-PCOS patients, patients with PCOS have higher serum levels of Kisspeptin, kisspeptin levels were independently correlated with PCOS (42). Elevated serum levels of Kisspeptin in PCOS patients may lead to an overactive hypothalamic-pituitary-gonadal axis (43). In the ovaries and corpus luteum, Kisspeptin levels are regulated by the sympathetic nervous system. The use of the β-adrenergic agonist isoproterenol increases Kisspeptin expression levels, while the β-adrenergic antagonist propranolol can reverse this increase (44, 45).

The vagus nerve is an essential component of the parasympathetic nervous system, with its ascending fibers terminating in four nuclei in the dorsal medulla: the dorsal motor nucleus of the vagus (DMV), solitary nucleus (NTS), trigeminal spinal nucleus, and ambiguous nucleus. There is no direct link between the gonads and the vagus nucleus in the central nervous system (46). The vagus nerve reaches the ovaries via peripheral nerves, with the left vagus nerve passing through the esophagus, bifurcating before inserting into the stomach, and entering the right celiac ganglion.The right vagus nerve runs parallel to the esophagus and is formed by the small ganglion into the right vagus plexus, which makes a connection with the celiac plexus and joins the RCG. The vagus nerve establishes connections with SON and OPN through the anterior vertebral ganglia. SON and OPN convey information from both vagus nerves to the ovaries, allowing indirect reception of central nervous system information by the ovaries (47). Because the vagus nerve innervates the gastrointestinal system, Through the synaptic connection of neurons in the prevertebral ganglion of the celiac plexus, information can be transmitted between the central nervous system, the stomach and the ovary, suggesting vagal innervation may play a role in the metabolism of the ovary (48). The vagus nerve is cholinergic fiber, and ACh is its primary neurotransmitter. ACh is synthesized from choline and acetyl coenzyme A (AcCoA) through choline acetyltransferase (ChAT). Choline is transported to the presynaptic nerve terminal via Na-dependent choline transporters. Adult rat and human ovaries contain ChAT enzymes, indicating that, in addition to exogenous ACh arriving via the vagus nerve, the ovaries themselves can synthesize ACh. Research has shown that granulosa cells (GC) of developing follicles and corpus luteum cells can produce ACh, and ACh secretion is influenced by gonadotropins, providing nutrition for follicular development and ovulation through muscarinic receptors (49–51). A decrease in ACh levels can interfere with the growth of

antral follicles and reduce reproductive capacity (52). Acetylcholinesterase (AChE) is also present in the ovaries and can hydrolyze ACh into choline and acetate (53). Studies have suggested that a splice variant of AChE, AChE-R, can induce necrotic apoptosis in granulosa cells, leading to follicular atresia and corpus luteum dissolution. Disrupting AChE enzyme activity may be a novel pathway affecting ovarian function (54). Administration of the AChE inhibitor huperzine A (HupA) to the ovaries of rats increased ovarian ACh levels and secondary follicle numbers after 4 weeks, significantly increasing corpus luteum numbers, indicating improved ovulation rates (55).

3 Autonomic nerves and PCOS in animal models

3.1 Estradiol valerate induced rat model

The use of animal models enriches the pathophysiological research of PCOS and serves as a crucial tool in exploring the mechanisms and treatments for PCOS. In studies investigating the relationship between PCOS and the ANS in animal models, estrogen-based modeling is the most commonly employed method. This involves a single intramuscular injection of long-acting estrogen, estradiol valerate (EV), at doses of 2-4 mg per rat to induce PCOS models (56). Exposure to EV during the critical developmental window causes irreversible changes in ovarian function, typically occurring between 14 and 24 days of age in Sprague-Dawley (SD) rats (57). EV induction results in disrupted estrous cycles, anovulation, and significant polycystic changes in the ovaries. Hormone levels such as follicle-stimulating hormone (FSH), LH, and testosterone (T) are altered. Because T can be rapidly converted into estradiol (E2) within the ovaries, the T levels in EV-induced rats may vary, showing reductions (58, 59), elevations (60), or similarities to control groups (61). The fundamental principle of EV induction is that high estrogen levels increase the pituitary gland's sensitivity to GnRH, leading to elevated LH levels and FSH suppression, thereby establishing a typical endocrine environment characteristic of PCOS and inducing non-obese PCOS animal models (62).

Changes in autonomic nervous activity occur in EV-induced rats with PCOS. Heart rate variability testing in PCOS rats reveals reduced cardiac vagal tone and increased sympathetic activity compared to normal rats (63). Studies have shown that, 60 days after EV injection, the ovaries of rats exhibit polycystic changes. At 30 days, there is a significant increase in the ovarian neurotransmitter NE released by sympathetic nerves. This is accompanied by selective downregulation of β-adrenergic receptors in the ovarian follicular membrane, indicating that activation of sympathetic neurons that innervate the ovaries precedes the onset of ovarian polycystic changes (64). Modulating the ANS that innervates the ovaries can impact the development of PCOS in rats. Neosaxitoxin is an algal toxin that binds to the outer pore of voltage-gated sodium channels, specifically blocking neuronal voltage-dependent Na^+ channels. This interaction produces an effective and reversible blockade of nerve conduction. Studies have demonstrated that Neosaxitoxin has minimal hemodynamic effects (65, 66). Ovarian cells are excitable, and endocrine-type voltage-dependent Na^+ channels, sensitive to Neosaxitoxin, are present in the ovaries (67). Animal experiments indicate that local administration of Neosaxitoxin to the ovaries via a micro-osmotic pump can reduce Na^+ levels in a short period. After 28 days of Neosaxitoxin application, a decrease in NE levels was also observed. This suggests that Neosaxitoxin chronically inhibits sympathetic nerve activity induced by EV in rats. Such intervention increases the number of corpora lutea, reduces ovarian follicular cysts, and lowers plasma T levels, restoring normal estrous cycles in rats (68).

The drug guanethidine selectively acts on postganglionic adrenergic nerve endings, antagonizing NE release. Administering guanethidine to EV-induced rats prior to successful modeling prevents ovulatory disturbances and hyperandrogenism (69). Beyond pharmacological interventions, neural regulation is also an intervention approach. Given the communication between peripheral and central nervous systems, one study removed the right ovarian tissue of EV-induced PCOS rats, eliminating the assumed influence of the right ovary on the hypothalamus. Subsequently, they removed the left SON and found that, compared to sham-operated rats, rats with severed nerves exhibited reduced numbers of cystic follicles and restored ovulation (70). Kilohertz-frequency alternating current (KHFAC) modulation is an emerging bioelectronic application that allows reversible regulation of nerve activity compared to nerve excision. In a study, researchers surgically removed the right ovary of rats and implanted electronic devices into the left SON to suppress nerve firing rates. After 2-3 weeks of KHFAC modulation, irregular or absent estrous cycles in PCOS rats were reversed, corpus luteum numbers increased, and ovarian NE concentrations decreased (71).

Vagal nerve transmission is also involved in the development of PCOS. In 24-day-old PCOS rats induced by EV, unilateral or bilateral vagotomy improved estrous cycles, restored ovulation, reduced ovarian androgen production, and decreased ovarian NE. However, there was no change in NE concentration in the celiac superior mesenteric ganglia complex (CSMG), which serves as a hub for the ovaries to receive information from the central system. This suggests that the vagus nerve can regulate ovarian function by directly affecting ovarian NE activity, independent of gonadotropin influences (72, 73). It has also been pointed out that the vagus nerve plays an asymmetric role in regulating the ovarian NE concentration, and the left vagotomy leads to a decrease in NE concentration, while the right vagotomy does not change NE concentration (74).

The ANS may influence PCOS rats through various mechanisms. Animal experiments have shown that low-frequency electroacupuncture (EA) stimulation can increase ovarian blood flow in PCOS rats. However, severing the SON and OPN nerves eliminates the increased ovarian blood flow, indicating that ovarian blood flow response to EA is mediated by the ANS. Ovarian blood flow affects follicular development. Thus, regulating ovarian blood flow may be one of the mechanisms by which the ANS affects PCOS (75). Using splenic macrophage culture medium to stimulate intact ovaries in PCOS rats, researchers found increased release of pro-

inflammatory molecules such as TNFα and NO by splenic macrophages, elevated Bax/Bcl2 ratios, increased apoptosis, and a high-inflammatory state. This state was reversed when the SON was severed. Additionally, markers of sympathetic nervous activity, such as NGF and kisspeptin, were downregulated, possibly indicating activation of the splenic sympathetic nervous system anti-inflammatory pathway. This suggests that the ANS also plays a role in regulating the immune function of inflammation in PCOS (76).

3.2 Androgen induced rat model

Exposure to excess androgens is a significant risk factor for PCOS. Overexposure to androgens during embryonic development can predispose individuals to PCOS, and increased intrauterine androgen levels in PCOS patients greatly increase the risk of passing PCOS genetically to their female offspring (77). Androgen exposure is also a commonly used method to induce PCOS models. Subcutaneous injection of T or dihydrotestosterone (DHT) in female rats can induce hyperandrogen PCOS models with reproductive dysfunction, and an increase in ovarian norepineergic fiber density, i.e. a change in ovarian sympathetic innervation, was observed after the development of cystic follicles in the model rats (78). The sympathetic nervous system is also involved in the metabolic disturbances induced by androgens in rats. There may be communication between sympathetic nerve cell bodies and fat cells, influencing lipid metabolism (79). Research has shown that PCOS rat models exhibit increased body weight, decreased sympathetic innervation in brown adipose tissue, and reduced thermogenic activity. EA can activate sympathetic innervation in brown adipose tissue and reduce body weight (80). Combining EA with exercise can improve the ovarian morphology of DHT-induced PCOS rats, increase healthy antral follicles, and decrease elevated phenotypic markers of sympathetic neurons, such as neuropeptide Y and NGF, in the mesenteric fat tissue. This provides support for the theory that sympathetic nervous activity is involved in the metabolic regulation of visceral fat in PCOS rats (81). Apart from lipid metabolic dysregulation, animal experiments have found that DHT-induced PCOS rats have increased mean arterial pressure. Treatment with adrenergic antagonist drugs like prazosin and propranolol or renal denervation can lower the mean arterial pressure in these rats, indicating that the activation of sympathetic and renal nerves were involved in the increase of blood pressure in PCOS rats (82).

3.3 Stress induced rat models

Sympathetic nervous system activity can be activated by stress responses. Cold stress can stimulate the release of glutamate in the paraventricular nucleus of the hypothalamus, and glutamate, through its N-methyl-D-aspartate receptor, further mediates the release of thyrotropin-releasing hormone. In this process, NE activity in the SON increases. Injecting glutamate receptor antagonists into the paraventricular nucleus reverses the activation of ovarian sympathetic nerves (83). Activation of the sympathetic nervous system promotes the development of PCOS. Rats subjected to stress exhibit halted follicular development, increased cystic follicles, elevated plasma T levels, irregular estrous cycles, infrequent ovulation, reduced fertility, resulting in PCOS-like phenotypes (84, 85). Research indicated that repeated cold exposure over four weeks can lead to sinusoid follicles with thickened follicular membranes in the ovaries of rats, which progress to cysts or type III follicles after eight weeks of stress exposure (86). Local administration of AChE inhibitor Huperzine-A (Hup-A) in the ovaries, which increases ovarian ACh levels, reduces ovarian cysts, increases corpus luteum numbers, and restores normal T and E2 plasma levels in stress-induced PCOS rats (87). Chronic mild unpredictable stress activates noradrenergic neurons in the locus coeruleus of the brainstem. The locus coeruleus is connected to pre-synaptic cell bodies in the ovarian sympathetic nerve pathway, leading to increased plasma and ovarian NE levels. This induces PCOS in rats similar to cold stress. The traditional Chinese medicine Xiao-yao-san intervention effectively improves abnormal follicular development, reduces granulosa cell apoptosis and autophagy in CUMS-induced PCOS rats. The treatment mechanism is believed to involve the reduction of locus coeruleus dopamine β-hydroxylase and c-FOS levels and the downregulation of NE and β2-adrenergic receptor expression in ovarian tissue (88).

4 Measurement of autonomic nervous system activity

Unlike in animal experiments where the role of the ANS is primarily judged through nerve blockade, in clinical practice, various non-invasive or minimally invasive instruments are often used to measure the autonomic nervous activity of patients. As the relationship between the ANS and the pathophysiological development of PCOS is continuously explored in basic research, the detection of autonomic nerve function in patients with PCOS is gradually being applied in clinical practice. Common clinical measurement methods include heart rate variability testing (HRV), muscle sympathetic nerve activity (MSNA), skin sympathetic response (SSR), and heart rate recovery (HRR) after exercise. HRV is the variation in the difference between each heartbeat cycle (the interval between the two consecutive R-waves of the electrocardiogram, R-R interval), resulting from the regulation of the sinoatrial node by the ANS. HRV measurement has become widely used for assessing ANS function and is currently one of the most commonly used non-invasive quantitative electrocardiographic evaluation method (89).

Analysis of HRV is often based on linear theoretical approaches, including time domain analysis and frequency domain analysis. Time domain analysis is a statistical measure of the discrete trend of R-R intervals of two adjacent heart beats, and the results of time domain analysis providing a comprehensive assessment of the ANS regulation of heart rate. Commonly used time domain indicators in clinical include the standard deviation of all N-N intervals (SDNN), the standard deviation of adjacent normal R-R intervals (SDANN),

and the root mean square of successive differences (RMSSD). SDNN reflects overall HRV, SDANN is associated with long-term HRV, and RMSSD represents short-term HRV (90, 91).The frequency domain analysis method is obtained by calculating the power spectrum through Fourier transform, which is more sensitive and accurate, reflecting the energy change with frequency change in HRV. Among the frequency domain indicators, low frequency power (LF) is jointly regulated by the cardiac sympathetic and vagal nervous systems, primarily reflecting sympathetic nervous system activity. High frequency power (HF) is mainly influenced by cardiac parasympathetic nervous activity. The LF/HF ratio is used to assess the overall balance between the sympathetic and parasympathetic nervous systems, with higher values indicating a dominance of sympathetic activity and lower values indicating parasympathetic dominance. Ultra low frequency power (ULF) and very low frequency power (VLF) components are less frequently observed and typically require the analysis of continuous data spanning over 24 hours or more. These components represent diurnal rhythms, temperature regulation, and hormonal influences. Total power (TP) corresponds to the total energy of the four spectral bands (LF, HF, VLF, and ULF) and reflects the dominant influence of sympathetic nervous activity or overall ANS activity (92, 93). The nonlinear calculation of autonomic nervous function includes SD1 and SD2,where SD1 represents the short axis of the HRV scatter plot, indicating the width of the plot at half its length, and SD2 represents the long axis of the scatter plot, measuring the length along the 45-degree line (94). Commonly used clinical HRV parameters were shown in Table 1.

MSNA is a measurement of the skin response of the sympathetic nervous system involving both MSNA frequency and MSNA burst incidence. MSNA frequency refers to the number of integrated bursts per minute, while MSNA burst incidence is the number of bursts per 100 heartbeats. MSNA reflects vasomotor activity and is a key regulator of cardiovascular homeostasis. It regulates blood pressure and blood flow through a cascade of neural vascular signals originating from the central nervous system. This neural-driven stimulus recruits efferent sympathetic neurons and can be used to assess sympathetic nervous system activation (95). Microneurography is the gold standard for assessing sympathetic vasoconstriction and vasodilation outflow, and can directly assess MSNA in humans at the neuronal level. The measurement method is to insert a tungsten microelectrode with a tip diameter of a few microns directly into the neuromuscular bundle through the skin, and find a site that can record the burst of neural activity and record MSNA directly. To facilitate identification, nerves chosen for recording need to be located close to the body surface, while also being sufficiently large to support and stabilize the microelectrode tip. Commonly selected nerves include the radial nerve, median nerve, ulnar nerve in the upper limbs, and the tibial nerve and fibular nerve in the lower limbs (96). SSR is one of the electrophysiological methods used to assess sympathetic nervous

TABLE 1 Summary of the main heart rate variability parameters.

Time domain parameters			
Variable	Units	Description	Physiological origin
SDNN	ms	Standard deviation of all R–R intervals	The degree of heart rate variability
SDANN	ms	The standard deviation of the averages of R–R intervals during all 5-min periods	Sympathetic nervous activity
RMSSD	ms	Root mean square of the difference between adjacent R–R intervals	Parasympathetic nervous activity
NN50	beats	Number of pairs of adjacent R–R intervals difference by more than 50 ms	Parasympathetic nervous activity
pNN50	%	Percent of R–R intervals differing more than 50 ms from each other	Parasympathetic nervous activity
Frequency domain parameters			
Variable	Units	Description	Physiological origin
TP	ms^2	The band ranges less than or equal 0.4Hz, the amplitude of normal heartbeat interval	Sympathetic or autonomic nervous activity
HF	ms^2	The band ranges between 0.15 and 0.40Hz, the amplitude of the normal heartbeat interval in the high-frequency range	Parasympathetic nervous activity
HFnorm	nu	Normalized units of HF	Parasympathetic nervous activity
LF	ms^2	The band ranges between 0.04 and 0.15 Hz, the amplitude of the normal heartbeat interval in the low-frequency range	Sympathetic and parasympathetic nervous activity
LFnorm	nu	Normalized units of LF	Sympathetic nervous activity
VLF	ms^2	The band ranges between 0.003 and 0.04Hz, the amplitude of the normal heartbeat interval in the very low frequency range	Long-term regulation mechanisms, thermoregulation and hormonal mechanisms
ULF	ms^2	The band ranges less than or equal 0.003Hz, the amplitude of the normal heartbeat interval in the ultra low frequency range	Circadian oscillations, core body temperature, metabolism and the renin-angiotensin system
LF/HF		Low frequency power-to-high frequency power ratio	Sympathetic activity or autonomic nervous balance

(Continued)

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TABLE 1 Continued

Non-linear indices			
Variable	Units	Description	Physiological origin
SD1		Quick and high-frequency changes in heart rate variability	Parasympathetic nervous activity
SD2		Long term changes in heart rate variability	Sympathetic nervous activity

system function. When a nerve is stimulated, nerve impulses travel through large myelinated sensory fibers, passing through central synapses including the hypothalamus, brainstem, limbic system, and spinal intermediolateral column. Subsequently, they efference through pre-ganglionic and post-ganglionic sympathetic fibers, leading to the excitation of sweat gland activity in the skin, which can be recorded as a reflexive electrical potential in the skin, known as SSR (97). The SSR is usually stimulated by electrical stimulation of the median nerve or the posterior tibial nerve, and the recording electrode is placed in the palm of the hand or foot. The reference electrode is placed on the back of the hand or foot. The acting electrodes are placed in the palms and feet. Upper and lower limbs can be recorded simultaneously. The SSR latency reflects the conduction time of the nerve impulse causing sweating in the whole reflex arc, while the amplitude reflects the excitability of sweat glands with secretory activity and is a reliable indicator of peripheral sympathetic nerve activity (98, 99).

HRR after exercise is a commonly used indicator for evaluating cardiac ANS activity. It is defined as the rate at which heart rate decreases following maximal graded exercise testing. HRR is calculated as the difference between peak heart rate during exercise and heart rate at 1, 2, 3, 4, 5, and 7 minutes after exercise cessation (100). In current clinical studies, the test method of HRR value can be extreme dose, subextreme dose or symptom-restricted cardiopulmonary exercise test, and the postexercise position can be standing, sitting, supine, etc. During the early phase of post-exercise HRR, enhanced vagal activity plays a major role in the rapid decline of heart rate. Subsequently, increased parasympathetic activity and decreased sympathetic activity together contribute to the return of heart rate to baseline levels before exercise. Delayed HRR reflects compromised parasympathetic activity, reduced vagal tone, and relatively heightened sympathetic activity, resulting in a slower decline in heart rate (101). Additionally, delayed recovery of systolic blood pressure (SBP) following peak exercise may also have diagnostic value and may reflect excessive sympathetic nervous system activity (102). Respiratory motion is regulated by the ANS, and changes in respiratory rate can cause periodic changes in heart rate. The cardiovascular and respiratory centers regulate vagal nerve outflow through expiratory-driven reflexes, and reducing the respiratory rate may increase vagal nerve tension. Therefore, measuring respiratory cycles could also be an effective way to reflect whether autonomic nerve activity is balanced (103, 104). Devices like the respiratory inductance plethysmograph can non-invasively measure parameters such as respiratory rate, tidal volume, and minute ventilation, and have good prospects for clinical application (105).

5 Changes in autonomic nervous system function in PCOS patients

Various methods have been applied in clinical settings to investigate the potential abnormalities in ANS activity among PCOS patients, with HRV detection being the most commonly used method. Multiple clinical studies have shown that PCOS patients exhibit enhanced sympathetic nervous system activity and reduced parasympathetic nervous system activity compared to control groups of women with regular menstrual cycles (106–109). However, due to the heterogeneity of PCOS, which often involves metabolic abnormalities, these factors can also influence the ANS function in PCOS patients. For instance, Yildirir and colleagues found that PCOS women have lipid abnormalities, with lower serum levels of high-density lipoprotein cholesterol (HDL-C) and higher triglyceride levels and total cholesterol-to-HDL-C ratio compared to normal menstrual cycle controls (106). PCOS patients also tend to have higher body mass index (BMI) and blood pressure compared to control group women (107, 108). Studies have shown that in PCOS patients, indices representing parasympathetic nervous system activity, such as TP, HF, HFnorm, and SD1, are significantly higher in those with lower BMI (<25 kg/m^2) (108), and there is a significant negative correlation between BMI and SDNN, LF, and HF (109). When combined with glycemic parameters, Saranya found a significant positive correlation between LF/HF and BMI, waist-hip ratio (WHR), and fasting blood sugar (110). Regression analysis has shown that IR index (HOMA-IR) and atherosclerosis index are independently related to the LF-HF ratio (111). Stroop color-word conflict tests used as mental stress tests (112) have revealed that anovulatory PCOS women exhibit impaired sympathetic nervous regulation following stress compared to women with regular ovulatory cycles (113). Mishra compared PCOS patients with age-matched healthy female subjects and found no significant differences in weight, BMI, waist circumference, hip circumference, and WHR between the two groups. The HRV index of the two groups at baseline was comparable, but the autonomic driving force of PCOS women decreased after exercise, indicating that young PCOS women with normal weight also had potential autonomic dysfunction, which was exposed after exercise (114).

HRR has been employed to assess cardiac ANS activity in PCOS patients. Tekin used a control group of women without hyperandrogenemia, menstrual cycle, and ovulation regularity. These women did not differ significantly from PCOS women in terms of age, BMI, triglycerides, T, and LH levels. All subjects underwent symptom-limited exercise tolerance testing using a modified Bruce protocol. The results indicated that PCOS patients had decreased 1-minute HRR, increased SBP during exercise peak, and delayed SBP recovery after exercise, indicative of decreased autonomic activity (115). Giallauria and colleagues

demonstrated a negative correlation between HRR in PCOS women and BMI and insulin area under the curve (116). Similarly, Kaya and colleagues observed a decrease in 1-minute HRR in PCOS patients, along with elevated homocysteine levels, it suggests that PCOS patients have an increased risk of cardiovascular disease in the future. PCOS patients also had significantly higher total T levels, fasting insulin, and HOMA-IR compared to the control group. HOMA-IR and BMI were identified as independent determinants of abnormal HRR in PCOS patients. The study also indicated a negative correlation between the inflammatory marker C-reactive protein (CRP) and HRR, suggesting that IR, hyperandrogenism, autonomic dysfunction, and chronic low-grade inflammation may collectively play a role in the pathophysiology of PCOS (117).

Direct measurements using microneurography have confirmed elevated MSNA in PCOS patients, including both MSNA frequency and MSNA burst incidence (118–121). Sverrisdóttir and colleagues found a positive correlation between MSNA levels and T and cholesterol levels in PCOS (118). Lansdown and colleagues measured MSNA during isometric forearm contraction in subjects, and compared to matched control groups, PCOS patients exhibited enhanced MSNA. Functional magnetic resonance imaging of the right orbitofrontal cortex showed differential activation, which correlated with insulin sensitivity. Hyperinsulinemia in PCOS may affect sympathetic output (119). Lambert compared obese women with PCOS. The two groups had similar metabolic characteristics, including BMI, waist circumference, hip circumference, high-density lipoprotein and low-density lipoprotein, cholesterol, triglyceride, glucose, insulin sensitivity and blood pressure. Nevertheless, PCOS patients had increased MSNA and heightened sympathetic drive (120). Shorakae also found in clinical studies that a significant correlation between MSNA and PCOS status after adjusting for age and BMI, demonstrating that metabolic abnormalities in PCOS can influence ANS activity, but elevated MSNA remains independently associated with PCOS (121). Furthermore, the mean latency of SSR in PCOS patients was significantly delayed, and the mean amplitude was reduced,also indicating the presence of autonomic dysfunction in PCOS patients (122).The changes of autonomic nerve parameters in PCOS patients were shown in Table 2.

In summary, PCOS has high heterogeneity, and different metabolic levels can affect autonomic nerve activity in PCOS patients. These women may already have autonomic nerve abnormalities before PCOS can be diagnosed, and autonomic nerve function detection has a good clinical application prospect for early diagnosis of PCOS. Women with PCOS are at increased risk for cardiovascular disease, and autonomic abnormalities can be effective predictors of future cardiovascular complications in women with PCOS. This also suggests that in terms of treatment, improving autonomic nervous function may help delay the occurrence and development of PCOS, in addition, autonomic nerve may be used as an evaluation indicator of patient treatment effect.

TABLE 2 Summary of research results on cardiovascular autonomic parameters.

Reference	PCOS/ Controls number	PCOS patients age	Controls age	Effect of experimental group compared with control group
(80)	30/30	27.9 ± 6.1	31.4 ± 6.5	LFnorm↑、LF/HF↑、HF↓、HFnorm↓
(81)	35/32	29.91 ± 0.73	31.06 ± 0.68	LF↑、LF norm↑、LF/HF↑、HF norm↓
(82)	30/30	28.03 ± 5.33	27.27 ± 5.69	TP↓、HF↓、HFnorm↓、VLF↑、LFnorm↑、LF/HF↑、SD1↓ 、SD2↓
(83)	23/23	26.7 ± 4.8	25.4 ± 4.6	SDNN↓、RMSSD↓、LF↓、HF↓
(84)	31/30	23.129 ± 4.129	24.733 ± 2.935	LFnorm↑、HFnorm↓、LF/HF↑、SDNN↓、RMSSD↓、NN50↓、pNN50↓
(85)	35/32	23.1 ± 4.1	24.6 ± 2.9	TP↓、HFnorm↓、LFnorm↑、LF/HF↑、SDNN↓、RMSSD↓、NN50↓、pNN50↓
(87)	30/23	22.80 ± 5.80	22.65 ± 5.89	(post-stress)LFnorm↓、LF/HF↓
(88)	27/25	19.00-23.00	19.00-20.00	(after exercise)LF↓
(89)	26/24	25.5 ± 3.9	26.0 ± 3.8	TP↓、SDNN↓、SDANN↓、RMSSD↓、pNN50↓、HF↓、LF↓、VLF↓、HRR1↓、
(90)	75/75	21.7 ± 2.1	21.9 ± 1.8	HRR↓
(91)	68/68	24.2 ± 4.8	24.4 ± 3.9	HRR↓
(92)	20/18	29.9	27.4	MSNA↑
(93)	20/20	29.8 ± 4.8	29.7 ± 5.0	MSNA↑
(94)	19/21	31.3 ± 1.6	28.2 ± 1.6	MSNA↑
(95)	49/23	30 ± 6	29 ± 8	MSNA↑
(96)	37/33	21.56 ± 3.37	21.20 ± 1.85	SSR↓

↑, The experimental group was higher than the control group, and the difference was statistically significant; ↓: The experimental group was lower than the control group, and the difference was statistically significant.

6 Potential mechanisms of autonomic nervous effect on PCOS

6.1 Interacting with adipokines

Adipose tissue is not only an energy storage for the body but also the largest endocrine organ. Through paracrine and autocrine pathways, it releases various adipokines targeting different organs and tissues. These adipokines regulate biological processes such as glucose and lipid metabolism, energy expenditure, inflammatory response, and immune reactions in the body (123–125). In PCOS, adipocyte dysfunction is observed (126). Studies indicate that compared to healthy women, PCOS patients have significantly lower serum concentrations of adiponectin (APN), and higher levels of leptin (LEP) and chemerin (CHEM). The diagnosis of PCOS is an independent predictor of serum levels of LEP, APN, and CHEM. The levels of LEP, APN, and CHEM can be used as independent biomarkers for the diagnosis of PCOS (127). The central nervous system receives information from adipokines and sends out metabolic balance signals through autonomic nervous circuits, suggesting the involvement of autonomic nerves in PCOS through regulating adipokines (128).

Secreted by mature white adipose tissue, APN possesses antioxidative, anti-inflammatory properties, and stimulates energy expenditure (129). As an endogenous insulin sensitizer, APN enhances glucose absorption by increasing fatty acid oxidation and reducing hepatic glucose synthesis, thereby improving insulin sensitivity. Sepilian et al. reported reduced serum APN levels in PCOS patients, showing a significant negative correlation with IR (130). APN also plays a role in ovarian steroidogenesis. Binding of APN and its receptors (AdipoR1 and AdipoR2) in the ovaries upregulates FSH levels, induces the synthesis of progesterone and E2, and inhibits LH and androgen levels in ovaries (131–133). Subcutaneous injection of APN in dehydroepiandrosterone-induced PCOS mice lowered their elevated weight and androgen levels, restoring ovulation (134). The synthesis of APN is regulated by the ANS. Animal studies show that cold exposure physiologically activates the sympathetic nervous system, reducing serum and white adipose tissue APN expression, reversible by β-adrenergic receptor antagonists (135). In PCOS patients, reduced APN concentration is an independent factor for the diminished HRR in PCOS women (136). Shorakae et al., using menstrually regular overweight or obese women as controls, found that after adjusting for age and BMI, the APN levels in PCOS women were lower than those in the control group, with significantly higher sympathetic nerve activity, suggesting that hyperactive sympathetic nerves may be a driving factor for reduced APN levels in PCOS (137).

LEP is an adipokine secreted mainly by white adipose tissue. It crosstalk with steroid-producing cells and can be transmitted to the brain as a metabolic signal, affecting the HPOA and regulating ovulation. A higher level of LEP will inhibit E2 synthesis and interfere with follicle development (126, 138). LEP is also involved in the immune inflammatory response of the body. Compared with women of normal reproductive age, the level of circulating LEP in women with PCOS is increased and positively correlated with Interferon-γ level. In cell experiments, interferon-gamma treatment increases the apoptosis of human granular cells (139). A meta-analysis involving 991 women with PCOS and 898 healthy control women showed that LEP levels were significantly higher in patients with PCOS than in the control group (140). A meta-analysis showed that non-obese PCOS patients had significantly higher circulating LEP levels than non-obese healthy women (141). In animal experiments, DHT-induced excess androgens in PCOS rats increased neuropeptide Y expression by down-regulating insulin and LEP signaling in the hypothalamus. This increases the rats' food intake and promotes obesity (142). Higher LEP levels in women with PCOS may be related to a number of factors, among which insulin has been shown to induce more LEP secretion in white adipose tissue and increase circulating LEP levels (143, 144). LEP receptors are enriched in the hypothalamus (145), a region rich in neurons that control energy homeostasis and autonomic nervous function, and LEP binding to its receptors can increase the sympathetic nerve activity that innervates many organs (146). Microinjection of LEP into the hypothalamic arcuate nucleus and paraventricular nucleus of rats can induce sympathetic nerve excitation (147), and LEP may increase sympathetic nerve activity partly through local activation of melanocortin 3/4 receptor (148). SHU9119 blocking melanocortin 3/4 type receptors in the paraventricular nucleus of the hypothalamus reduced sympathetic nerve activity in LEP treated rats (149). Studies have shown that LEP interacts with sympathetic nerve fibers in adipose tissue and is involved in the lipolysis of white adipose tissue (150), and may be involved in more energy metabolism processes.

CHEM is mainly secreted by white adipose tissue and is involved in biological processes such as immune regulation, adipogenesis and energy metabolism (151). CHEM and its chemokine-like receptor 1 are richly expressed in the ovary and have an inhibitory effect on the production of ovarian steroid hormones (152). In human primary granulosa cells, CHEM treatment inhibited the expression of aromatase and cytochrome P450 in follicles, further impairs the secretion of E2 and progesterone (153). In DHT-induced PCOS rats, hyperandrogenemia increases the expression of chemerin and chemokine-like receptor 1 in the ovary and may therefore affect the immune microenvironment of the ovary, leading to local ovarian inflammation (154). A retrospective study reported increased CHEM expression in patients with PCOS, and serum CHEM concentrations can reflect the severity of polycystic changes (155). Metabolic factors such as obesity and IR affect CHEM expression in PCOS patients. Studies have shown that compared with healthy women, serum CHEM expression is higher in PCOS patients, and serum CHEM levels are higher in PCOS women with higher BMI than PCOS women with lower BMI (156, 157). CHEM, on the other hand, are known to be inhibitors of insulin signaling and glucose catabolism, weakening gene expression in cells involved in glucose and lipid homeostasis (158). Clinical studies have observed significant increases in chemerin levels in serum, subcutaneous, and omental adipose tissue in patients with PCOS, further increases after insulin infusion, and declines after metformin treatment (159). CHEM

expressed in the hypothalamus and pituitary gland and have a potential role in controlling neuroendocrine events, and intraventricular injection of chemokine-9 increases plasma adrenaline levels and sympathetic nerve activity, which is mediated by the CMKLR1 receptor (160). Chemotactic proteins have been shown to alter sympathetic contractions to control blood pressure (161). This may indicate that the autonomic nerve exerts its influence on the body through its interaction with adipokines.

6.2 Participating in inflammatory pathways

Inflammation is one of the key factors affecting the pathology of PCOS. Studies have shown that compared with normal women, PCOS patients have significantly higher levels of serum and ovarian inflammatory markers (IL-2, IL-6, IL-18, IL-8, IFN-γ, TNF-α, etc.) and lower concentrations of anti-inflammatory cytokines (IL-10) (162, 163). The imbalance between pro-inflammatory and anti-inflammatory cytokines breaks down physiological homeostasis and generates inflammation, resulting in a microenvironment of low-degree chronic inflammation in PCOS patients, leading to the occurrence and development of PCOS (164). IR and hyperandrogenemia are considered to be the main causes of PCOS. Studies have shown that inflammation in tissues of PCOS patients interacts with impaired insulin metabolism (165). Local inflammatory environment can induce abnormal insulin signal transduction to cause IR, which is accompanied by hyperglycemia, and glucose can lead to increased levels of pro-inflammatory cytokines. This induces local and systemic proinflammatory states and impairs insulin signaling (166). High levels of androgens can activate the NF-κB inflammatory signaling pathway, resulting in the increase of CRP, TNF-α, IL-6 and other inflammatory factors, and induce the body's inflammatory response. Inflammatory factors such as IL-6 can also activate the JAK/STAT3 signaling pathway, thereby inhibiting GLUT-4 secretion and preventing normal glucose metabolism (167). Chronic inflammation also affects follicular development, leading to ovarian dysfunction, and abnormally elevated levels of pro-inflammatory factors have been shown to inhibit the proliferation and differentiation of PCOS follicles, resulting in delayed follicular maturation (168).

In the inflammatory response, signals of the inflammatory state are communicated to the central nervous system, and the brain integrates the information and plays an important role in immune regulation, in which the autonomic nervous system plays an important role. Inflammatory signals are transmitted from the afferent fibers of vagus nerve to the nucleus tractus solitaris, and then activate the paraventricular nucleus of hypothalamus to secrete antidiuretic hormone and adrenocorticotropin releasing hormone, which activate the anterior pituitary gland and release adrenocorticotropin, corticotropin activates adrenocortical cells, which further release adrenocortical hormones. The final release of the most potent anti-inflammatory hormone, glucocorticoids, exerts long-lasting anti-inflammatory effects, namely the hypothalamic-pituitary-adrenalin axis (HPA) anti-inflammatory pathway (169, 170). Inflammation also activates the cholinergic anti-inflammatory pathway, where inflammatory signals are transmitted to the brain through the afferent vagus nerve and then integrated in the central part of the brain, and then these integrated immunoinflammatory signals are transmitted to the efferent vagus nucleus. Finally, it is activated at the efferent fibers of the vagus nerve, triggering the release of neurotransmitter ACh from external nerve endings. On the surface of immune cells (macrophages), ACh effectively inhibits the release of pro-inflammatory factors in macrophages by binding to the specific nicotinic Ach receptor, and weakens the inflammatory response, thus playing a role in protecting the immune system (171, 172). The splenic sympathetic anti-inflammatory pathway also plays an important role. After the inflammatory message is transmitted to the spleen, the vagus nerve in turn drives the splenic sympathetic nerve to release NE in the spleen (173), which activates β2 adrenergic receptors expressed by specific subsets of T cells that are capable of synthesizing and releasing Ach. Ach binds to α7 nicotinic receptors in macrophages, thereby inhibiting the release of pro-inflammatory cytokines (174). The regulation of inflammatory processes by ANS may be an important mechanism involved in the development of PCOS.

6.3 Affecting the digestive system

The autonomic nervous system is a key component of a two-way communication system between the central nervous system and the gut, where the gut microbiome, the gut, and the central nervous system form a microbiome-gut-brain axis that plays a crucial role in several aspects of physiology, including regulation of eating and appetite, glucose homeostasis, and intestinal motility (175, 176). ANS neurons are found in the celiac, superior and inferior mesenteric ganglia, which governs intestinal function. These neurons transmit information about intestinal homeostasis, including intestinal fluid exchange, pancreatic body secretion, mucosal barrier function, etc. to the CNS (177, 178). Gastrointestinal hormones cholecystokinin, glucagon-like peptide-1, and casein are secreted from intestinal mucosal cells in response to nutrient intake signals and can act on the afferent subgroup of the vagus nerve (179, 180). Stress caused by food intake can also stimulate the sensory neurons of the vagus nerve, through which information reaches the brain. Triggers the activation of outgoing vagus signals in the brain, which in turn are involved in the neural control of food intake (181). Glucagon-like peptide-1 receptor vagus afferent activation improves glucose tolerance and its inhibition increases blood glucose levels (182). There is two-way communication between the neuroendocrine system and the gut microbiome, with a network of specialized target cells/transducers in the gut wall acting as an interface between the microbiome and the host cavity. In response to external and physical demands, the brain regulates these specialized cells in this network through the branches of ANS. The microbiome maintains continuous two-way communication with this interface through

multiple microbial signaling pathways that are regulated in response to perturbations in the microbiome or brain (183, 184). The disturbance of intestinal microbiota and gastrointestinal hormone secretion in PCOS patients can affect the gastrointestinal system and the central nervous system through the vagus nerve (185, 186), and its specific role needs to be further studied.

7 Clinical evidence of autonomic nerve regulation for PCOS

Given the role of the ANS in PCOS, regulating autonomic activity may be an effective approach to managing PCOS. In terms of surgical treatment, ovarian wedge resection can increase ovulation in PCOS patients, part of the reason may be that the procedure destroys the sympathetic nerve that innervates the ovaries (187). Clinical research has shown that two PCOS patients with concomitant refractory hypertension experienced reduced MSNA and whole-body NE overflow three months after bilateral renal neuroablation. Not only did their blood pressure decrease, but their insulin sensitivity also significantly improved, and one woman regained her menstrual cycle (188). Rational exercise is an effective method for treating PCOS. Clinical studies have demonstrated that after 16 weeks of aerobic exercise, obese PCOS patients can experience increased vagal nerve regulation (RMSSD, HF, HFnu, 2UV%), reduced sympathetic modulation (LF, LFnu), and lower resting heart rate and SBP (189). Other research has indicated that aerobic exercise training can lower body weight and insulin area under the curve in overweight and obese PCOS women, meanwhile levels of the inflammatory markers CRP and white blood cells were reduced, moreover, the improvement of the above metabolic and inflammatory indexes is correlated with the improvement of HRR after exercise (190). However, when PCOS women underwent HRV measurements after 4 months of anaerobic exercise, there were no significant changes in the parameters (191). Clinical research also suggests that aerobic exercise is more beneficial for PCOS patients compared to anaerobic exercise.

OSA refers to the recurrent occurrence of apnea and/or hypopnea during sleep, resulting in chronic intermittent hypoxemia, hypercapnia, and disruptions in sleep architecture. As previously mentioned, PCOS women have a higher prevalence of OSA (192), the pathogenesis of OSA is associated with increased sympathetic nervous system activity (193, 194). Continuous positive airway pressure (CPAP) therapy is the most commonly used method to treat OSA (195). In young obese women with PCOS who received CPAP, subjects showed improved insulin sensitivity and reduced plasma 24-hour NE levels with no change in body weight, as found by HRV testing sympathetic output is reduced, and treatment transforms cardiac autonomic nerve activity to lower sympathetic tone and higher parasympathetic tone (196). Clinical studies have exposed subjects to repeated passive heat exposure through hot water baths, which improved flow-mediated vasodilation, protected vascular endothelium from ischemia-reperfusion-related injury, lowered total cholesterol levels, and fasting blood sugar. After treatment, subjects exhibited decreased MSNA and total T levels, with MSNA decreasing earlier than T, indicating that changes in sympathetic nervous system activity precede changes in androgen production. This provides support for the theory that sympathetic nervous system activity drives ovarian androgen production (197). Acupuncture may regulate sympathetic nervous system activity by stimulating ergot receptors and somatic afferents in muscles to modulate sympathetic activity. Compared to untreated control groups, low-frequency EA reduced MSNA burst frequency and free T in overweight PCOS women (198). Therefore, the ANS may offer new therapeutic targets for PCOS, but larger-scale and long-term studies are needed before applying these treatments in clinical practice. Based on our previous discussion of the mechanism, potential treatments include altered gut microbiota that normalizes signals to the autonomic nervous system, and probiotics are a good option (199). Transcutaneous auricular vagus nerve stimulation is a nerve regulation technique that can enhance the excitability of vagus nerve by stimulating the vagus nerve of the auricular appendicular cavity, which helps to restore the balance of autonomic nerve function (200). It is the most widely used and safest way of transcutaneous nerve stimulation. Nerve impulse is gradually transmitted to the brain region of the central nervous system through the vagus nerve along the dorsal raphe nucleus, parbrachial nucleus, hippocampus, prefrontal cortex, etc., thus achieving the regulatory effect on organs (201). This therapy has been used in the treatment of a variety of autonomic nervous disorders such as depression, obesity, hypertension and abnormal glucose tolerance, and has achieved good clinical efficacy (202, 203). With reduced vagus nerve activity in patients with PCOS, taVNS may be a potential treatment for PCOS (204). Since IR is a hallmark of PCOS and insulin may be involved in activation of the sympathetic nervous system, insulin sensitizers may be considered to have therapeutic benefits in reducing sympathetic output in PCOS. Studies have shown that metformin therapy can normalize cholinergic response in obese rats through M3 muscarinic acetylcholine receptors in pancreatic beta cells and improve ANS function in obese rats, suggesting that metformin may act on the ANS to treat PCOS (205). Intervention of PCOS based on adjustment of autonomic nerve were shown in Figure 1.

8 Discussion

The ANS plays a significant role in the development and progression of PCOS, affecting aspects such as follicular growth and development, steroid hormone secretion, and glucose and lipid metabolism regulation. Mechanisms include the activation of the central nervous system by afferent nerves, direct influences on ovarian function by regulating ovarian blood flow and hormone secretion, and involvement in the body's inflammatory immune response. MSNA and HRV are commonly used methods in clinical

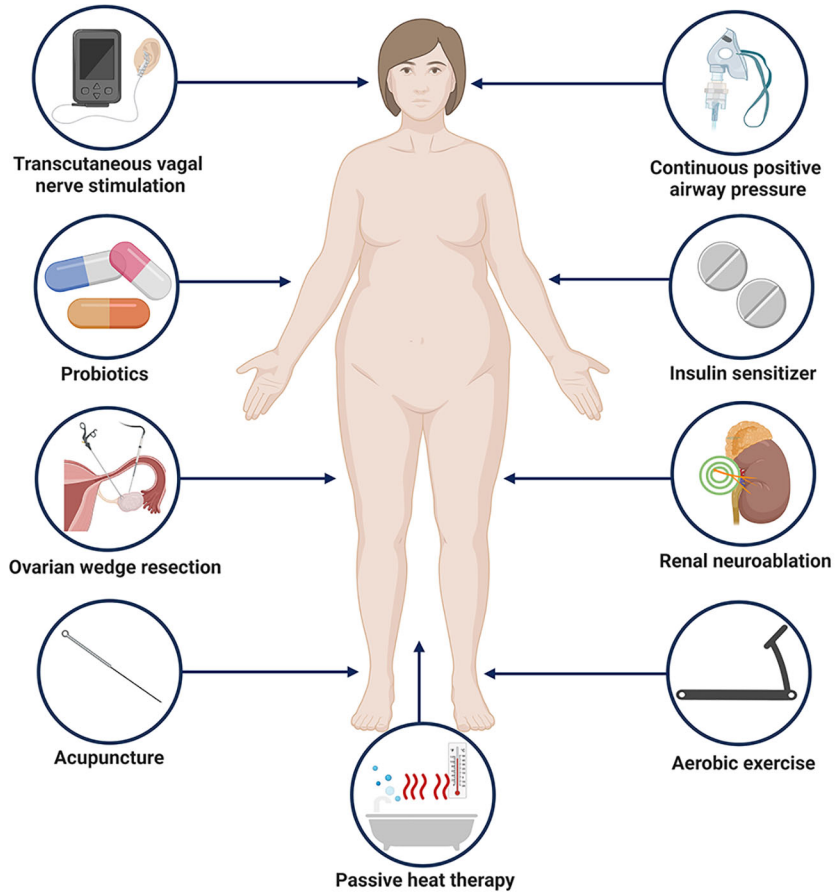

FIGURE 1
Potential approaches to PCOS intervention based on regulation of autonomic nerves. Created with BioRender.com.

practice to assess autonomic function in PCOS patients. PCOS women often exhibit enhanced sympathetic nervous system activity and reduced vagal nerve activity. Interventions based on regulating the ANS have already been explored for PCOS management. However, we contend that there remain several issues in this field that warrant further investigation. As previously mentioned, the ANS works in parallel with the HPOA, regulating follicular development and ovulation. The fluctuation of female hormones affects the remodeling of central neurons and the sensitivity of adrenergic receptors, thereby influencing autonomic nervous function. Furthermore, different sex hormones may play varied roles, but the specific mechanisms of action are not yet clear. Future research could explore the distinct mechanisms by which varying levels of sex hormones modulate autonomic nervous function in patients with PCOS and their potential targets. We also wish to highlight that studies based on rodent models have provided substantial evidence of the ANS's involvement in the development and progression of PCOS. This inspires us to pursue more explorations in future clinical work, including the identification of more serological markers that reflect autonomic activity with high specificity, investigating targeted therapeutic approaches from the relatively independent perspective of the ANS, and conducting more randomized controlled trials to evaluate the clinical efficacy of autonomic-related treatments in PCOS patients. Moreover, research on the concept and implementation of vagus nerve modulation in treating PCOS is scant, representing a potentially promising area of study. However, it must be noted that as the ANS controls cardiopulmonary functions, many therapeutic approaches may carry the risk of cardiopulmonary side effects. Therefore, exploring ways to mitigate these risks is also of significant interest.

Author contributions

YYu: Writing – original draft, Writing – review & editing. TC: Writing – original draft, Writing – review & editing. ZZ: Writing – original draft. FJ: Writing – original draft. YaL: Visualization, Writing – review & editing. YR: Visualization, Writing – review & editing. XL: Writing – review & editing. YL: Conceptualization, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Blood nesfatin-1 levels in patients with polycystic ovary syndrome: a systematic review and meta-analysis

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Background: Previous studies have investigated the relationship between nesfatin-1 level and polycystic ovary syndrome (PCOS). However, these studies have produced conflicting results. Thus, in this meta-analysis, we aimed to clarify the association between blood nesfatin-1 levels and PCOS, and the ability of nesfatin-1 as a biomarker in PCOS.

Methods: Meta-analysis was performed using STATA 12.0 software. We computed standard mean difference (SMD) and 95% confidence interval (CI) regarding the comparison of blood nesfatin-1 in patients with PCOS and controls.

Results: The present meta-analysis showed no significant difference in blood nesfatin-1 level between patients with PCOS and controls with a random effects model (SMD = 0.03; 95%CI: -0.71, 0.77; I^2 = 97.1%, *p* value for Q test < 0.001). Subgroup analysis for different ethnicities reported no significant difference in blood nesfatin-1 level between patients with PCOS and controls in both Caucasian and Asian populations. Subgroup analysis for different sample types reported no significant difference in serum nesfatin-1 level between patients with PCOS and controls. Subgroup studies reported no significant difference in blood nesfatin-1 level between PCOS and controls in both obese and non-obese populations.

Conclusion: In conclusion, there is no significant relationship between blood nesfatin-1 levels and PCOS.

KEYWORDS

meta-analysis, nesfatin-1, polycystic ovary syndrome, serum, systematic review

1 Introduction

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders without exact etiology currently, affecting approximately 6%-10% of women worldwide (1). PCOS patients are most characterized by sex hormone imbalance, with hallmark features of acne, hirsutism, infertility, irregular menstrual cycle, and polycystic appearing ovaries on ultrasound (2). The Rotterdam diagnostic criteria for PCOS are now internationally endorsed and are based on two of three features: oligo- or anovulation, hyperandrogenism (clinical or biochemical), and polycystic ovaries (3). Additionally, the evidence indicates that PCOS is associated with several endocrine and metabolic disorders, including insulin resistance, and dyslipidemia (4, 5). A recent narrative review proposed that the levels of nesfatin-1, myonectin, omentin, and neudesin were decreased in PCOS patients, while the levels of the other considered agents (e.g., preptin, gremlin-1, neuregulin-4, xenopsin-related peptide, xenin-25, and galectin-3) were increased (6).

Nesfatin-1 is widely expressed in both the central nervous system and peripheral tissue with the role of regulating metabolism, appetite, gut motility, and feeding behavior (7, 8). As a multifunctional biomolecule, nesfatin-1 plays an important role in the diagnosis and treatment of many diseases, including coronary artery disease (9), multiple sclerosis (10), type 2 diabetes mellitus (11). Studies have shown that nesfatin-1 is related to the inhibition of lipid-related diseases, because it can reduce fat accumulation and increase lipid decomposition in the lipid metabolism (12).

As a newly discovered cytokine in 2006, previous studies have investigated the relationship between nesfatin-1 level and PCOS. However, these studies have produced conflicting results. Some studies revealed higher levels of nesfatin-1 in patients with PCOS relative to healthy controls, while others reported opposite findings. Thus, in this meta-analysis, we aimed to clarify the association between blood nesfatin-1 levels and PCOS, and the ability of nesfatin-1 as biomarker in PCOS.

2 Methods

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines (13) and Meta-analyses of Observational Studies in Epidemiology (MOOSE) guidelines (14).

2.1 Literature search

Two reviewers (MW and JT) independently searched these databases (PubMed, Web of Science, EMBASE, Medline and Google Scholar) from the inception of the databases to June 30, 2023. We only included studies written in English. The search terms were ("nesfatin-1" OR "nesfatin" OR "markers" OR "biomarkers") AND ("polycystic ovary syndrome" OR "PCOS"). Articles were discussed and decided by the three authors (MW, JT and QZ) after the appearance of inconsistent selections.

2.2 Study selection

Inclusion criteria: 1) study investigated blood nesfatin-1; 2) study investigated PCOS; 3) study written in English; 4) studies used control group. The control group had no clinical or biochemical evidence of PCOS.

Exclusion criteria: 1) reviews, meta-analysis and case reports; 2) letters book chapters, animal studies and published abstracts; 3) study which did not provide sufficient information about blood nesfatin-1 level in PCOS.

2.3 Data extraction

Two reviewers screened titles and abstracts of all articles. We extracted these data from included articles: first author, publication year, country, sample size, mean age, body mass index (BMI), blood nesfatin-1 concentrations, sample type and detection method.

2.4 Statistical analysis

Meta-analysis was performed using STATA 12.0 software. We computed standard mean difference (SMD) and 95% confidence interval (CI) regarding the comparison of blood nesfatin-1 in patients with PCOS and controls. Heterogeneity across studies was explored with I^2 and Q test. A random effects model was used for $I^2 \geq 50\%$ and p value for Q test ≤ 0.05. A fixed-effects model was used for $I^2 < 50\%$ and p value for Q test > 0.05. Meta-regression analysis was adopted to investigate the source of heterogeneity. Subgroup studies for different ethnicities and different sample types were conducted to investigate the source of the heterogeneity. Obesity in adults was defined by the World Health Organization (WHO) (15) as BMI > 30kg/m^2 for obese. Subgroup studies depending on the presence and absence of obesity was conducted to investigate the source of the heterogeneity. Sensitivity analysis was adopted to evaluate the stabilization of meta-analysis. Begg's test was adopted to evaluate publication bias.

3 Results

3.1 Characteristics of included studies

Figure 1 showed the flow chart of the literature search and selection process. Table 1 showed characteristics of included studies. Mean value and standard deviation (SD) of blood nesfatin-1 in patients with PCOS and controls were collected from 13 studies (16–28) (patients with PCOS: n = 757, controls: n = 569).

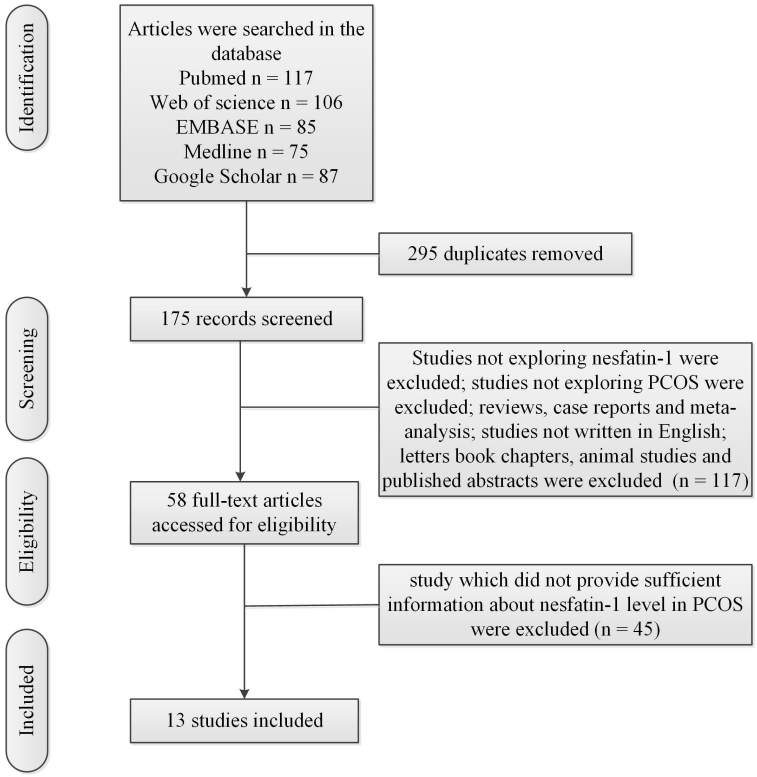

FIGURE 1
Flow chart of the literature search and selection process.

3.2 Meta-analysis results

The present meta-analysis showed no significant difference in blood nesfatin-1 level between patients with PCOS and controls with a random effects model (SMD = 0.03; 95%CI: -0.71, 0.77; I^2 = 97.1%, *p* value for Q test < 0.001; Figure 2). Meta-regression analysis showed that publication year and age were not responsible for heterogeneity across studies (publication year: *p* value = 0.369; age: *p* value = 0.632). Subgroup analysis for different ethnicities reported no significant difference in blood nesfatin-1 level between patients with PCOS and controls in both Caucasian and Asian populations (Caucasian: SMD = 0.30; 95%CI: -0.68, 1.28; Asian: SMD = -0.41; 95%CI: -2.03, 1.21; Figure 3). Subgroup analysis for different sample types reported no significant difference in serum nesfatin-1 level between patients with PCOS and controls (SMD = 0.20; 95%CI: -0.63, 1.03; Figure 4). Subgroup studies depending on presence and absence of obesity reported no significant difference in blood nesfatin-1 level between PCOS and controls in both obese and non-obese populations (Figure 5). Sensitivity analysis reported no changes in the direction of effect when any one study was excluded (Figure 6). Begg's test and funnel plots showed no significant risk of publication bias (Begg's test: *p* = 0.125; Figure 7).

4 Discussion

Our literature search yielded a comprehensive selection of 13 studies involving a substantial cohort of approximately 1300 participants, which enabled us to obtain more precise and potentially more accurate estimates of standardized mean differences (SMD) compared to the individual primary studies. Additionally, this extensive pool of data provided us with the opportunity to explore the potential factors contributing to any observed heterogeneity among these studies. Our literature search yielded a comprehensive selection of 13 studies involving a substantial cohort of approximately 1300 participants, which enabled us to obtain more precise and potentially more accurate estimates of SMD compared to the individual primary studies. Additionally, this extensive pool of data provided us with the opportunity to explore the potential factors contributing to any observed heterogeneity among these studies (SMD = 0.03; 95%CI: -0.71, 0.77). Furthermore, subgroup analysis revealed no differences in nesfatin-1 levels between Caucasian and Asian populations suffering from PCOS (Caucasian: SMD = 0.30; 95%CI: -0.68, 1.28; Asian: SMD = -0.41; 95%CI: -2.03, 1.21). PCOS cases exhibit a variable phenotypic spectrum, and previous studies have suggested that nesfatin-1 has effects on obesity (29, 30). Therefore, nesfatin-1 levels in PCOS may vary depending on the presence or absence of obesity. However, our present study reported no significant difference in blood nesfatin-1 levels between PCOS and controls in both obese and non-obese populations. Salman et al. (27) reported a sensitivity 93.5%, specificity of 79% and accuracy of 86.2% for serum nesfatin -1 level as predictor of PCOS using receiver operating characteristic (ROC) curve analysis. More studies were essential to explore the change of blood nesfatin-1 levels in PCOS.

TABLE 1 Study characteristics of included studies.

Reference	Country	Subjects	Age (years)	BMI (kg/m^2)	Results (mean ± SD)	Samples	Methods
Deniz et al. 2012 (16)	Turkey	Con: 30	23.16 ± 3.66	24.43 ± 0.50	2.22 ± 1.14 ng/mL	plasma	ELISA (Phoenix Pharmaceuticals)
		PCOS:30	23.56 ± 4.80	25.03 ± 0.86	0.88 ± 0.36 ng/mL		
Ademoglu et al. 2014 (17)	Turkey	Con: 28	26.2 ± 4.9	21.0 ± 2.8	275.55 ± 1.74 pg/mL	serum	ELISA (USCN Life Science)
		PCOS: 55	25.1 ± 5.6	27.4 ± 6.8	371.43 ± 2.50 pg/mL		
Binnetoğlu et al. 2014 (18)	Turkey	Con: 28	28 ± 6.17	22.81 ± 3.6	6.24 ± 3.69	plasma	ELISA (EIAab Science)
		PCOS: 37	25 ± 78	25.17 ± 4.9	6.56 ± 3.78		
Alp et al. 2015 (19)	Turkey	Con: 35	28.14 ± 6.766	22.34 ± 3.222	2.43 ± 0.846 ng/mL	serum	ELISA (Cloud-Clone)
		PCOS: 55	25.95 ± 5.612	24.03 ± 5.067	2.29 ± 0.558 ng/mL		
Sahin et al. 2015 (20)	Turkey	Con: 48	21.5 ± 4.5	29.7 ± 5.6	6.5 ± 2.9 ng/mL	serum	ELISA (USCN Life Science)
		PCOS: 54	22.2 ± 4.2	30.0 ± 7.5	10.2 ± 5.0 ng/mL		
Taskin et al. 2015 (21)	Turkey	Con: 26	26.85 ± 5.06	22.16 ± 2.47	154262700.5 ± 100199116.3 ng/mL	serum	ELISA (SunRed Biotechnology)
		Obese PCOS: 28	25.61 ± 4.58	35.81 ± 4.60	70015207.1 ± 46135532.1 ng/mL		
		Non-obese PCOS: 32	24.72 ± 4.30	23.83 ± 3.55	89883096.7 ± 49192130.5 ng/mL		
Ali et al. 2021 (22)	Iraq	Con: 40	29.5 ± 5.2	29.3 ± 5.1	6.3 ± 3.0 ng/mL	serum	ELISA
		PCOS: 45	29.3 ± 5.7	30.1± 4.2	11.1± 3.5 ng/mL		
Demir Caltekin et al. 2021 (23)	Turkey	Con: 40	28.23 ± 5.09	24.7 ± 3.7	36.8 ± 20.7 ng/mL	serum	ELISA ((Bioassay Technology Laboratory)
		PCOS: 44	26.41 ± 5.036	24.07 ± 2.97	17.08 ± 13.8 ng/mL		
Varli et al. 2021 (24)	Turkey	Con: 42	29.0 ± 3.7	23.7 ± 5.0	1830424848 ± 930447656.7 ng/mL	serum	ELISA (SunRed Biotechnology)
		PCOS: 41	27.7 ± 3.6	24.8 ± 4.2	1495249730 ± 877148222.1 ng/mL		
Wang et al. 2022 (25)	China	Con: 150	29 ± 12.1	NA	1.10 ± 0.97 mg/mL	serum	ELISA (NA)
		PCOS: 200	28.5 ± 10.1	NA	1.89 ± 0.99 mg/mL		
Hamed et al. 2022 (26)	Egypt	Con: 24	30.13 ± 3.26	25.43 ± 1.44	316.10 ± 59.87 pg/mL	serum	ELISA (Sinogeneclon Biotech)
		PCOS: 60	28.42 ± 4.34	31.32 ± 4.80	362.37 ± 85.06 pg/mL		
Salman et al. 2022 (27)	Iraq	Con: 48	28.12 ± 6.0	29.68 ± 4.7	0.858 ± 0.271 ng/ml	serum	NR
		PCOS: 46	27.23 ± 5.4	30.69 ± 3.1	0.439 ± 0.127 ng/ml		
Mahmood et al. 2023 (28)	Iraq	Con: 30	NR	NR	736.405 ± 259.222 pg/mL	serum	ELISA

BMI, body mass index; Con, control; ELISA, enzyme-linked immunosorbent assay; NA, not available; PCOS, polycystic ovary syndrome.

Nesfatin-1, a peptide derived from the precursor nucleobindin2 (NUCB2), plays a significant role in regulating feeding behavior and energy metabolism (31). The etiology of PCOS involves multiple aspects, including ovarian and adrenal hyperandrogenism, neuro-endocrine and hypothalamic-pituitary dysfunction, disorders of peripheral insulin resistance, and overweight or obesity (32, 33). Many studies have suggested that nesfatin-1 has a direct influence on obesity, including food intake, glucose metabolism, weight loss, and cardiac functions (22, 34). A study by Algual et al. reported lower serum nesfatin-1 levels in individuals with metabolic

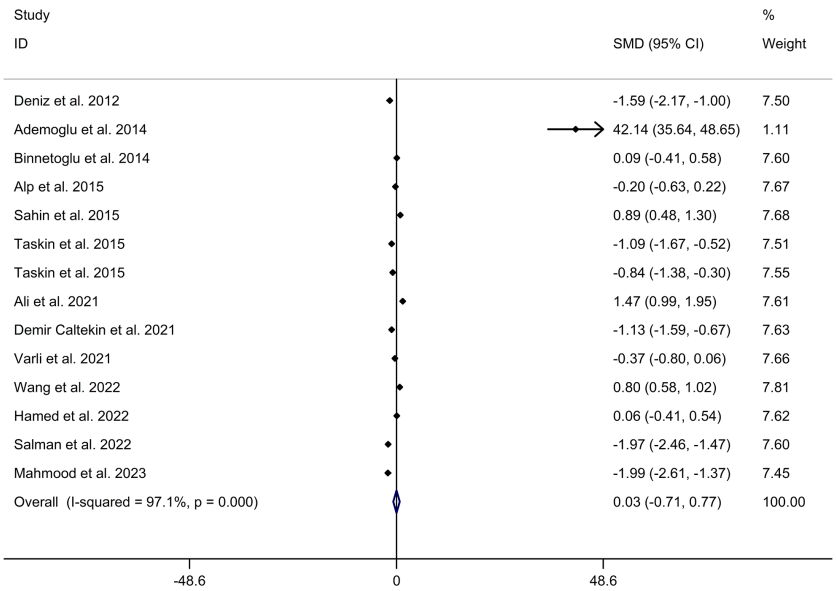

FIGURE 2
Forest plot for comparison in blood nesfatin-1 level between patients with PCOS and controls. CI, confidence interval; PCOS, polycystic ovary syndrome; SMD, standard mean difference.

syndrome compared to the control group (35). However, other studies have shown that serum nesfatin-1 concentrations were significantly lower in obese subjects compared to non-obese subjects (36, 37). These inconsistent results may contribute to the lack of significant difference in blood nesfatin-1 levels between PCOS subjects and controls. It has been observed to have an anorexic effect by reducing meal frequency and increasing the time interval between meals (19). In a study involving PCOS model rats, it was found that nesfatin-1 serum levels decreased significantly compared to the normal control group (38). These results were consistent with the analysis of ovarian nesfatin-1 mRNA and protein levels using RT-PCR and western blot

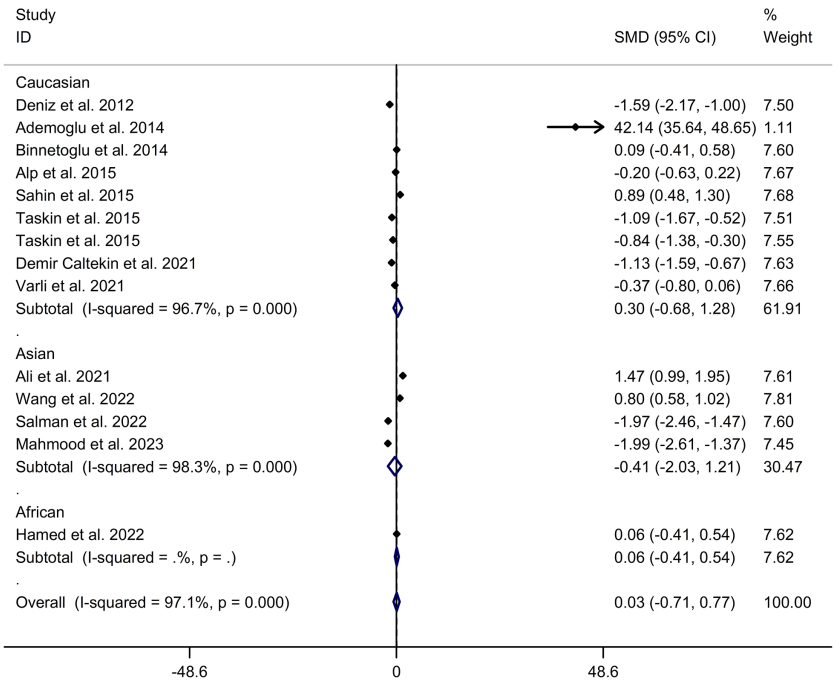

FIGURE 3
Subgroup analysis for comparison in blood nesfatin-1 level between patients with PCOS and controls with different ethnicities. CI, confidence interval; PCOS, polycystic ovary syndrome; SMD, standard mean difference.

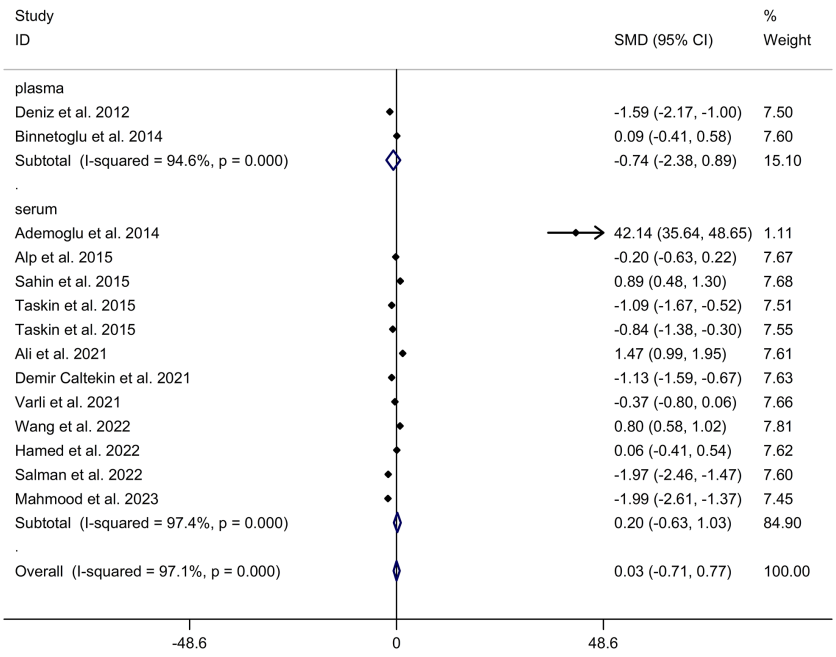

FIGURE 4
Subgroup analysis for comparison in nesfatin-1 level detected by different sample types between patients with PCOS and controls. CI, confidence interval; PCOS, polycystic ovary syndrome; SMD, standard mean difference.

techniques (38). Additionally, the study revealed a positive correlation between nesfatin-1 and follicle-stimulating hormone (FSH), estradiol (E_2), and progesterone (P) (38). This suggests that the decrease in nesfatin-1 levels in PCOS may disrupt follicular cell development through the inhibition of FSH in folliculogenesis (38). For PCOS patients, elevated serum nesfatin-1 concentrations were directly associated with serum levels of prolactin (26). This may be attributed to the co-localization of nesfatin-1 and prolactin-releasing peptide producing neurons in adrenal medullary A1 and A2 catecholamine cell groups, as well as the co-expression of nesfatin-1 and prolactin-releasing peptide (39, 40). However, it is worth noting that some studies have reported the opposite findings. A previous study demonstrated that intravenous injection of nesfatin-1 significantly decreased blood sugar in

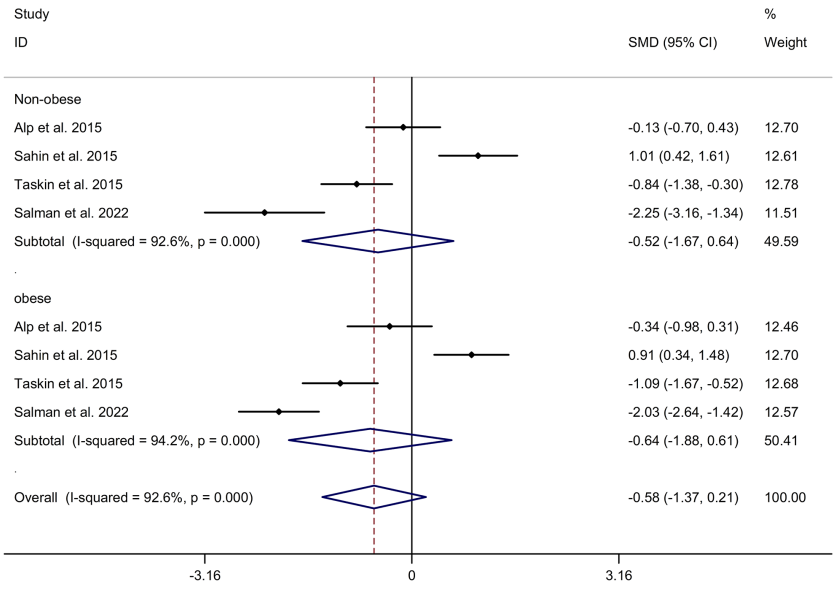

FIGURE 5
Subgroup analysis depending on presence and absence of obesity for comparison in nesfatin-1 level between patients with PCOS and controls. CI, confidence interval; PCOS, polycystic ovary syndrome; SMD, standard mean difference.

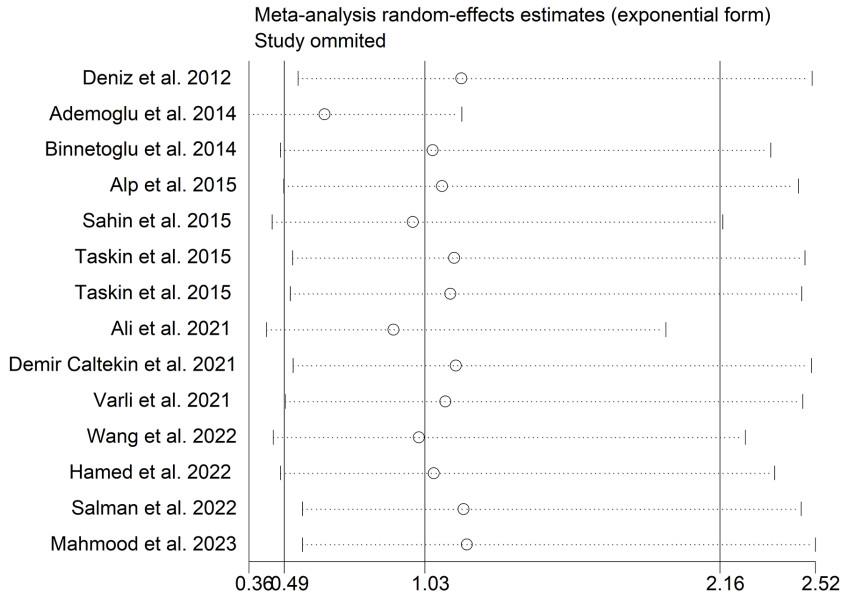

FIGURE 6
Sensitivity analysis for comparison in blood nesfatin-1 level between patients with PCOS and controls. PCOS, polycystic ovary syndrome.

hyperglycemic db/db mice, indicating that nesfatin-1 has hypoglycemic effects by accelerating insulin secretion through increased calcium ion influx via L-type channels in mouse pancreas islet beta-cells (41, 42). Caltekin et al. also revealed lower nesfatin-1 levels in PCOS patients compared to healthy individuals, suggesting that PCOS shares similarities with diabetes and gestational diabetes mellitus (GDM) due to weight and insulin resistance (23). A separate Japanese study provided evidence supporting a relationship between nesfatin-1 and the insulin receptor (43). However, further research is necessary to elucidate the precise mechanisms underlying the association between nesfatin-1 and PCOS.

In the current meta-analysis, several limitations should be acknowledged. Firstly, the number of included studies was limited, comprising only 13 studies, and most of these studies had small sample sizes. Secondly, the study solely focused on articles published in the English language, potentially introducing bias. This exclusion of non-English literature may have restricted the

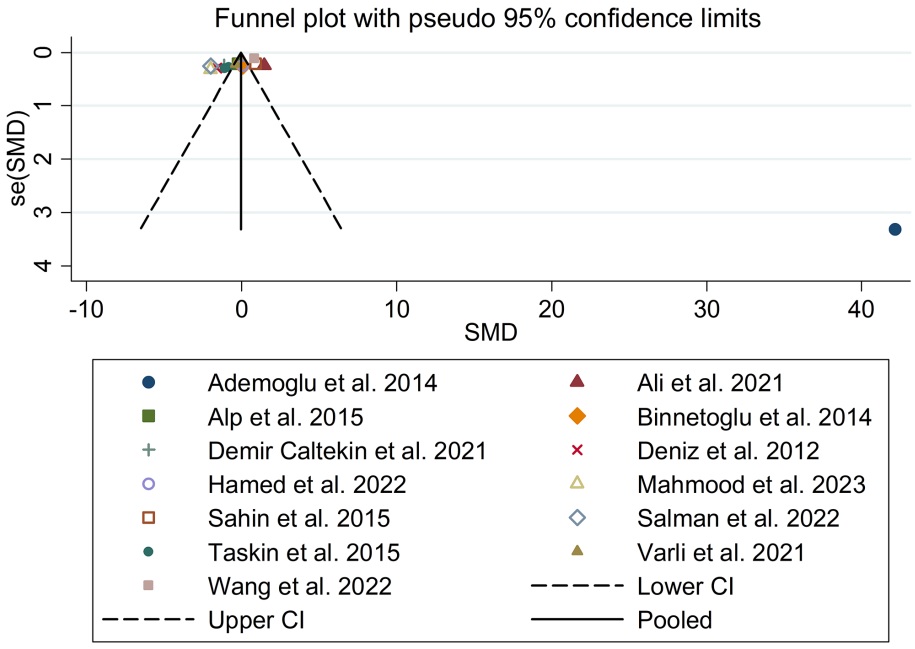

FIGURE 7
Funnel plot for comparison in blood nesfatin-1 level between patients with PCOS and controls. PCOS, polycystic ovary syndrome.

generalizability of the findings. Thirdly, complete access to detailed data sets, including potential confounders such as BMI, fasting blood glucose, insulin levels, homeostasis model assessment-insulin resistance (HOMA-IR) index, luteinizing hormone (LH), follicle stimulating hormone (FSH), smoking, and physical activity, was not available. These confounders may have influenced the results.

From this meta-analysis, it is concluded that there is no significant relationship between blood nesfatin-1 levels and PCOS. However, the precise role of nesfatin-1 in the pathogenesis of PCOS remains poorly understood. Consequently, further examination of our findings necessitates additional prospective evidence-like clinical studies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

MW: Data curation, Formal Analysis, Investigation, Writing – original draft. JT: Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft. QZ: Investigation, Methodology, Writing – review & editing. HT: Formal Analysis, Investigation, Methodology, Writing – review & editing. LT: Investigation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1275753/full#supplementary-material

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Effects of different gonadotropin preparations in GnRH antagonist protocol for patients with polycystic ovary syndrome during IVF/ICSI: a retrospective cohort study

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Purpose: To compare the effects of recombinant FSH alfa (rFSH-alfa), rFSH-beta, highly purified human menopausal gonadotropin (HP-hMG) and urinary FSH (uFSH) in women with polycystic ovarian syndrome who have undertaken the GnRH antagonist protocol during IVF/ICSI treatment.

Method: A single-center retrospective cohort study including women with PCOS who received the GnRH antagonist protocol from January 2019 to July 2022 was conducted. Patients were divided into rFSH-alfa group, HP-hMG group, uFSH group, and rFSH-beta group, and the number of oocytes retrieved, clinical pregnancy rate of the fresh cycle (primary outcomes), embryo quality, and severe OHSS rate (secondary outcomes) were compared.

Results: No statistical differences were found among the four groups in fresh cycle clinical pregnancy rate (p=0.426), nor in the subgroup analyses. The HP-hMG group had a smaller number of oocytes retrieved and a higher high-quality D3 embryo rate than the three FSH groups (p<0.05). No statistical differences were found among the four groups in the severe OHSS rate (p=0.083).

Conclusion: For women with PCOS undergoing the GnRH antagonist protocol, the clinical pregnancy rates of fresh IVF/ICSI-ET cycle are similar for all four types of Gn. With a lower risk of OHSS and a similar number of high-quality and available embryos, HP-hMG may have an advantage in the PCOS population.

KEYWORDS

polycystic ovary syndrome, GnRH antagonist protocol, IVF, ICSI, gonadotropin

1 Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder that affects a great number of females of reproductive age and is the most common cause of anovulatory infertility (1). For treatment, *in-vitro* fertilization/intra-cytoplasmic sperm injection (IVF/ICSI) is usually considered a third-line medical therapy when other ovulation induction therapies have failed (2). However, due to the increased antral follicular count (AFC) and anti-Müllerian hormone (AMH) in PCOS patients, there is an increased sensitivity and response to controlled ovarian stimulation (COS) and therefore a higher risk of ovarian hyperstimulation syndrome (OHSS) (3, 4).

One of the important ways to improve these problems is to explore the best protocol for COS. Previous studies (5, 6) have shown that the gonadotrophin-releasing hormone (GnRH) antagonist protocol is beneficial to patients with PCOS due to its lower risk of OHSS and is recommended for this population according to the ESHRE guideline (7). As a common type of ovulation stimulant, gonadotropins (Gn) play an important role in COS. Recombinant follicle-stimulating hormone (FSH) alfa (rFSH-alfa), recombinant FSH beta (rFSH-beta), highly purified urinary FSH (uFSH), and highly purified human menopausal gonadotropin (HP-hMG) are four common types of Gn preparation that have been used in COS. Previous studies (8–13) have compared these preparations among the general population but haven't reached an agreement. Some studies (9–11) suggest that the efficacy is comparable or without clinical significance in live birth rate, clinical pregnancy rate, and number of retrieved oocytes, while others find out that the rFSH-alfa (12, 13) or the HP-hMG (8) may have a better efficacy than the others. However, few articles have focused on the specific population that suffered from PCOS and undertook the GnRH antagonist protocol for COS.

This study was designed to compare the effects of the four Gn preparations, including rFSH-alfa, rFSH-beta, uFSH, and HP-hMG, in women with PCOS who have undertaken the GnRH antagonist protocol. It aimed to provide evidence for the selection and optimization of COS protocols for patients with PCOS.

2 Materials and methods

2.1 Study design

A single-center retrospective cohort study was conducted at West China Second University Hospital, Sichuan University. Women with PCOS who received the GnRH-antagonist protocol for their first IVF/ICSI cycle from January 2019 to July 2022 were included. The study was approved by the Ethics Committee of West China Second University Hospital.

PCOS was diagnosed according to the Rotterdam criteria (14). Infertility was defined as the failure to be pregnant after at least 12 months of regular unprotected sexual intercourse (15). Demographic characteristics (including age, weight, and body mass index (BMI)), clinical characteristics (including type of infertility, duration of infertility, baseline sex hormones, AMH, and AFC), and treatment information (including ovarian stimulation information and IVF/ICSI cycle information) were collected from the electronic medical record management system.

Exclusion criteria included: 1) with infertility factors other than anovulation; 2) with other endocrine diseases (such as thyroid diseases and diabetes mellitus) or immune diseases (such as systemic lupus erythematosus and antiphospholipid syndrome); 3) with a history of recurrent pregnancy loss; 4) with chromosomal abnormalities; 5) cycles with preimplantation genetic testing; 6) without complete clinical information.

2.2 Treatment protocol

All the patients received the GnRH antagonist protocol. COS was started on day 2 of the menstrual cycle with rFSH-alfa (GONAL-F; Merck Serono, Italy), HP-hMG (Menopur; Ferring, Germany), uFSH (Lizhu Pharmaceutical Trading Co., China), or rFSH-beta (Puregon; Organon, The Netherlands). The starting dose was 75-300 IU according to the type of Gn, age, BMI, and AMH, and the daily dose during COS remained unchanged unless the serum estradiol (E2) did not increase after 7 days of COS. The pituitary gonadotrophin suppression was started with a GnRH antagonist (Injection Cetrotide acetate, Aeterna Zentaris, Canada) at a dose of 0.25mg/d on the day 6 of COS (fixed protocol), or the day the dominant follicle reached 14mm diameter or serum E2 reached 300pg/ml (flexible protocol). As soon as two follicles ≥ 18mm or three follicles ≥ 17mm diameter were detected, patients received their last GnRH antagonist injection, and final follicular maturation (ovulation trigger) was performed by human chorionic gonadotropin (hCG; Lizhu Pharmaceutical Trading Co., China) at a dose of 8000-10000 IU according to the patient's weight. For patients with a high risk of OHSS, 4000-5000 IU of hCG was used. For patients with more than 19 follicles ≥11mm diameter detected on the trigger day, 0.2 mg GnRH agonist (Decapeptyl, Ferring, Germany) was used. Endometrium thickness was measured by transvaginal ultrasound on the trigger day before the injection of hCG for a rough assessment of endometrial receptivity.

Oocytes were retrieved by transvaginal ultrasound-guided aspiration 36-38h after the trigger, and oocyte maturity was assessed. IVF and/or ICSI were performed depending on the medical history and male factors. Fertilization was assessed on day 1 after the oocyte retrieval. Embryo quality was evaluated daily after the fertilization assessment, and high-quality embryos and available embryos were identified on day 3 and day 5 after oocyte retrieval. The assessment of embryo quality was done independently by at least two embryologists and was summarized and negotiated until a consensus was reached. Ultrasound-guided fresh embryo transfer (ET) was conducted on day 3 or day 5 following the embryo quality assessment, and the remaining available embryos were frozen. For patients with at least one high-quality embryo, single embryo transfer was performed on the best embryo. For patients without high-quality embryos, single embryo transfer or double embryo transfer was performed on the best one or two embryos. All operations were performed in accordance with the standard guidelines of the Chinese Medical

Association by experienced embryologists. All patients received luteal phase support with intramuscular progesterone (60 mg per day) or vaginal progesterone gel (90 mg per day) combined with oral dydrogesterone (20 mg per day).

Pregnancy was assessed by measurement of serum β-hCG concentrations 2 weeks after ET and confirmed by transvaginal ultrasound 4 weeks after ET.

2.3 Data collection and outcomes

Baseline information included age, BMI, infertility duration, infertility type, AMH, baseline serum E2, progesterone (P), luteinizing hormone (LH), FSH, testosterone (T), and AFC. Baseline sex hormones, AMH, and transvaginal ultrasound for AFC were examined on day 2 of the menstrual cycle before the start of COS. All measurements of hormones were performed in the same laboratory using competitive chemiluminescent immunoassay (CLIA, Siemens ADVIA CENTAUR). The normal ranges of sex hormones in the follicle phase are shown in Supplementary Table S1.

The primary outcomes were the number of oocytes retrieved and the clinical pregnancy rate (per fresh ET cycle). The secondary outcomes included high-quality and available D3 embryo rate (per normal fertilized oocyte) and count, high-quality and available blastocyst rate (per formed blastocyst), and severe OHSS rate (per ovulation induction cycle). Besides, total Gn dose, metaphase II oocytes (MII) count and rate, normal fertilized rate, fresh ET cancellation rate, duration of Gn use, and trigger day information including sex hormones, number of follicles ≥14mm, and single endometrium thickness (half of endometrium thickness) were also collected.

Clinical pregnancy was defined as the presence of a gestational sac under ultrasound 4 weeks after ET. D3 embryos with ≥6 cells and ≤20% fragmentation were regarded as high-quality embryos, and those with ≥4 cells and ≤35% fragmentation were regarded as available. The quality of blastocysts (day 5) was assessed based on the Gardner and Schoolcraft scoring system. High-quality blastocysts included grades AA, AB, BA, and BB blastocysts, while available blastocysts included grades BC and CB and high-quality blastocysts.

2.4 Statistical analysis

Patients were divided into 4 groups: rFSH-alfa group, HP-hMG group, uFSH group, and rFSH-beta group. A Kolmogorov-Smirnov test was used to estimate the normality of distribution for continuous variables. Normally distributed variables were presented as mean ± standard deviation (SD) and analyzed by one-way ANOVA, using Dunnett t-test as appropriate. Non-normally distributed variables were presented as median (25^{th}-75^{th} percentiles) and analyzed by Kruskal-Wallis one-way ANOVA, using Bonferroni method as appropriate. Categorical variables were presented as number of cases (percentage) and analyzed by chi-square or Fisher's exact test as appropriate. P-value of less than 0.05 was regarded as statistically significant. Subgroups were divided based on the median of the interested parameters. All analyses were performed using the SPSS version 26.0 (SPSS Inc., Chicago, IL, UPL).

3 Results

3.1 Baseline characteristics

A total of 771 patients were included in this retrospective study and were divided into rFSH-alfa group (n=375), HP-hMG group (n=105), uFSH group (n=173), and rFSH-beta group (n=118) according to the type of Gn they used.

Baseline characteristics are shown in Table 1. There were no statistical differences among the four groups in age (p=0.301), duration of infertility (p=0.574), type of infertility (p=0.397), baseline FSH (p=0.085) and baseline E2 (p=0.524). The BMI of uFSH group was higher than others (p<0.05) and HP-hMG group was higher than rFSH-alfa group (p<0.05). The AMH and baseline LH of the uFSH group were lower than the rFSH-alfa group (p<0.05). The baseline P of the rFSH-beta group was lower than the rFSH-alfa group (p<0.05). The AFC of the HP-hMG and uFSH groups was lower than the rFSH-alfa group (p<0.05), and the uFSH group was lower than the rFSH-beta group (p<0.05).

3.2 Outcomes of ovarian stimulation

The ovarian stimulation characteristics are shown in Table 2. There were no statistical differences in the type of GnRH antagonist protocol (p=0.379). The starting Gn dose of the uFSH group was the highest, while the two rFSH groups were the lowest (p<0.001). The total Gn doses of the HP-hMG and uFSH groups were higher than the two rFSH groups (p<0.05). Statistically, the duration of Gn use in the HP-hMG and uFSH groups was different from the two rFSH groups (p<0.05). As for the indicators on the trigger day, there were no statistical differences in endometrium thickness (p=0.501). The uFSH group had the smallest number of follicles≥14mm (p<0.05). The rFSH groups had the highest trigger day E2, while the HP-hMG group had the lowest (p<0.05). The HP-hMG group had lower trigger day LH than the rFSH-alfa group and uFSH group (p<0.05). The uFSH group had a lower trigger day P than the two rFSH groups (p<0.05). The HP-hMG group had smaller numbers of oocytes retrieved and MII oocytes than the three FSH groups (p<0.05). The rFSH-alfa group had a higher MII oocyte rate than the rFSH-beta group (p<0.05). There were no statistical differences among the four groups in the severe OHSS rate (p=0.083).

3.3 Outcomes of IVF/ICSI treatment

The IVF/ICSI treatment outcomes were shown in Table 3. The normal fertilized rate of the uFSH group was lower than that of the rFSH-alfa group and the HP-hMG group (p<0.05). The uFSH group had a smaller number of high-quality D3 embryos than the

TABLE 1 Baseline characteristics.

	rFSH-alfa (n=375)	HP-hMG (n=105)	uFSH (n=173)	rFSH-beta (n=118)	P-value
Age (y)	29.0 (27.0-32.0)	29.0 (26.0-32.0)	29.0 (26.0-32.0)	30.0 (27.0-31.0)	0.301
BMI (kg/m^2)	22.26 ± 2.92	23.35 ± 3.22*	24.44 ± 3.21*†	22.41 ± 3.22‡	<0.001
Duration of infertility (y)	3.0 (2.0-5.0)	3.0 (2.0-5.0)	3.0 (2.0-5.0)	2.0 (2.0-4.0)	0.574
Type of infertility [n (%)]					0.397
Primary infertility	258 (68.8)	80 (76.2)	121 (69.9)	78 (66.1)	
Secondary infertility	117 (31.2)	25 (23.8)	52 (30.1)	40 (33.9)	
AMH (ng/mL)	10.12 (6.91-14.54)	8.44 (6.12-12.18)	6.83 (4.38-12.43) *	10.74 (6.54-16.22) ‡	<0.001
Baseline sex hormone					
FSH (IU/L)	6.30 (5.30-7.70)	6.60 (5.70-7.70)	6.30 (5.40-7.50)	6.30 (5.60-7.98)	0.085
LH (IU/L)	8.60 (5.60-13.55)	7.50 (5.80-9.95)	6.70 (4.30-10.10) *	8.15 (5.42-11.22)	0.020
E2 (pg/mL)	40.66 (31.48-53.75)	45.55 (34.38-55.80)	39.20 (28.90-52.30)	43.55 (34.25-52.15)	0.524
P (ng/mL)	0.52 (0.38-0.69)	0.42 (0.33-0.61)	0.44 (0.32-0.60)	0.44 (0.29-0.59) *	0.004
T (ng/ml)	0.39 (0.30-0.49)	0.47 (0.37-0.57) *	0.41 (0.30-0.56)	0.37 (0.26-0.51) †	<0.001
LH/FSH	1.42 (0.93-2.13)	1.15 (0.86-1.60)	1.17 (0.66-1.78) *	1.26 (0.81-1.94)	0.010
AFC	21.0 (18.0-25.0)	20.0 (17.0-23.0) *	20.0 (15.0-23.0) *	21.0 (17.2-25.0) ‡	<0.001

Data are presented as mean ± SD, median (25th-75th percentiles) or number (percentage).
*p<0.05 compared to rFSHα group; †p<0.05 compared to HP-hMG group; ‡p<0.05 compared to uFSH group.
BMI, Body Mass Index; AMH, anti-mullerian hormone; LH, luteinizing hormone; E2, estradiol; P, progesterone; T, testosterone; AFC, antral follicle count.

two rFSH groups (p<0.05), and the rFSH-alfa group had a larger number of available D3 embryos than the HP-hMG group and uFSH group (p<0.05). The HP-hMG group had a higher high-quality D3 embryo rate than the rFSH-alfa group and the uFSH group (p<0.05), and the highest available D3 embryo rate, high-quality blastocysts rate, and available blastocyst rate (p<0.05). The rFSH-beta group had a lower high-quality blastocyst rate than the rFSH-alfa group (p<0.05), but a higher available blastocyst rate than the rFSH-alfa group and uFSH group (p<0.05). The two rFSH groups had a higher ET cancellation rate than the HP-hMG group (p<0.05), and the rFSH-alfa group had a higher ET cancellation rate than the uFSH group (p<0.05). There were no statistical differences among the four groups in clinical pregnancy rate (p=0.426).

3.4 Outcomes of subgroup analyses

As was shown in Table 4, when dividing the subgroups by age, BMI, weight, LH/FSH, AMH, and AFC, there was no significant difference among the four groups in clinical pregnancy rate in each subgroup. As was shown in Table 5, when dividing the subgroups by age, AMH, and AFC, the number of retrieved oocytes was always lower in the HP-hMG group than in the rFSH groups (p<0.05).

4 Discussion

This is a single-center retrospective cohort study concerning the effects of four different types of Gn on women with PCOS undergoing the GnRH antagonist protocol. In this study, we mainly used the number of retrieved oocytes and the clinical pregnancy rate to assess the efficacy of COS and the fresh IVF/ICSI-ET cycle. We found that the lowest number of oocytes retrieved was observed in the HP-hMG group and the highest number in the two rFSH groups. The HP-hMG group had the highest high-quality embryo rate, while the rFSH-alfa group had the highest high-quality embryo number. Though there was no significant difference among the four groups in clinical pregnancy rate, it seemed that the HP-hMG group had the highest clinical pregnancy rate numerically.

The four Gn preparations are different in production and composition. The two rFSH preparations are synthesized by the same recombinant DNA technology but differ in the glycosylation and purification procedures. They are considered to be 99% pure FSH, without LH activity (10, 16). The hMG and uFSH are human-derived preparations from the urine of postmenopausal women and contain <5% copurified proteins. The hMG contains FSH and LH activity in a 1:1 ratio, and the uFSH mainly contains FSH, with a little LH activity due to the purification process (10). The LH activity of hMG derives from LH itself and/or hCG, and in this study, Menopur is an HP-hMG preparation whose LH activity mainly derives from hCG content (17). The differences among the four Gn preparations in composition and production lead to differences in biological activity and effect, which may influence the efficacy of COS and IVF/ICSI-ET.

In the PCOS population, the number of retrieved oocytes during COS tends to be excessive (18). In this study, we found that the use of HP-hMG led to a significantly lower number of

TABLE 2 Outcomes of ovarian stimulation.

	rFSH-alfa (n=375)	HP-hMG (n=105)	uFSH (n=173)	rFSH-beta (n=118)	P-value
GnRH antagonist protocol					0.379
Fixed	278 (74.1)	85 (81.0)	124 (71.7)	87 (73.7)	
Flexible	97 (25.9)	20 (19.0)	49 (28.3)	31 (26.3)	
Starting dose of Gn (IU)	150.00 (125.00-200.00)	200.00 (156.25-225.00) *	225.00 (225.00-300.00) *†	175.00 (150.00-200.00) †‡	<0.001
Duration of Gn use (d)	10.0 (9.0-11.0)	10.0 (9.0-11.0) *	10.0 (9.0-11.0) *	9.0 (9.0-10.0) †‡	<0.001
Total Gn dose (IU)	1500.00 (1275.00-1856.25)	2137.50 (1750.00-2550.00) *	2250.00 (1875.00-2775.00) *	1500.00 (1275.00-1818.75) †‡	<0.001
Trigger day					
No. of follicles ≥14mm	11.5 (9.0-14.0)	10.0 (8.0-13.0)	9.0 (7.0-11.0) *†	11.0 (8.0-13.8) ‡	<0.001
E2 (pg/mL)	5175.50 (3197.30-7630.10)	3176.30 (2396.35-3988.10) *	3438.00 (2277.60-5200.30) *†	4775.05 (3052.38-6981.08) †‡	<0.001
P (ng/mL)	1.06 (0.77-1.39)	1.04 (0.79-1.31)	0.86 (0.66-1.08) *	1.03 (0.68-1.41) ‡	<0.001
LH (IU/L)	2.20 (1.20-4.10)	1.65 (1.00-2.75) *	2.40 (1.60-3.80) †	2.05 (1.10-3.58)	0.004
Single Em thickness (mm)	5.20 (4.50-6.00)	5.20 (4.50-5.88)	5.10 (4.50-6.00)	5.00 (4.50-5.88)	0.523
No. of oocytes retrieved	17.0 (12.0-21.2)	11.5 (9.0-15.0) *	12.0 (9.0-18.0) *†	16.0 (10.0-21.8) †	<0.001
No. of MII oocytes	14.0 (10.0-18.0)	10.0 (7.2-12.0) *	12.0 (7.0-16.0) *†	12.5 (8.0-18.0) †	<0.001
MII oocyte rate [n(%)]	5665/6652 (85.2)	1044/1243 (84.0)	2144/2582 (83.0)	1611/1948 (82.7) *	0.014
Severe OHSS rate [n(%)]	12 (3.2)	0 (0.0)	1 (0.6)	3 (2.5)	0.083

Data are presented as median (25^{th}-75^{th} percentiles) or number (percentage).
*p<0.05 compared to rFSHα group; †p<0.05 compared to HP-hMG group; ‡p<0.05 compared to uFSH group.
GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; E2, estradiol; P, progesterone; Em, endometrium; MII, metaphase II oocytes; OHSS, ovarian hyperstimulation syndrome.

TABLE 3 Outcomes of ART treatment.

	rFSH-alfa (n=375)	HP-hMG (n=105)	uFSH (n=173)	rFSH-beta (n=118)	P-value
ART method [n (%)]					0.012
IVF	310 (82.7)	92 (87.6)	148 (85.5)	102 (86.4)	
ICSI	17 (4.5)	11 (10.5)	10 (5.8)	5 (4.2)	
IVF+ICSI	48 (12.8)	2 (1.9) *	15 (8.7)	11 (9.3)	
Normal fertilized rate [n(%)]	4120/6652 (61.9)	787/1243 (63.3)	1464/2582 (56.7) *†	1153/1948 (59.1)	<0.001
No. of high-quality D3 embryos	4.0 (2.0-7.2)	4.0 (2.0-6.0)	3.0 (1.0-5.0) *	4.5 (2.0-7.0) ‡	<0.001
High-quality D3 embryos rate [n(%)]	1971/4120 (47.8)	426/787 (54.1) *	669/1464 (45.7) †	582/1153 (50.5)	0.001
No. of available D3 embryos	9.0 (6.0-13.0)	7.0 (6.0-9.0) *	6.0 (4.0-10.0) *	8.0 (5.0-12.0)	<0.001
Available D3 embryos rate [n(%)]	3820/4120 (92.7)	775/787 (98.5) *	1339/1464 (91.5) †	1079/1153 (93.6) †	<0.001
High-quality blastocysts rate [n(%)]	679/2199 (30.9)	190/447 (42.5) *	195/658 (29.6) †	151/598 (25.3) *†	<0.001
Available blastocysts rate [n(%)]	1509/2199 (68.6)	426/447 (95.3) *	480/658 (72.9) †	505/598 (84.4) *†‡	<0.001
Fresh ET cancellation rate [n(%)]	288 (76.8)	54 (51.4) *	101 (58.4) *	85 (72.0) †	<0.001
Clinical pregnancy rate [n(%)]	50/87 (57.5)	32/51 (62.7)	38/72 (52.8)	15/33 (45.5)	0.426

Data are presented as median (25^{th}-75^{th} percentiles) or number (percentage).
*p<0.05 compared to rFSHα group; †p<0.05 compared to HP-hMG group; ‡p<0.05 compared to uFSH group.
ART, assisted reproductive technology; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; D3, day 3.

TABLE 4 Subgroup analyses for clinical pregnancy rate.

Subgroups based on median		P value
Age	≤ 29	0.320
	> 29	0.580
BMI	≤ 22.6	0.275
	> 22.6	0.082
LH/FSH	≤ 1.26	0.865
	> 1.26	0.360
AMH	≤ 9.5	0.870
	> 9.5	0.146
AFC	≤ 20	0.626
	> 20	0.649

retrieved oocytes compared to other Gn preparations. The uFSH group also retrieved fewer oocytes than the rFSH groups. The result for HP-hMG is in agreement with previous studies (19) but the result for uFSH is not (9, 10). It may be because previous studies didn't focus on the PCOS population or the GnRH antagonist protocol (9, 10, 19). Besides, AMH and AFC have been suggested as predictors of the number of oocytes retrieved (20). In this study, the baseline AFC and AMH of the uFSH group were statistically lower than the two rFSH groups, which may affect the result. The baseline AFC of the HP-hMG group was also different from the rFSH-alfa group, but this difference seemed not to be enough to explain the difference in the oocyte retrieved number.

More oocytes retrieved may be related to a higher risk of severe OHSS, as reported in previous studies (21–23). In this study, though without statistical difference, the severe OHSS rate is numerically consistent with the number of oocytes retrieved. Therefore, the use of HP-hMG may lead to a lower risk of severe OHSS than the three FSH groups. According to our subgroup analyses divided by medians of age, AMH, and AFC, this tendency existed in all the subgroups, especially in patients with higher AMH and/or AFC. It has also been indicated that more oocytes retrieved may be correlated with a higher E2 level on the trigger day (24, 25). Our results also show this trend. E2 is mainly produced by mature follicles that are more than 8mm diameter (26), and may be able to reflect the number and size of mature follicles to some extent. The excessive E2 level during the COS is generally considered a risk for OHSS (27), and it may have a concentration-dependent effect on the pregnancy and birth outcomes (such as clinical pregnancy rate, live birth weight, and preeclampsia) in the fresh IVF-ET cycle (25, 28, 29). Therefore, clinicians tend to withhold Gn or cancel the fresh cycle if excessive E2 levels are observed (30). This was shown in our results as the consistency of the number of oocytes retrieved, trigger day E2, fresh cycle cancellation rate, and severe OHSS rate.

Apart from OHSS, the number of oocytes retrieved is also considered a positive predictor of live birth. Previous studies suggested that the fresh live birth rate seemed to be maximized when the retrieved oocytes reached a plateau whose lower limit ranged from 6 to 11 and the upper limit ranged from 15 to 20 (18, 21, 22, 31–33). The cumulative live birth rate, however, was indicated to increase continuously with the number of oocytes retrieved and stabilize after the number of 20 (22, 31). Therefore, according to our results, the HP-hMG group seems to be more beneficial for the fresh cycle, and the rFSH groups seem to be better for the cumulative live birth rate. However, the optimal range derived from these studies has a large variation due to differences in COS protocols, populations, and grouping, so this finding needs further validation. The exploration of the optimal range also needs to take the risk of OHSS into account. Besides, it is worth mentioning that, though with the lowest number of oocytes retrieved, the HP-hMG group has the highest proportion of high-quality and available embryos, and it obtains a statistically similar number of high-quality D3 embryos as the rFSH groups. It may be due to the HCG-driven LH activity of Menopur, which may produce hormone changes beneficial for embryo quality (34). Therefore, for patients with PCOS, HP-hMG might have an advantage. Previous studies concerning the effectiveness of different Gn preparations showed a better live birth rate and cumulative live birth rate in people using rFSH-alfa for COS (12, 13). However, studies focused on the PCOS population and the GnRH antagonist protocol should be conducted to explore this issue. Therefore, further follow-up and more research are needed.

In this study, we did not find a significant difference in clinical pregnancy rates in the fresh cycle between the four preparations, nor in subgroup analyses divided by medians of age, BMI, LH/FSH, AMH, or AFC. This result is consistent with a meta-analysis in 2019

TABLE 5 Subgroup analyses for No. of oocytes retrieved.

Subgroups based on median		rFSH-alfa	HP-hMG	uFSH	rFSH-beta	P value
Age	≤ 29	17.0 (12.0-23.0)	11.0 (8.0-14.0) *	14.0 (8.0-20.0) *†	16.5 (10.0-22.0) †	<0.001
	> 29	16.0 (12.0-20.0)	12.0 (9.2-16.0) *	13.0 (10.8-18.0) *	16.0 (10.0-20.0) †	<0.001
AMH	≤ 9.5	14.0 (10.0-18.0)	11.0 (8.0-16.0) *	12.0 (8.5-17.0) *	14.0 (9.0-20.0) †‡	<0.001
	> 9.5	18.0 (14.0-24.0)	12.0 (9.8-14.2) *	17.5 (12.0-24.0) †	17.0 (10.0-22.0) †	<0.001
AFC	≤ 20	15.0 (11.0-20.0)	11.0 (9.0-14.0) *	12.0 (9.0-16.5) *	15.0 (9.0-21.0) †	<0.001
	> 20	18.0 (14.0-24.0)	13.0 (10.0-16.0) *	16.0 (9.8-22.5) *†	16.5 (12.0-20.0) †	<0.001

Data are presented as median (25^{th}-75^{th} percentiles) or number (percentage).
*p<0.05 compared to rFSHα group; †p<0.05 compared to HP-hMG group; ‡p<0.05 compared to uFSH group.

that focused on the PCOS population (35). However, numerically, the clinical pregnancy rate in the HP-hMG group was the highest, while in the rFSH-beta group it was the lowest. The small sample size of the fresh cycle might limit the exploration, and a high-quality randomized clinical trial (RCT) is still needed to validate this trend.

We also found that the total dose of rFSH during the COS process was less than the urinary preparations, which may be because the urinary preparations are more acidic and therefore somewhat less potent than the recombinant preparations (36). Besides, due to the better stability and liquid formulations of the recombinant preparations, pen injection devices have been used for administration, which allows more precise dose adjustment of 25IU or 12.5IU, possibly leading to a smaller dosage (37). In addition, due to the low batch-to-batch variability, rFSH-alfa (GONAL-F) is able to be provided filled-by-mass, while other preparations, including rFSH-beta, are still filled-by-bioassay (38). Therefore, though without statistical difference, the total dose and clinical efficacy of the rFSH-alfa preparation may be more stable than the rFSH-beta preparation (39). Considering that the price per unit of the urinary preparations is usually slightly lower than that of the rFSH preparations in most countries, the economic analysis needs to take into account the specific conditions of different countries and regions.

This study was conducted at West China Second University Hospital, Chengdu, China. On the one hand, this is an authoritative hospital in China, and the embryo laboratory in our center is built in strict accordance with national standards, with regular quality control of equipment, environment, and technical operations to ensure the stability of medical quality and scientific results. On the other hand, the patients in our center come from a wide range of areas, and their baseline characteristics are representative of the Chinese and East Asian populations. It is worth mentioning that, compared to Caucasian patients, a lower BMI has been reported in East Asian patients with PCOS, which our data of 22.6 kg/m^2 in median is close to (40). Therefore, we believed that more well-designed studies in the future in different regions, taking into account differences in ethnicity, cultural environment, dietary habits, and so on, would help to provide evidence for Gn use in COS in a wider population.

The study does have some limitations. Firstly, it is a single-center retrospective cohort study and may have some bias, especially in the inclusion of patients. Secondly, the differences in sample size between groups may have affected the statistical differences in some indicators. Thirdly, some baseline characteristics were not all statistically identical, especially the difference between the uFSH group and the rFSH groups, which may be confounding factors. Fourthly, only fresh cycles were included in this study, for they are temporally close to the COS process and have a high likelihood of being affected by gonadotropins. Besides, in order to focus on fresh cycle outcomes, the small sample size for clinical pregnancy rate may have limited the results. In the future, frozen cycles may be included to explore the effect of different Gn preparations on the cumulative pregnancy rate and cumulative live birth rate. Fifthly, not all the patients received single embryo transfer. Double embryo transfer was performed for patients without high-quality embryos, which tended to lessen the impact of embryo quality on clinical pregnancy rates. Therefore, well-designed RCTs are still needed for further exploration of pregnancy outcomes in the future.

5 Conclusion

In conclusion, for women with PCOS undergoing the GnRH antagonist protocol, use of the four types of Gn leads to a similar clinical pregnancy rate in the fresh IVF/ICSI-ET cycle, but it seems that the use of HP-hMG leads to the highest clinical pregnancy rate numerically. In addition, the use of HP-hMG leads to a lower number of retrieved oocytes than others and therefore seems to have a lower risk of OHSS. Overall, HP-hMG may have an advantage in the PCOS population. The results in this study need to be proven by further follow-up and well-designed RCTs or prospective studies in the future.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study involving humans was approved by Ethics Committee of West China Second University Hospital and written informed consent was waived. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

ZH: Writing – original draft, Formal Analysis. RZ: Writing – original draft, Data curation. RG: Writing – review & editing. MC: Writing – review & editing, Data curation. XL: Writing – review & editing, Data curation. QZ: Writing – review & editing, Data curation. LQ: Writing – review & editing, Conceptualization. XZ: Writing – review & editing, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1309993/full#supplementary-material

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The effects of behavioral intervention on anthropometric, clinical, and biochemical parameters in patients with polycystic ovary syndrome: a systematic review and meta-analysis

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Objective: To evaluate the effects of behavioral intervention for polycystic ovary syndrome (PCOS).

Methods: Electronic databases were searched, including Pubmed, Medline, EMBASE, and the Cochrane Central Register of Controlled Trials from inception to 1 April 2023. Inclusion criteria for this study required a diagnosis of PCOS. Interventions of interest included behavioral intervention and routine treatment compared with routine treatment. The studies included in the analysis were designed as randomized controlled trials (RCTs). We conducted meta-analyses following the recommended guidelines. The data was analyzed using either the random effects model or fixed effects model. The results of the studies were expressed as either mean differences (MD) or standardized mean differences (SMD) along with their corresponding 95% confidence intervals (CIs).

Results: Eight RCTs were identified, including data from 744 patients (415 in the intervention group and 329 in the control group). The results indicate an improvement in the effectiveness of behavioral interventions for weight loss (MD: -1.07; 95% CI: -2.1 to 0.03; I^2 = 0%; P=0.04), body mass index (BMI) (MD: -1.12; 95% CI: -1.92 to -0.33; I^2 = 73%; P=0.006), waist circumference (MD: -3.97; 95% CI: -5.64 to -2.29; I^2 = 0%; P<0.00001), quality of life about weight (MD: 0.58; 95% CI: 0.15 to 1.02; I^2 = 0%; P=0.008), depression (SMD: -1.12; 95% CI: -2.35 to -0.07; I^2 = 92%; P=0.04), and triglycerides (MD: -0.16; 95% CI: -0.27 to -0.05; I^2 = 27%; P=0.004). However, there were no significant differences in menstrual cycles, hirsutism, emotions, and infertility. The study also found that behavioral interventions had no significant effect on systolic and diastolic blood pressure, high-density lipoprotein, low-density lipoprotein, homeostasis model assessment of insulin resistance, testosterone, total cholesterol, fasting glucose, fasting insulin, hemoglobin A1C, and sex hormone binding globulin.

Conclusion: Behavioral intervention supplementation contributes to weight loss, reduction in BMI and waist circumference, and improvement in depression among patients with PCOS. However, no significant improvement was observed in the biochemical index and quality of life. The long-term effects of behavioral intervention for PCOS remain unclear due to limitations in the quality of the studies involved and the short duration of treatment.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO, identifier CRD42023442875.

KEYWORDS

behavioral intervention, polycystic ovary syndrome, weight loss, body mass index, waist circumference, meta-analysis

1 Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects women of reproductive age (1). The estimated prevalence of PCOS worldwide is reported to be between 10% and 20% (2). Additionally, many cases remain undiagnosed (3). The main characteristics of PCOS include infrequent or absent menstrual periods, infertility, high levels of androgens (male hormones), excessive hair growth, obesity, and insulin resistance (4). The exact cause and pathogenesis of PCOS are still not yet fully understood, and there is currently no definitive cure for the condition (5). PCOS can significantly impact patients' quality of life and lead to psychological issues such as low self-esteem and depression (6). PCOS patients often experience a range of symptoms, including menstrual and ovulation disorders, infertility, metabolic syndrome, emotional distress, and reproductive problems. Additionally, many PCOS patients are overweight or obese (7). The metabolic and reproductive characteristics of PCOS tend to deteriorate with obesity (8).

PCOS, as a complex multisystem disorder, has traditionally been treated with medications and surgeries. However, recent research has led to recognition and attention to treatment strategies such as lifestyle modification, psychological evaluation and interventions, long-term medication management, and multidisciplinary collaboration (9). Lifestyle interventions play a crucial role in the treatment of PCOS and are recommended as a first-line strategy to improve the health outcomes of PCOS patients (9). Current lifestyle recommendations focus on eating a healthy diet and regular physical activity (10). Adherence to these recommendations often requires major behavioral pattern change (11). Behavioral interventions are commonly employed to modify behavioral patterns (12), These interventions typically include text messages, mobile health applications, cognitive-behavioral interventions, supervised training, encouragement courses, psychoeducational group programs, psychological care, motivational interviewing, peer support, and educational group meetings (13, 14). Behavioral intervention has been successfully used in the treatment of various diseases, including obesity, coronary heart disease, psychological health, and depression (13, 15–17). Currently, behavioral intervention has been applied and reported as a treatment option for PCOS patients (18). However, there is inconsistent evidence regarding the effects of behavioral interventions on body composition, clinical manifestations, and biochemical indicators in patients with PCOS (19–32). Some studies suggest that behavioral interventions can lead to increased improvements in PCOS patients (20–28, 30, 31), while others have proved that behavioral interventions have no significant effect (29, 32). Additionally, the sample sizes of these studies have been relatively small.

Previous systematic reviews have primarily focused on the effectiveness and safety of lifestyle modifications, physical activity, and cognitive-behavioral interventions for patients with PCOS (33–35). However, limited attention has been given to behavioral interventions. Therefore, the main objective of this study is to conduct a systematic review and meta-analysis of published RCTs to comprehensively assess the positive effects of behavioral interventions on PCOS. By doing so, we aim to provide evidence-based recommendations for the treatment of patients with PCOS.

2 Methods

The present systematic review and meta-analysis was conducted with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (36). The research focuses on the PICOS question: What are the effects of behavioral interventions on anthropometric measurements (weight loss, BMI, waist circumference), clinical outcomes (quality of life, psychological status), and biochemical indexes (high-density lipoprotein (HDL), low-density lipoprotein (LDL), blood pressure (BP), homeostasis model assessment of insulin resistance (HOMA-IR), testosterone (T), total cholesterol (Tch), triglycerides (TG), fasting glucose, fasting insulin, sex hormone binding globulin (SHBG), and

hemoglobin A1C (HbA_{1C}) in women with PCOS compared with conventional treatment after four weeks to 12 months of intervention? Prior to data extraction, the systematic review was registered in the PROSPERO database (CRD42023442875). Due to the lack of data, the minimum number of studies for the meta-analysis was decreased to two.

2.1 Inclusion criteria

Studies were included in the systematic review if they met the following criteria: (1) Participants: women with a definite diagnosis of PCOS; (2) Intervention: behavioral interventions, such as text messages, mobile health applications, cognitive-behavioral interventions, supervised training, encouragement courses, psychoeducational group programs, psychological care, motivational interviewing, peer support, and educational group meetings, compared with routine treatment without any behavioral intervention.; (3) Outcomes: outcomes included anthropometric measurements, clinical measures or biochemical markers, at least one of following statistics: weight loss, BMI, waist circumference, quality of life, psychological status, BP, HDL, LDL, HOMA-IR, T, Tch, TG, fasting glucose, fasting insulin, HbA1C, and SHBG; (4) Study designs: randomized controlled trials (RCTs).

2.2 Exclusion criteria

Excluded from the analysis were conference summaries, animal experiments, cohort studies, retrospective studies, non-randomized controlled intervention studies, studies with overlapping data, studies with unavailable full text or data, studies with unreported target outcomes, and non-English language literature.

2.3 Outcome indexes

The study's primary outcome indicators were weight loss, BMI, and waist circumference. These outcomes are essential for evaluating the effectiveness of interventions or treatments aimed at improving clinical symptoms in patients with PCOS. BMI is a standardized measure that consider both weight and height, enabling a more standardized assessment of a patient's body composition. The study's secondary outcome measures in this study aim to provide a more comprehensive understanding of the effects of the condition on a patient's life. These secondary outcome measures encompass clinical manifestations of PCOS, such as quality of life, psychological status, and blood pressure. The quality of life of patients with PCOS is evaluated using the disease-specific polycystic ovary syndrome questionnaire (PCOSQ) (37). The questionnaire comprises 26 items that measure five areas: emotions, hirsutism, weight, infertility problems, and menstrual problems. It enables researchers to investigate the effects of PCOS on a patient's emotional well-being, self-image, fertility, and menstrual regularity. Furthermore, the study aims to examine various metabolic indicators associated with PCOS, including HOMA-IR, T, TG, LDL, HDL, Tch, fasting glucose, fasting insulin, HbA_{1C}, and SHBG. Assessing these metabolic indicators can provide insights into the hormonal and metabolic dysregulation commonly observed in patients with PCOS, enabling a better understanding of the condition's underlying mechanisms. This study aims to comprehensively evaluate the effectiveness of interventions or treatments for PCOS patients by incorporating both primary and secondary outcome measures. The evaluation will consider physical aspects such as weight loss and body composition, as well as psychological well-being and metabolic health.

2.4 Search strategies

The systematic search was performed in Pubmed, MEDLINE, EMBASE, and Cochrane Central Register of Controlled Trials (CENTRAL) from inception until April 1, 2023. We applied: "polycystic ovary syndrome" "PCOS" "polycystic ovarian syndrome" "behavioral therapy" "behavioral modification" "behavioral intervention" "behavior change interventions" "Randomized Controlled Trial" as search terms. The search strategy is available in Supplementary Information. Only English language studies were considered, and human filters were applied. Potential eligible studies were manually searched for additional data by reviewing relevant conference proceedings and reference lists.

2.5 Data extraction

Using Zotero to remove duplicate studies from the identified articles. Two review authors independently collected information and screened the abstracts. Full texts were retrieved for further analysis. The characteristics of included studies were extracted according to Cochrane guidelines by two authors. Inconsistencies were resolved through discussion with the third author. The selection process was documented with a flowchart of PRISMA (Figure 1).

A data collection sheet was standardized based on the consensus of clinical and methodological experts. Two review authors independently collected the following data: basic characteristics such as author, year, and country; subject characteristics including age and number of people in each group; and outcome indicators such as weight loss, BMI, waist circumference, quality of life, psychological status, HDL, LDL, HOMA-IR, T, Tch, TG, BP, fasting glucose, fasting insulin, HbA_{1C}, and SHBG (Table 1). For continuous variables, we extracted mean and standard deviation (SD) values at baseline and after treatment. We sent emails to study authors for more detailed information and outcome data.

2.6 Data synthesis and analysis

All related statistical analysis was conducted by using the software Review Manager 5.4. MD was applied for continuous data such as weight loss and BMI. Standardized mean difference (SMD) was commonly used when different scales were taken for the same outcome. If MD was not mentioned, it was derived from either the

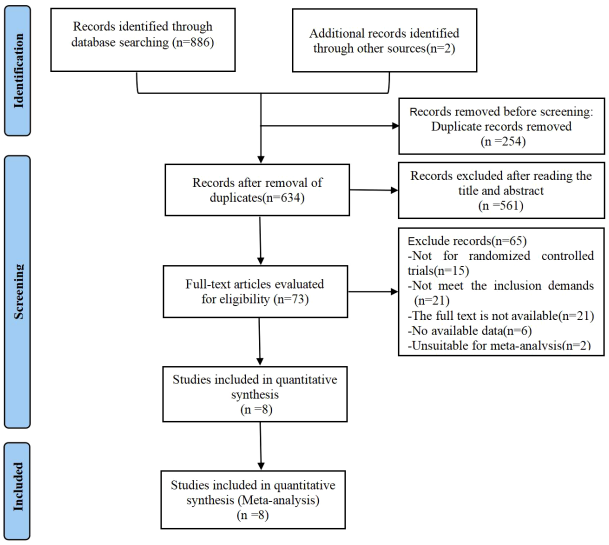

FIGURE 1
PRISMA flow diagram of the study process. PRISMA, Preferred Reporting Items for Systematic review and Meta- analysis.

standard error, interquartile range, or the 95% confidence interval. The review expressed effect sizes for each outcome measure as the weighted mean difference (WMD) and 95% CI between the behavioral interventions and routine treatment controls. Heterogeneity between studies was evaluated using I^2 statistical analysis (I^2 statistics>75% assigned as highly heterogeneous) and 95% confidence interval. The fixed effects model was applied when I^2<50%; otherwise, the random effects model was used for further data analysis. Subgroup and sensitivity analyses were used to explore sources of heterogeneity. If necessary, we utilized Engauge Digitizer 4.1 to extract data from images. Publication bias was assessed using Begg's and Egger's tests when more than ten trials were included in the analysis.

2.7 Assessment of risk of bias and evidence quality

The risk of bias of the included studies was conducted independently by two authors using the Cochrane Collaboration's tools and criteria (38). The domains typically evaluated using the Cochrane Collaboration's risk of bias tool include sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other sources of bias, with the risk of bias for each domain classified as low, high, or unclear. Disagreements between data extractors were resolved by discussion with a third author.

3 Results

3.1 Studies selection and the flow chart

A total of 888 articles were retrieved from various databases, including 292 from PubMed, 139 from Embase, 264 from Cochrane library, 191 from Medline, and 2 from the references. After screening by Zotero, 634 articles were left after removing duplicates (n=254). Among these, 561 articles were excluded after screening of the titles and abstracts for irrelevance. Seventy-three articles were selected for full-text revision, and sixty-five of these were excluded for following reasons: (1) Not for randomized controlled trials(n=15); (2) Not meet the inclusion demands(n=21); (3) The full text is not available (unpublish trails or unable to find full text) (n=21); (4) No available data(n=6); (5) Unsuitable for meta-analysis(n=2). Finally, this systematic review analyzed eight RCTs with 744 patients with PCOS, as shown in Figure 1 (25–32).

3.2 Characteristics of included studies

The basic characteristics of the eight RCTs including 744 subjects were listed in Table 1. The mean age of control group was 29.1 ± 6.4 years, while the behavioral intervention group was 29.9 ± 5.8 years. There was no significant difference in the age of the study participants. The mean baseline BMI was 31.0 ± 6.4 kg/m^2 in the control groupand 31.8 ± 6.7 kg/m^2 in the intervention group. There was no significant difference in the baseline BMI between the two groups of subjects.

These eight studies were published between 2015 and 2022. Two of the studies were performed in China (26, 27), and the remaining six were performed in Iran (25), Sweden (29), the United Kingdom (28), Pennsylvania (30), Canada (31), and Australia (32), respectively. Two studies evaluated the effectiveness of behavioral interventions using mobile health applications (26, 27). These interventions aimed to modify individuals' behavior patterns in order to achieve positive health outcomes. Additionally, four studies evaluated the impact of encouragement courses as a behavioral intervention (25, 26, 29, 32). These interventions focused on patients participating in team-based courses, where healthcare professionals provided health education and behavior guidance to promote behavior change and improve overall health outcomes. Furthermore, two studies utilized supervised encouragement training as the central approach to modify behavior patterns and ultimately achieve the desired outcomes (30, 31). The aim of these interventions was to encourage individuals to adopt regular exercise routines to promote healthy behavior change. The studies had a follow-up duration ranging from four weeks to 12 months, allowing for longitudinal assessment and monitoring of participants' progress in changing their behavior patterns. Regarding outcome indicators, four studies reported weight loss as a measurable outcome of the behavioral interventions. Additionally, six studies used BMI as the outcome measure, while four studies used waist circumference. Some studies also measured secondary outcomes such as quality of life, psychological status, HDL, LDL, HOMA-IR, T, Tch, TG, BP, fasting glucose, fasting insulin, HbA_{1C}, and SHBG (Table 1).

3.3 Risk bias in included studies

The risk of bias in the included studies was summarized in Figures 2, 3 according to the Cochrane risk of bias tool. All included

TABLE 1 Characteristics of the included studies.

Study	Country region	Publish year	Recruitment	Population				Sample size	Intervention	Control	Duration	Outcomes
				Mean age (y)		BMI						
Author				Intervention	Control	Intervention	Control					
Abdollahi et al (25).	Iran	2018	2012.4-2015.1	28.0 (4.4)	27.0 (4.6)	27.6 (5.9)	29.2 (4.9)	74	cognitive behavioral therapy (encouragement courses)	routine treatments	4w	②⑩
Wang et al. (26)	china	2022	2008.10-2010.3	24.72 (4.20)	24.94 (4.31)	25.99 (3.87)	25.25 (3.95)	122	Transtheoretical model-based mobile health application	routine treatments	12m	②③⑩
Oberg et al. (29)	Sweden	2019	2012.4-2015.1	31.0 (5.1)	29.9 (5.7)	33.5 (5.13)	34.3 (4.93)	68	behavioral modification intervention (encouragement courses)	routine treatments	12m	①②③⑪⑫⑭
Legro et al. (30)	Pennsylvania	2015	2008.10-2010.3	28.6 (3.77)	29.8 (3.7)	35.3 (4.5)	35.1 (4.2)	149	lifestyle modification and/or oral contraceptive pills (encouragement courses)	routine treatments	16w	③④⑧⑪
Benham et al. (31)	Canada	2020	2017.12-2019.9	29.3 (4.3)	29.1 (5.4)	31.35 (8.6)	31.6 (8.2)	47	encourage supervision training	routine treatments	6m	①②③⑤⑥⑦⑧⑨⑫⑬
Guo et al. (27)	China	2022	2021.3-2021.6	24.95 (4.02)	25.98 (4.05)	25.86 (2.64)	25.45 (2.42)	80	Transtheoretical model based intervention	routine treatments	6m	②③
Thomson et al. (32)	Australia	2016	2006.4-2007.2	30.3 (6.2)	30.3 (6.2)	36.4 (5.6)	36.4 (5.6)	43	encourage supervision training	routine treatments	20w	①④⑩
Mani et al. (28)	UK: United Kingdom	2018	2012.7-2013.7	33.4 (7.1)	33.3 (8.1)	34.2 (7.2)	33.2 (6.2)	161	A structured education program	routine treatments	12m	①②④⑤⑥⑦⑧⑨⑪⑫⑬⑭

outcomes, ①weight loss; ②BMI, body mass index; ③waist circumference; ④PCOSQ, polycystic ovary syndrome questionnaire; ⑤HOMA-IR, homeostasis model assessment of insulin resistance; ⑥HDL/LDL, high-density lipoprotein/low-density lipoprotein; ⑦TG, triglycerides; ⑧Tch, total cholesterol; ⑨BP, blood pressure; ⑩depression; ⑪T, testosterone; ⑫fasting glucose/fasting insulin; ⑬HbA1C, hemoglobin A1C, ⑭SHBG, sex hormone binding globulin.

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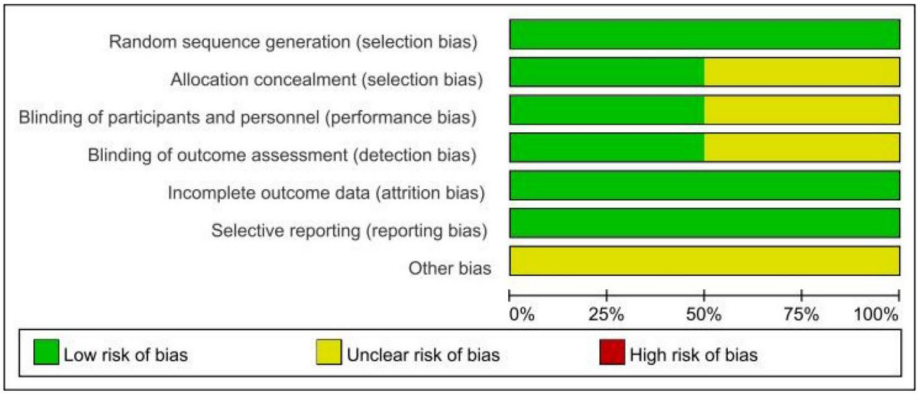

FIGURE 2
Overall risk of bias assessment.

trials reported adequate randomized sequence generation. Two studies used random number tables (30, 32), five studies used block randomization (25–27, 29, 31), and one study only mentioned randomization but did not provide further details (30). Four trials were assessed as being at unclear risk of selection bias because allocation concealment details were not provided (28–30, 32). Four trials were assessed as having an unclear risk of performance bias because they did not require blinding of participants or researchers (28, 29, 31, 32). Similarly, four studies mentioned the blinding of outcome assessors (25–27, 30), but the other four studies did not mention whether blinding of outcome assessors was carried out. All trials were preregistered in a clinical trial registry, which might have efficiently controlled reporting bias. Furthermore, these studies did not offer precise information regarding the existence of other potential sources of bias.

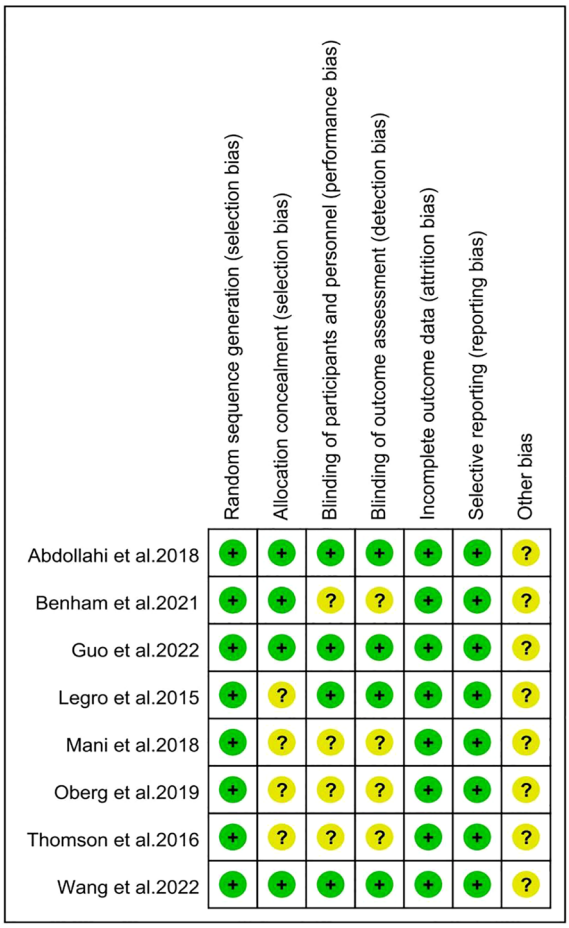

FIGURE 3
Risk of bias summary for individual studies. The symbol '?' indicates unclear risk of bias, while the symbol '+' indicates low risk of bias.

3.4 Data synthesis and meta-analysis

3.4.1 Primary outcomes

3.4.1.1 Weight loss

Four studies (28, 29, 31, 32)showed a range of weight loss with 131 subjects in the behavioral intervention group and 107 subjects in the control group. The meta-analysis of RCTs using fixed effects models revealed that behavioral interventions were significantly more effective in reducing weight in patients with PCOS compared to the controls (MD: -1.07; 95% CI: -2.1 to -0.03; I^2 = 0%; P=0.04; Figure 4). Three studies (25–27) were excluded from the meta-analysis as they did not report weight at the end of the study. However, these studies provided information on changes in BMI. The BMI of the intervention groups showed a significant decrease (MD: -2.42; 95% CI: -3.33 to -1.52; I^2 = 35%; P<0.00001; Figure 5). One study (30) reported a significant impact on weight reduction in the intervention group. However, it should be noted that the intervention group also received oral weight loss medication in addition to behavioral interventions. Therefore, this study was not included in the current meta-analysis.

3.4.1.2 Body mass index

BMI was reported in six RCTs (25–29, 31), including 224 participants in the intervention group and 211 participants in the control group. The random effects model was used for meta-analysis, and our results showed that behavioral intervention was associated

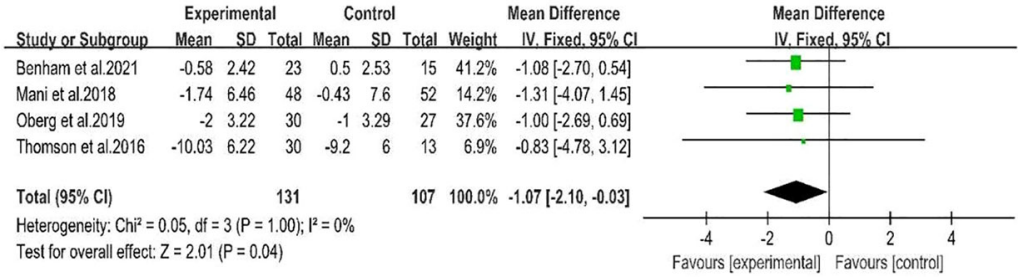

FIGURE 4
Forest plot of mean difference standardized of range of weight loss.

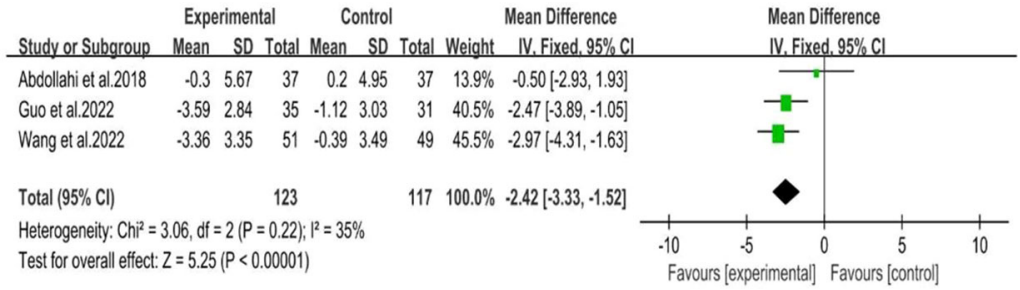

FIGURE 5
Forest plot of the BMI (the studies not mentioned weight loss).

with a significant decrease in BMI (MD: -1.12; 95% CI: -1.92 to -0.33; I^2 = 73%; P=0.006; Figure 6).

Subgroup analyses were conducted based on random-effects models according to the specific method of behavioral interventions, including mobile health applications, supervised training, and encouragement courses, due to the high heterogeneity.

Based on the specific methods of behavioral intervention, there are two subgroups distinguished by the use of mobile health applications. Behavioral intervention was conducted through mobile health applications in two studies (26, 27). And the intervention group showed a significant decrease in BMI when compared to the control group (MD: -2.73; 95% CI: -3.71 to -1.76; I^2 = 0%; P<0.00001; Table 2).

However, supervised training and encouragement courses were used to modify behavior patterns in four studies (25, 28, 29, 31), but there was no significant difference in BMI between the groups (MD: -0.45; 95% CI: -0.84 to -2.29; I^2 = 0%; P=0.02; Table 2).

3.4.1.3 Waist circumference

There were four studies reported Waist circumference (26, 27, 30, 31), including 196 participants in the intervention group and 144 participants in the control group. The results showed that behavioral intervention significantly improved waist circumference (MD: -3.97; 95% CI: -5.64 to -2.29; I^2 = 0%; P<0.00001; Figure 7).

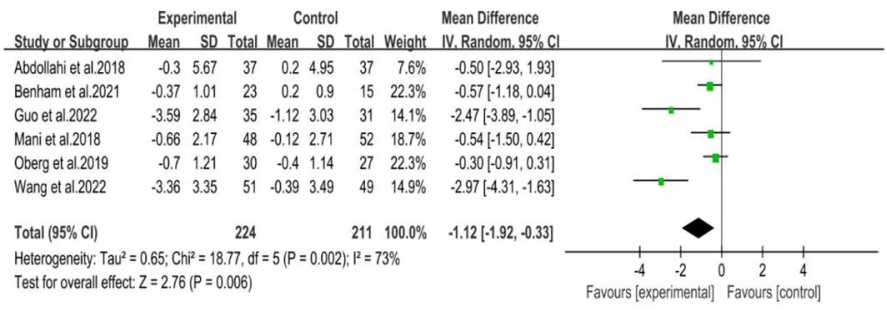

FIGURE 6
Forest plot of mean difference standardized of BMI.

TABLE 2 Subgroup analyses of BMI.

Subgroups	Study included number	Heterogeneity		Effect model	Meta analysis	
		P value	I^2 (%)		Relative effect (95%CI)	P value
Mobile health applications	2	0.62	0	Fixed	-2.73 (-3.71, -1.76)	<0.00001
Encouragement courses and supervision training	4	0.94	0	Fixed	-0.45 (-0.84, -2.29)	0.02

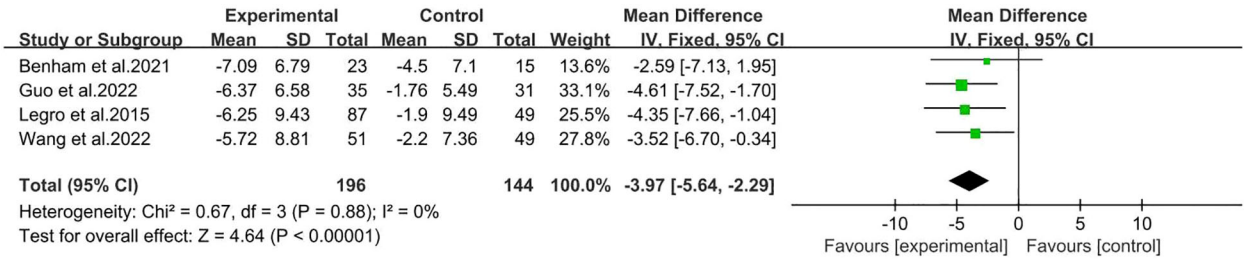

FIGURE 7
Forest plot of mean difference standardized of Waist circumference.

3.4.2 Secondary outcomes

3.4.2.1 Clinical parameters

3.4.2.1.1 Psychological status: depression

Three studies (25, 26, 32)with a total of 118 participants in the intervention group and 99 in the control group displayed depression. The behavioral intervention group showed a significant change in depression (SMD: -1.12; 95% CI: -2.35 to -0.07; I^2 = 92%; P=0.04; Table 3).

The meta-analysis revealed high heterogeneity among the studies. However, due to the limited number of available studies, it was not possible to explore the sources of heterogeneity through subgroup analysis.

3.4.2.1.2 Quality of life

Overall, three literatures (28, 30, 32)assessed the quality of life, involving 161 patients supplemented with behavioral interventions and 103 patients in the control group. Quality of life was assessed by PCOSQ. There was no significant difference in the quality of life related to menstrual problems between the two groups (MD: 0.17; 95% CI: -0.11 to 0.46; I^2 = 0%; P=0.23; Table 3). The similar results can also be observed in the hirsutism domain (MD: -0.26; 95% CI: -0.53 to 0.00; I^2 = 46%; P=0.05; Table 3), the emotions domain (MD: 0.11; 95% CI: -0.13 to 0.36; I^2 = 0%; P=0.35; Table 3), and the infertility problems domain (MD: 0.24; 95% CI: -0.06 to 0.54; I^2 = 22%; P=0.11; Table 3). However, the results indicated that behavioral interventions had a significant positive impact on the quality of life of patients in terms of weight, as measured by the PCOSQ (MD: 0.58; 95% CI: 0.15 to 1.02; I^2 = 0%; P=0.008; Table 3). One study (30) used diet pills in combination with behavioral interventions for weight loss was excluded from the analysis.

3.4.2.1.3 Blood pressure

Only two RCTs have reported on blood pressure (28, 31). The behavioral intervention group did not show a significant difference in systolic blood pressure compared to the routine treatment group (MD: 1.31; 95% CI: -2.36 to 4.98; I^2 = 0%; P=0.48; Table 3). Additionally, there was no significant difference in diastolic blood pressure between the two groups (MD: -0.32; 95% CI: -3.04 to 2.40; I^2 = 0%; P=0.82; Table 3). Overall, the findings suggest that the behavioral intervention is not more effective than routine treatment in improving both systolic and diastolic blood pressure.

3.4.2.2 Metabolic parameters

3.4.2.2.1 Triglycerides

Three studies including 158 patients in intervention group and 111 patients in the control group provided data of TG (28, 30, 31). We observed a significant decrease in TG in the behavioral intervention group compared to the control group (MD: -0.16; 95% CI: -0.27 to -0.05; I^2 = 27%; P=0.004; Table 3).

3.4.2.2.2 HOMA–IR

There were three studies including 93 patients in intervention group and 89 patients in the control group reported HOMA–IR (28, 29, 31). The fixed effects model displayed no discernible difference between the intervention and control groups (MD: -0.14; 95% CI: -0.47 to 0.19; I^2 = 0%; P=0.41; Table 3).

3.4.2.2.3 Testosterone

Testosterone levels were mentioned in three studies (28–30), involving 171 participants in the intervention group and 131 in the control group. According to the random effects model, there was no

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TABLE 3 Behavioral intervention compared with control for PCOS patients.

Outcome		Study included number	Heterogeneity		Effect model	Meta analysis	
			P value	I^2		Relative effect (95%CI)	P value
Anthropometric measurements	Weight loss	4	1.00	0%	Fixed	-1.07 (-2.10, -0.03)	0.04
	Body mass index	6	0.002	73%	Random	-1.12 (-1.92, -0.33)	0.006
	Waist circumference	4	0.88	0%	Fixed	-3.97 (-5.64, -2.29)	<0.00001
Clinical outcomes	Depression	3	<0.00001	92%	Random	-1.21 (-2.35, -0.07)[a]	0.04
	Menstrual problems	3	0.93	0%	Fixed	0.17 (-0.11, 0.46)	0.23
	Hirsutism	3	0.16	46%	Fixed	-0.26 (-0.53, 0.00)	0.05
	Emotion	3	0.79	0%	Fixed	0.11 (-0.13, 0.36)	0.35
	Infertility	3	0.28	22%	Fixed	0.24 (-0.06, 0.54)	0.11
	Weight	2	0.40	0%	Fixed	0.58 (0.15, 1.02)	0.008
Biochemical indexes	High-density lipoprotein	2	0.08	67%	Random	0.03 (-0.12, 0.18)	0.69
	Low-density lipoprotein	2	0.76	0%	Fixed	-0.04 (-0.20, 0.12)	0.65
	Systolic blood pressure	2	0.49	0%	Fixed	1.31 (-2.36, 4.98)	0.48
	Diastolic blood pressure	2	0.42	0%	Fixed	-0.32 (-3.04, 2.40)	0.82
	HOMA-IR	3	0.61	0%	Fixed	-0.14 (-0.47, 0.19)	0.41
	Triglycerides	3	0.25	27%	Fixed	-0.16 (-0.27, -0.05)	0.004
	Total cholesterol	2	0.55	0%	Fixed	-0.04 (-0.23, 0.16)	0.71
	Testosterone	3	0.02	76%	Random	0.30 (-0.18, 0.78)[a]	0.22
	Fasting glucose	3	0.06	64%	Random	0.00 (-0.21, 0.21)	0.99
	Fasting insulin	3	0.41	0%	Fixed	-0.56 (-2.79, 1.68)	0.63
	HbA1C	2	0.95	0%	Fixed	-0.34 (-0.68, -0.00)[a]	0.05
	SHBG	2	0.63	0%	Fixed	0.05 (-0.27, 0.26)[a]	0.78

HOMA-IR, homeostasis model assessment as an index of insulin resistance; HbA1C, hemoglobin A1C; SHBG, sex hormone binding globulin; [a], standardized mean difference.

statistically significant difference in testosterone levels between the two groups (SMD: 0.30; 95% CI: -0.18-0.78; I^2 = 76%; P=0.22; Table 3). We found a high degree of heterogeneity, and further subgroup analysis was conducted according to the study duration. Two studies were conducted for four months, and the results showed a significant decrease in testosterone levels in the control group (SMD: 0.52; 95% CI: 0.15 to 0.89; I^2 = 37%; P=0.005). Another study was conducted for one year found that behavioral intervention had no significant effect on testosterone levels. Further research is necessary for additional meta-analysis.

3.4.2.2.4 Fasting glucose and fasting insulin

Three studies reported fasting glucose levels (28, 29, 31). The behavioral intervention group consisted of 101 patients, while the control group consisted of 94 patients. These studies also measured the fasting insulin levels, with 93 patients assigned to the behavioral intervention group and 89 patients assigned to the control group. Fasting glucose calculated using the random effects model. The analysis showed no statistically significant difference in fasting glucose between the two groups (MD: 0.00; 95% CI: -0.21 to 0.21; I^2 = 64%; P=0.99; Table 3). A fixed-effects model was used to analyze fasting insulin levels, revealing no significant difference between the intervention and control groups (MD: -0.56; 95% CI: -2.79 to 1.68; I^2 = 0%; P=0.63; Table 3).

3.4.2.2.5 High-density lipoprotein and low-density lipoprotein

High-density lipoprotein and low-density lipoprotein were measured in two studies with study duration of six months and 12 months respectively (29, 32). The random effects model showed no significant difference in HDL levels between the behavioral intervention group and the control group (MD: 0.03; 95% CI: -0.12 to 0.18; I^2 = 0%; P=0.69; Table 3). Fixed-effects modeling revealed no significant difference in LDL levels between the two groups (MD: -0.04; 95% CI: -0.20 to 0.12; I^2 = 0%; P=0.65; Table 3).

3.4.2.2.6 Total cholesterol

Two studies provided data on total cholesterol with 70 patients in intervention group and 66 patients in the control group (28, 31). In the fixed effects models, there was no significant difference in total cholesterol in the behavioral intervention group compared with the control group (MD: -0.04; 95% CI: -0.23 to 0.16; I^2 = 0%; P=0.71; Table 3).

3.4.2.2.7 Hemoglobin A_{1C}

Only two studies reported HbA_{1C} levels (28, 31), which were evaluated using a fixed-effects model. The results showed no significant difference between the group that received the behavioral intervention and the control group (SMD: -0.34; 95% CI: -0.68 to -0.00; I^2 = 0%; P=0.05; Table 3).

3.4.2.2.8 Sex hormone binding globulin

Two studies mentioned SHBG (28, 29). One study reported significantly elevated SHBG levels (29), while the other study showed no clinically significant changes. More adequate data is needed for meta-analysis on this topic. Fixed effects model resulted that estradiol levels were not significantly different in two groups (SMD: 0.05; 95% CI: -0.27 to 0.26; I^2 = 0%; P=0.78; Table 3).

In summary, we primarily found that behavioral interventions improved outcomes like weight loss, BMI, waist circumference, psychological status and TG. While other clinical manifestations and metabolic indexes were not significantly altered.

3.4.3 Adverse reaction

The four studies (26, 29–31) included in the Meta-analysis mentioned adverse reactions. Two of the studies (26, 29) showed no adverse events. Adverse events were recorded in the remaining two studies, one (31) of which did not report trial-related adverse events, and the other (30) reported adverse events mainly related to specific other treatment modalities. Behavioral interventions may be a safe treatment for PCOS.

4 Discussion

In conclusion, lifestyle interventions recommended for patients with PCOS include exercise, adopting a balanced and nutritious dietary pattern, and behavioral changes (39). Furthermore, studies have demonstrated that lifestyle modifications are beneficial treatment methods for women with PCOS (20, 40, 41). Although there is some understanding of the effects of behavioral interventions on PCOS, only a few trials have confirmed these findings.

This study aimed to investigate the impact of different behavioral interventions on various aspects of health in patients diagnosed with PCOS. The study focused on analyzing the effects of these interventions on weight loss, BMI, waist circumference, clinical manifestations of PCOS and biochemical indicators. This meta-analysis included eight studies involving 744 reproductive-aged PCOS patients, we observed beneficial effect of behavioral interventions on various aspects of health in patients with PCOS, including weight loss, improvement in BMI, and reduction in waist circumference. This statement is consistent with Jiskoot et al.'s findings that behavioral interventions are crucial for achieving long-term weight loss and improving mental health (20, 42, 43). However, a meta-analysis report showed that cognitive-behavioral interventions alone did not have a significant effect on reducing weight in patients with PCOS (44). This difference may be related to the differences in study populations, intervention durations, and types of behavioral interventions.

In addition, our data also suggest that behavioral interventions can improve the patients' quality of life in terms of depressive symptoms and weight. Previous studies in patients with PCOS have similarly reported that cognitive-behavioral interventions can reduce depressive scores in PCOS (35, 44, 45). Moreover, research has shown that using mobile applications and text messages as intervention measures can improve patients' mental and physical health (22, 46), which is consistent with our study findings. Furthermore, in our study, subgroup analysis based on study duration revealed a significant decrease in testosterone in the control group. Possible reasons for this are the relatively high initial levels in the control group and the short duration of the study. More studies and longer follow-up time are needed to further clarify the effect of behavioral intervention on testosterone.

Our analysis suggests that behavior interventions through short message service (SMS), mobile health applications, supervised training, and encouragement courses can have beneficial impacts by inducing changes in patients' behavior patterns. However, the effectiveness of behavior interventions in achieving other outcomes such as menstrual health, infertility, and emotional life quality has not been confirmed. Furthermore, there is insufficient evidence to support the effectiveness of behavior interventions in reducing systolic and diastolic blood pressure, HDL, LDL, HOMA-IR, T, Tch, TG, fasting glucose, fasting insulin, HbA_{1C}, and SHBG. On the contrary, some studies have indicated that behavior interventions can have favorably affect patients' menstrual cycles and fertility (47), which contradict our findings. This discrepancy may be attributed to the quality and quantity of included studies, and more definitive conclusions can be drawn through further relevant research.

Patients with PCOS have a significantly higher prevalence of overweight and obesity compared to non-PCOS patients (23). Most individuals with PCOS are overweight and obesity throughout their entire lifespan, and obesity exacerbates the reproductive, metabolic, and psychological symptoms of PCOS (48). Weight loss can bring about significant improvements in psychological symptoms (depression and quality of life), reproductive function (menstrual cycles and fertility), and metabolic symptoms (insulin resistance, metabolic syndrome, etc.) of patients, even if they remain in the overweight or obese range.

This meta-analysis has several advantages. It includes retrieval of multiple databases without any time restrictions. One of the strengths of our study is that it simultaneously investigates the common complications associated with PCOS patients, such as obesity, depression, and biochemical markers. Additionally, we also assessed the quality of life and blood pressure.However, there are several limitations to this study that must be considered when interpreting the results. The main limitation of this study is the limited number of published literatures evaluating the impact of behavioral interventions in patients with PCOS. Therefore, it was not possible to perform subgroup analysis based on all interventions and associated outcomes. Additionally, the original data of individual studies were not available and some studies were of low quality. Some trials lacked specific

descriptions of whether behavioral interventions led to changes patients' behavior, and some trials lasted less than 6 months, which is typically necessary for behavioral changes to occur in patients (49). Heterogeneity is a significant issue. There was significant heterogeneity in study participants, outcome measures, and intervention content, which could potentially affect the study results. Due to the limited number of studies and sample size, we were unable to conduct subgroup and sensitivity analyses to explore the sources of heterogeneity. Furthermore, the use of a self-reported questionnaire to assess outcome may have introduced bias in some studies. In the future, more well-designed clinical trials are needed to investigate the effects of behavioral interventions on PCOS patients. Long-term follow-up is also necessary to observe the long-term effectiveness of behavioral interventions in PCOS patients.

5 Conclusion

Our analysis indicates that using interventions such as text messages, mobile health applications, supervised training, and encouragement courses can improve weight loss, BMI, waist circumference, and depressive symptoms in patients with PCOS. However, because the intervention duration was short and there was no long-term follow-up, it is not possible to determine the long-term benefits for patients. Therefore, further well-designed studies are still needed to clarify and confirm the effects of behavioral interventions in patients with PCOS.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

MX: Data curation, Formal analysis, Writing – original draft. YY: Data curation, Formal analysis, Writing – review & editing. JZ: Conceptualization, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1297841/full#supplementary-material

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The effect of β-cell dysfunction on reproductive outcomes of PCOS undergoing IVF or ICSI embryo transfer cycles: a retrospective cohort study

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Objective: To investigate the effects of β-cell dysfunction on IVF outcomes in women with PCOS.

Methods: This retrospective cohort study includes 1,212 women with PCOS undergoing their first IVF cycle between September 2010 and December 2019. Beta-cell dysfunction was measured by homeostasis model assessment of β-cell function (HOMA-β) index.

Results: In quartiles of HOMA-β, the incidence of miscarriage dramatically increased from 10.2% (Q1) to 31.1% (Q4) ($P_{for\ trend}$ <0.001). Likewise, the incidence of miscarriage in quartiles of HOMA-β also showed a similar trend ($P_{for\ trend}$ <0.001). After adjusting for confounding factors, logistic regression analyses showed that high HOMA-IR values were independently associated with a high risk of miscarriage, with the odds ratios (OR) and 95% confidence intervals for quartiles 2–4 versus quartile 1 were 1.30 (0.69-2.46), 1.82 (0.97-3.43), and 3.57 (1.86-6.85), respectively ($P_{for\ trend}$ <0.001). When analyzed jointly, women in the highest HOMA-IR and highest HOMA-β group exhibited the highest risk for miscarriage compared with all other groups. Furthermore, higher HOMA-IR values were associated with higher risks of miscarriage among PCOS women regardless of HOMA-β values.

Conclusions: β-cell dysfunction is independently associated with increased miscarriage rate and decreased live birth rate in women with PCOS. It also plays a synergistic role with IR in terms of the reproductive outcomes, while the influence of IR overweighs that of β-cell dysfunction.

KEYWORDS

β-Cell dysfunction, insulin resistance, hyperinsulinemia, polycystic ovary syndrome, IVF outcome

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders characterized by oligo-anovulation, hyperandrogenism and polycystic ovarian morphology, affecting 5%–18% women of reproductive age (1). Most women with PCOS may experience irregular menstruation, metabolic disorders, hirsutism and infertility (2). Therefore, subfertility has become a growing problem and there is an increased use of in vitro fertilization (IVF) as a last resort in women with PCOS (3). Moreover, compared to non-PCOS, PCOS is associated with increased risk of adverse pregnancy outcomes, including miscarriage (1.7-fold higher), gestational hypertension (2-fold higher), preeclampsia (4-fold higher), gestational diabetes (3-fold higher) and premature delivery (2-fold higher) (4–7). Hitherto, the pathogenesis of adverse pregnancy outcomes of PCOS has not yet been fully elucidated, making it more difficult to perform interventions and improve pregnancy outcomes.

Approximately 45%-65% of women with PCOS have insulin resistance (IR), which is considered an initiating factor and plays a key role in the development of PCOS (8, 9). It has been confirmed that IR is closely related to adverse pregnancy outcomes (especially increased risk of miscarriage) in women with PCOS undergoing IVF treatment (10, 11). In contrast to IR, relatively fewer studies have explored the effects of β-cell function (insulin secretion) on metabolic and pregnancy outcomes in women with PCOS. Our previous study indicated that both IR and β-cell dysfunction independently affected cardiometabolic abnormalities including obesity, central obesity, dyslipidemia and high blood glucose in women with PCOS. IR was also correlated with a higher prevalence of cardiometabolic abnormalities than β-cell dysfunction (76.7% vs. 61.2%) (12). These results indicate the different roles of IR and β-cell dysfunction in the development of cardiometabolic disorders in PCOS. However, it remains unknown whether these two pathological states exhibit different effects on pregnancy outcomes. Thus, in the present retrospective cohort study, we aimed to investigate the effects of β-cell dysfunction on IVF outcomes in women with PCOS.

Patients and methods

Participants

Initially, a total of 1,515 infertile women with PCOS undergoing their first in-vitro fertilization embryo transfer (IVF-ET) cycle from September 2010 to December 2019 at the Reproductive Center of the First Affiliated Hospital of Wenzhou Medical University were enrolled in this study. The diagnostic criteria included two out of three following features according to the 2003 Rotterdam diagnostic criteria (13): (1) menstrual abnormalities, including oligomenorrhea or amenorrhea; (2) clinical and/or biochemical hyperandrogenism, including hirsutism (Ferriman-Galwey score >6) or testosterone concentration >2.6 nmol/L; and (3) polycystic ovarian morphology under B-ultrasound as indicated by the number of follicles with a diameter of 2-9 mm ≥12 and/or ovarian volume ≥10 ml. The exclusion criteria were as follows: women older than 40 years of age (n=7); women with a history of thyroid dysfunction n=19), hyperprolactinemia (n=33), hydrosalpinx (n=109), endometriosis (n=39), adenomyosis (n=30), chromosome abnormality (n=20), pituitary microadenoma (n=1), recurrent spontaneous abortion (n=24) and uterine malformation (n=10); and women lost to follow-up during pregnancy (n=11). Finally, 1,212 women with PCOS were included in the study analyses. This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (Wenzhou, China) (2021R05), and approved a waiver of patient consent for the reason that all data were deidentified in this retrospective study.

Study procedures

All patients received a standardized ovarian stimulation protocol (GnRH antagonist protocol or long GnRH agonist protocol), oocyte retrieval, fertilization, and embryo transfer. The GnRH antagonist protocol or long GnRH agonist protocol used in our reproductive center has been previously described (14). Good-quality embryos at cleavage stage were defined according to the Istanbul consensus with <10% fragmentation, stage-specific cell size and no multimucleation (15). Due to the elective single-embryo transfer policy, no more than two embryos have been transferred since June 2016. Luteal supportive therapy was administered orally with dydrogesterone (20 mg daily) and vaginally with progesterone (90 mg daily), starting on the day of oocyte retrieval, and was continued until 8 weeks of gestation.

Definitions of β-cell dysfunction and insulin resistance

Beta-cell function was estimated by the homeostasis model assessment of β-cell function (HOMA-β) index as follows: HOMA-β=(20×FINS)/(FBG-3.5) (16). Beta-cell dysfunction was defined as HOMA-β in the top quartile (HOMA-β >186.86). IR was estimated by HOMA-IR index as follows: HOMA-IR=fasting blood glucose (FBG, mmol/L) x fasting insulin (FINS, mIU/L)/22.5 (17). The top quartile of HOMA-IR, which was greater than 3.75 in the present study, was defined as insulin resistance.

Laboratory testing

Blood samples were acquired after overnight fasting for at least 8 hours. Levels of FBG, FINS and gonadal hormones were quantified by chemiluminescence. Concentrations of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were measured by a dry slide enzymatic colorimetric assay. Serum LH, FSH, E2, testosterone and AMH were measured using an ultrasensitive enzyme-linked immunosorbent assay (ELISA) (Unicel Dxl 800, Beckman Coulter, USA). Fasting plasma glucose, total cholesterol (TC),

serum triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were quantified by an autoanalyzer (AU 5800, Beckman, USA). The AMH data were limited due to the regular measurement of AMH in infertile women since June 2016 in our center. The intra-assay and inter-assay variations for the testing method were mentioned in our previous study (18). All measurements were performed at the First Affiliated Hospital of Wenzhou Medical University.

Definitions of IVF outcomes

Biochemical pregnancy was defined as the detection of β-hCG in urine or serum after embryo transfer (19). Clinical pregnancy was defined as the presence of a gestational sac with fetal heart activity under ultrasound examination 35 days after embryo transfer (19). Spontaneous loss of an intra-uterine pregnancy prior to 22 completed weeks of gestational age was defined as miscarriage (19). A live birth was defined as baby born after 22 weeks of gestational age (19). The definition of the clinical pregnancy rate was the number of clinical pregnancies per 100 embryo transfer cycles (19). The miscarriage rate was defined as the number of spontaneous fetal loss per 100 clinical pregnancy cycles (19). The live birth rate was defined as the number of deliveries per 100 embryo transfer cycles (19). All IVF outcomes were obtained through electronic medical records.

Statistical analysis

SPSS 23.0 software was used for all statistical analyses in the study. We divided the distribution of HOMA-β values into four groups from the lowest quartile (quartile 1, Q1) to the highest quartile (quartile 4, Q4). Participants were analyzed according to the quartile groups of HOMA-β. Demographic and biochemical variables with a skewed distribution were presented as the medians (interquartile ranges) according to quartiles of HOMA-β. *P* values for trends across all quartiles were calculated by linear regression analysis for continuous variables. Nonnormal distributed data were logarithmically transformed prior to linear regression analysis. Logistic regression analysis was performed to obtain the odds ratios for IVF outcomes based on quartiles of HOMA-β after adjusting for relevant variables (log-transformed). Meanwhile, *P* values for trends across quartiles were calculated by the Cochran–Mantel–Haenszel method. Odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) were calculated in three models for the logistic regression analyses. Adjustments were made for the following variables: no variable was adjusted in model 1; age and BMI were adjusted in model 2; and SBP, HOMA-IR, TC, TG, basal T levels and the number of transferred embryos were further adjusted in model 3 based on model 2. Interaction analysis of HOMA-IR and HOMA-β on IVF outcomes was adjusted for age, BMI, SBP, TG, TC, basal T levels and the number of transferred embryos. ORs (95% CIs) of miscarriage and live birth were used to compare the combined effects of HOMA-IR and HOMA-β between different groups. All *P* values were two-sided, and $P<0.05$ was considered statistically significant.

Results

Baseline characteristics according to quartiles of HOMA-β in PCOS

Among the 1,212 infertile women with PCOS in the present study, the average age and the infertility duration was 29.69 and 3.81 years, respectively. There were 590 participants diagnosed with primary infertility with a prevalence rate of 48.7%, while the overall prevalence of secondary infertility was 51.3%.

The baseline characteristics of women with PCOS according to the quartiles of HOMA-β were described in Table 1. Subjects with a lower HOMA-β presented elevation in basal LH, FSH, FBG and HDL, but decreases in BMI, blood pressure, basal T, TC, TG, LDL, HOMA-IR, compared to those with higher HOMA-β (Table 1). The IVF outcomes, including fertilization mode, the number of retrieved oocytes, mature oocytes, fertilized oocytes, embryos obtained, good-quality embryos, transferred embryos and endometrial thickness, showed no significant differences among the quartiles of HOMA-β.

TABLE 1 Baseline characteristics according to quartiles of HOMA-β in PCOS.

Variables	Quartiles of HOMA-β				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
	302	304	303	303	
Ovarian stimulation date[a], n (%)					0.24
Tertile 1	110 (27.2)	92 (22.8)	116 (28.7)	86 (21.3)	
Tertile 2	97 (24.0)	103 (25.5)	97 (24.0)	107 (26.5)	
Tertile 3	95 (23.5)	109 (27.0)	90 (22.3)	110 (27.2)	
Age (years)	30.00 (27.00-32.25)	29.00 (27.00-31.75)	30.00 (27.00-32.00)	29.00 (27.00-32.00)	0.13
Infertility duration (year)	3.00 (2.00-4.00)	3.00 (2.00-4.00)	3.00 (2.00-5.00)	3.00 (2.00-5.00)	0.11

(Continued)

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TABLE 1 Continued

Variables	Quartiles of HOMA-β				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
	302	304	303	303	
Infertility type, n(%)					0.86
Primary	145 (48.0)	158 (52.0)	137 (45.2)	150 (49.5)	
Secondary	157 (52.0)	146 (48.0)	166 (54.8)	153 (50.5)	
Fertilization mode, n(%)					0.92
IVF	200 (66.2)	197 (64.8)	199 (65.7)	202 (66.7)	
ICSI	80 (26.5)	79 (26.0)	76 (25.1)	75 (24.8)	
Half-ICSI	22 (7.3)	28 (9.2)	28 (9.2)	26 (8.6)	
BMI (kg/m2)	20.36 (18.80-22.87)	22.62 (20.89-24.65)	24.61 (22.08-27.34)	25.30 (23.05-27.34)	<0.001
SBP (mmHg)	110.00 (102.75-118.00)	110.00 (103.00-123.00)	118.00 (107.75-128.00)	120.00 (112.00-129.00)	<0.001
DBP (mmHg)	70.00 (66.00-79.00)	71.00 (68.00-79.00)	75.00 (70.00-81.00)	78.00 (70.00-83.00)	<0.001
Basal LH (IU/L)	6.78 (5.07-9.30)	7.57 (5.43-10.20)	7.18 (4.74-9.24)	6.20 (4.34-9.25)	0.10
Basal FSH (IU/L)	6.62 (5.65-8.10)	6.63 (5.73-7.60)	6.54 (5.51-7.56)	6.26 (5.29-7.30)	<0.001
LH/FSH ratio	0.99 (0.76-1.36)	1.10 (0.80-1.58)	1.04 (0.75-1.46)	1.09 (0.71-1.48)	0.65
Basal E2 (pmol/L)	148.50 (97.00-200.25)	161.00 (119.00-216.00)	159.00 (108.00-214.00)	150.00 (109.00-197.00)	0.83
Basal T (nmol/L)	1.64 (1.15-1.99)	1.86 (1.49-2.30)	1.79 (1.41-2.26)	2.09 (1.56-2.43)	<0.001
Basal AFC, n	25 (20-31)	27 (21-33)	25 (20-31)	27 (20-34)	0.24
AMH (ng/mL)*	8.04 (6.05-10.62)	8.56 (6.29-10.98)	8.90 (6.71-11.69)	7.80 (6.07-10.52)	0.99
FBG (mmol/L)	5.30 (5.00-5.60)	5.20 (5.00-5.50)	5.20 (5.00-5.60)	5.10 (4.80-5.40)	<0.001
FINS (mIU/L)	4.96 (3.69-6.41)	9.23 (7.79-10.85)	13.21 (11.27-16.04)	19.91 (15.55-25.44)	<0.001
TC (mmol/L)	4.49 (3.98-5.09)	4.72 (4.17-5.31)	4.76 (4.23-5.35)	4.89 (4.34-5.49)	<0.001
TG (mmol/L)	0.98 (0.72-1.34)	1.28 (0.85-1.63)	1.33 (0.95-1.91)	1.88 (1.28-2.45)	<0.001
HDL (mmol/L)	1.41 (1.23-1.65)	1.32 (1.15-1.52)	1.28 (1.15-1.45)	1.16 (1.00-1.36)	<0.001
LDL (mmol/L)	2.44 (2.06-2.93)	2.72 (2.24-3.26)	2.69 (2.20-3.20)	2.81 (2.39-3.35)	<0.001
HOMA-IR	1.14 (0.86-1.54)	2.15 (1.77-2.64)	3.08 (2.49-3.85)	4.46 (3.31-5.88)	<0.001
HOMA-β	55.81 (43.61-70.95)	105.43 (91.40-119.57)	156.34 (140.70-172.56)	248.18 (215.72-299.68)	<0.001
No. of retrieved oocytes	12.00 (8.00-16.00)	12.50 (8.00-17.00)	11.00 (7.00-15.00)	12.00 (8.00-16.00)	0.64
No. of mature oocytes	10.00 (6.00-13.00)	10.00 (7.00-14.00)	9.00 (6.00-13.00)	10.00 (6.00-13.00)	0.21
No. of fertilized oocytes	7.00 (4.00-10.00)	7.00 (5.00-11.00)	7.00 (4.00-10.00)	7.00 (4.00-10.00)	0.14
No. of embryos obtained	7.00 (3.00-9.00)	7.00 (4.00-10.00)	6.00 (4.00-10.00)	7.00 (3.00-9.00)	0.17
No. of good-quality embryos	2.00 (1.00-5.00)	3.00 (1.00-5.00)	3.00 (1.00-5.00)	2.00 (1.00-5.00)	0.69
No. of transferred embryos	2.00 (2.00-2.00)	2.00 (2.00-2.00)	2.00 (2.00-2.00)	2.00 (1.00-2.00)	0.10
Endometrial thickness (mm)	10.0 (8.45-11.00)	10.0 (8.50-12.00)	10.0 (9.00-11.00)	10.0 (8.00-12.00)	0.93

The quartile ranges of HOMA-β were < 82.28 (n=302), 82.28-129.54 (n=304), 129.55-188.75 (n=303), and > 188.75 (n=303).

*Quartile 1: n=64, Quartile 2: n=114, Quartile 3: n=82, Quartile 4: n=108.

[a]Tertiles of controlled ovarian stimulation starting date are as follows: tertile 1, September 1, 2010 to April 29, 2014; tertile 2, April 30, 2014 to November 7, 2017; tertile 3, November 8, 2017 to December 31, 2019.

HOMA-β, homeostasis model assessment of β cell function; PCOS, polycystic ovary syndrome; BMI, body mass index; LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, estradiol; T, testosterone; AFC, antral follicle count; AMH, anti-mullerian hormone; FBG, fasting plasma glucose; FINS, fasting insulin; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure.

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IVF outcomes based on quartiles of HOMA-β and different beta-cell function plus insulin levels in PCOS

Figure 1A showed the IVF outcomes based on quartiles of HOMA-β. In quartiles of HOMA-β, the miscarriage rate in Q4 was significantly higher than that in the other 3 quartiles (all *P* values <0.05). From the lowest quartile to the highest quartile of HOMA-β, the incidence of the miscarriage rate dramatically increased from 8.5% to 29.0% ($P_{\text{for trend}}$ <0.001). However, the live birth rate decreased from Q1 to Q4 ($P_{\text{for trend}}$ <0.05). No significant differences were observed in clinical pregnancy rate, biochemical pregnancy rate and ectopic pregnancy rate among the quartiles (*P* >0.05). The IVF outcomes in women with both IR and β-cell dysfunction (top quartile HOMA-IR and HOMA-beta) versus women without IR and without β-cells dysfunction were shown in Figure 1B. Compared with women without IR and β-cell dysfunction, the miscarriage rate in women with both IR and β-cell dysfunction was significantly higher (29.7% vs. 12.1%, *P* <0.001). Although the live birth rate in women with both IR and β-cell dysfunction was comparatively lower, no significant difference was found between the two groups (40.5% vs. 46.9%, *P* >0.05). Furthermore, the reproductive outcomes of women with and without resistance or with and without β-cell dysfunction were shown in Table 2. The miscarriage rate in women with IR or β-cell dysfunction was significantly higher than the controlled groups, while the live birth rate in women with IR was significantly lower than the non-IR group.

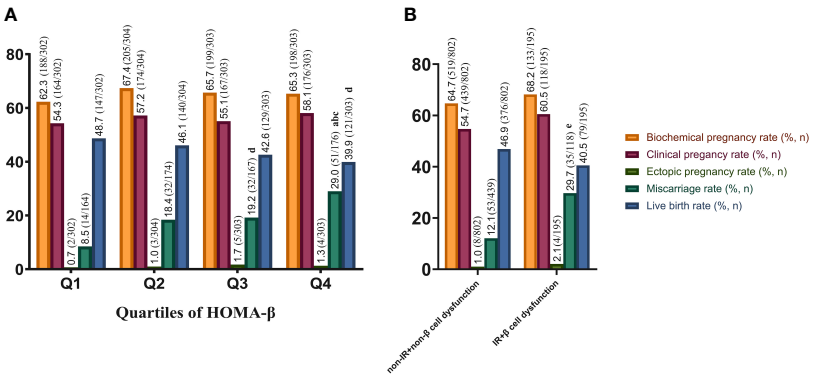
TABLE 2 Reproductive outcomes in women with and without β-cell dysfunction or with and without insulin resistance.

	β-cell dysfunction	Non-β-cell dysfunction	*P*-value
Biochemical pregnancy rate, n (%)	198/303 (65.3)	592/909 (65.1)	0.94
Clinical pregnancy rate, n (%)	176/303 (58.1)	505/909 (55.6)	0.44
Ectopic pregnancy rate, n (%)	4/303 (1.3)	10/909 (1.1)	0.76
Miscarriage rate, n (%)	51/176 (29.0)	78/505 (15.4)	<0.001
Live birth rate, n (%)	121/303 (39.9)	416/909 (45.8)	0.08
	IR	Non-IR	*P*-value
Biochemical pregnancy rate, n (%)	206/302 (68.2)	584/910 (64.2)	0.20
Clinical pregnancy rate, n (%)	184/302 (60.9)	497/910 (54.6)	0.06
Ectopic pregnancy rate, n (%)	6/302 (2.0)	8/910 (0.9)	0.12
Miscarriage rate, n (%)	60/184 (32.6)	69/497 (13.9)	<0.001
Live birth rate, n (%)	119/302 (39.4)	418/910 (45.9)	0.048

IR, insulin resistance.

Odds ratios for IVF outcomes based on quartiles of HOMA-β in PCOS

Table 3 lists the prevalence ratios for relevant IVF outcomes, including clinical pregnancy rate, biochemical pregnancy rate, ectopic pregnancy rate, miscarriage rate and live birth rate, according to quartiles of HOMA-β. With the first quartile of HOMA-β as the reference group, univariate logistic regression analysis showed significantly increased ORs for the prevalent miscarriage rate across HOMA-β categories (OR=4.37, 95% CI: 2.31-8.27) and the lowest odds ratio of live birth rate (OR=0.70, 95% CI: 0.51-0.97). After adjustment for traditional confounding factors (model 2), the ORs for the prevalent miscarriage rate, as compared with the lowest quartile, were 1.85 (95% CI, 0.90-3.80) for Q2, 2.13 (95% CI, 1.07-4.24) for Q3, and 3.09 (95% CI, 1.53-6.24) for Q4,



Note: IR= insulin resistance.
a. compared with Q1, *P* <0.001; b. compared with Q2, *P* <0.001; c. compared with Q3, *P* <0.05; d. compared with Q1, *P* <0.05; e. compared with the other group, *P* <0.001. In graph A, *P* for trend of miscarriage rate <0.001, *P* for trend of live birth rate =0.02. In graph B, *P* value of miscarriage rate between the two groups was <0.001.

FIGURE 1
IVF outcomes based on quartiles of HOMA-β **(A)** and different beta-cell function plus insulin levels **(B)** in PCOS. IVF, in-vitro fertilization; HOMA-β, homeostasis model assessment of β cell function; PCOS, polycystic ovary syndrome.

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TABLE 3 Odds ratios (OR) for IVF outcomes based on quartiles of HOMA-β in PCOS.

IVF outcomes	Quartiles of HOMA-β				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Biochemical pregnancy rate					
Model 1	1.00 (Reference)	1.26 (0.90-1.75)	1.16 (0.83-1.62)	1.14 (0.82-1.59)	0.54
Model 2	1.00 (Reference)	1.24 (0.88-1.75)	1.14 (0.80-1.63)	1.12 (0.77-1.62)	0.55
Model 3	1.00 (Reference)	1.31 (0.92-1.86)	1.19 (0.83-1.71)	1.16 (0.78-1.72)	0.39
Clinical pregnancy rate					
Model 1	1.00 (Reference)	1.13 (0.82-1.55)	1.03 (0.75-1.42)	1.17 (0.85-1.61)	0.47
Model 2	1.00 (Reference)	1.11 (0.80-1.55)	1.01 (0.71-1.42)	1.14 (0.80-1.62)	0.48
Model 3	1.00 (Reference)	1.20 (0.85-1.68)	1.06 (0.75-1.51)	1.23 (0.84-1.80)	0.35
Ectopic pregnancy rate					
Model 1	1.00 (Reference)	1.50 (0.25-9.01)	2.52 (0.49-13.07)	2.01 (0.37-11.04)	0.34
Model 2	1.00 (Reference)	1.46 (0.23-9.18)	2.53 (0.44-14.61)	1.96 (0.31-12.65)	0.34
Model 3	1.00 (Reference)	1.53 (0.24-9.78)	2.27 (0.40-13.04)	1.34 (0.21-8.63)	0.32
Miscarriage rate					
Model 1	1.00 (Reference)	2.41 (1.24-4.71)	2.54 (1.30-4.96)	4.37 (2.31-8.27)	<0.001
Model 2	1.00 (Reference)	1.85 (0.90-3.80)	2.13 (1.07-4.24)	3.09 (1.53-6.24)	<0.001
Model 3	1.00 (Reference)	1.94 (0.96-3.93)	1.78 (0.86-3.70)	2.66 (1.27-5.55)	<0.001
Live birth rate					
Model 1	1.00 (Reference)	0.90 (0.65-1.24)	0.78 (0.57-1.08)	0.70 (0.51-0.97)	0.02
Model 2	1.00 (Reference)	0.95 (0.68-1.32)	0.87 (0.61-1.23)	0.79 (0.55-1.13)	0.02
Model 3	1.00 (Reference)	1.04 (0.74-1.46)	0.92 (0.65-1.31)	0.91 (0.62-1.33)	0.04

Model 1 was unadjusted. Model 2 was adjusted for age and BMI. Model 3 was further adjusted for SBP, TG, HDL and basal T levels. All the confounding factors were log transformed. OR, odds ratio; IVF, in-vitro fertilization; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β cell function; PCOS, polycystic ovary syndrome.

respectively ($P_{\text{for trend}}$ <0.001). Following further adjustment for SBP, TG, TC, basal T levels and the number of transferred embryos (model 3), a 94%, 78%, and 166% increase in ORs for the risk of the prevalent miscarriage rate was found in the second, third and fourth quartile, respectively, compared with those in the top one ($P_{\text{for trend}}$ <0.001). In terms of live birth rate in quartiles of HOMA-β, there was significant decreasing trend from Q1 to Q4 in model 1, 2 and 3 ($P_{\text{for trend}}$ <0.05). Other IVF outcomes, such as the clinical pregnancy rate, biochemical pregnancy rate and ectopic pregnancy rate, were comparable in all 3 models in quartiles of HOMA-β.

Joint effects of HOMA-IR and HOMA-β on IVF outcomes

Figure 2 showed the unadjusted odds ratios of miscarriage and live birth by comparing nine groups of patients with various combinations of HOMA-β and HOMA-IR values. Women with both high HOMA-β and high HOMA-IR values (group 7) exhibited the highest OR for miscarriage compared to all other groups. Furthermore, a higher HOMA-IR value (groups 7, 8 and 9) was associated with a relatively higher OR of miscarriage among participants regardless of the levels of HOMA-β values. However, the odds ratios of live birth in different groups according to the values of HOMA-IR and HOMA-β showed no significant differences after adjusting for confounding factors.

Discussion

To the best of our knowledge, this is the first cohort study to explore the independent effect of β-cell dysfunction and the interaction effects of β-cell dysfunction and IR on the risks of IVF outcomes in women with PCOS. In the current study, we found that HOMA-β values were independently correlated with an increased risk of miscarriage.

Previous studies mainly have focused on the higher risk of miscarriage in PCOS women with insulin resistance (20, 21), while evidence regarding the effects of β-cell function on IVF outcomes is relatively sparse. Our results showed that high HOMA-β values were associated with an increased risk of miscarriage and a lower incidence of live birth, indicating that β-cell dysfunction (excess insulin secretion) independently exerted adverse reproductive

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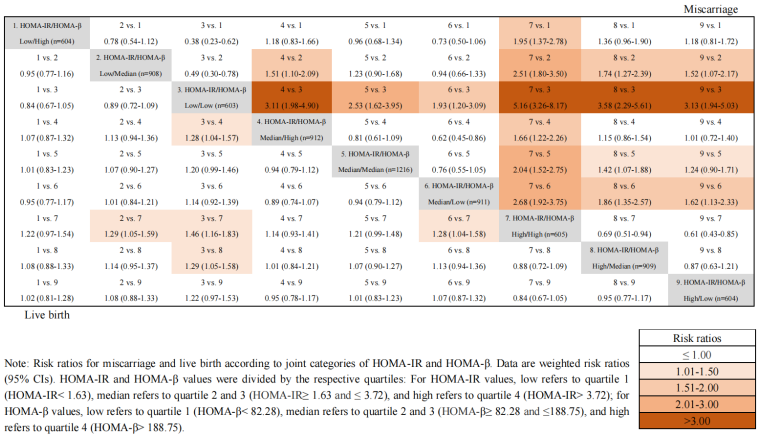

Note: Risk ratios for miscarriage and live birth according to joint categories of HOMA-IR and HOMA-β. Data are weighted risk ratios (95% CIs). HOMA-IR and HOMA-β values were divided by the respective quartiles: For HOMA-IR values, low refers to quartile 1 (HOMA-IR< 1.63), median refers to quartile 2 and 3 (HOMA-IR≥ 1.63 and ≤ 3.72), and high refers to quartile 4 (HOMA-IR> 3.72); for HOMA-β values, low refers to quartile 1 (HOMA-β< 82.28), median refers to quartile 2 and 3 (HOMA-β≥ 82.28 and ≤188.75), and high refers to quartile 4 (HOMA-β> 188.75).

FIGURE 2
Joint effects of HOMA-IR and HOMA-β on IVF outcomes. Odds ratios for miscarriage and live birth according to joint categories of HOMA-IR and HOMA-β. Data are weighted odds ratios (95% CIs). HOMA-IR and HOMA-β values were divided by the respective quartiles: For HOMA-IR values, low refers to quartile 1 (HOMA-IR< 1.63), median refers to quartile 2 and 3 (HOMA-IR ≥1.63 and ≤ 3.72), and high refers to quartile 4 (HOMA-IR> 3.72); for HOMA-β values, low refers to quartile 1 (HOMA-β < 82.28), median refers to quartile 2 and 3 (HOMA-β≥ 82.28 and ≤188.75), and high refers to quartile 4 (HOMA-β> 188.75).

effects on PCOS. The decrease in the HOMA-β values indicated a decrease in the sensitivity of human somatic cells to insulin receptors. Variation in β-cell capacity is attributed to the growth of the β-cell pool and insulin secretion ability (22). The balance between such a hyperdynamic β-cell pool and insulin resistance ensures a steady flow of nutrients in women with PCOS when attempting conception (23). The exacerbation of IR leads to growing demands for insulin secretion by pancreatic β-cells. However, a hyperbolic relationship exists between IR and insulin secretion, that is with continued deterioration of IR, the compensation in insulin secretion could be limited with continued deterioration of IR (24). The resultant unfavorable state uncovers potential defects of β-cell function, thereby precipitating the development of gestational diabetes or type 2 diabetes in women with PCOS (25). Previous studies have demonstrated that the variation trend of HOMA-IR and HOMA-β is consistent in women with PCOS (12, 26), which agree with our findings. Therefore, it could be indicated that PCOS women with β-cell dysfunction are still at higher risks of miscarriage and lower incidence of live birth even without IR.

Furthermore, considering the classic feedback loop between hyperinsulinemia and IR, our study further examined the joint effects of β-cell dysfunction and IR on the miscarriage and live birth in women with PCOS. Our results suggested that women with both high HOMA-β and high HOMA-IR values exhibited the highest odds ratios for miscarriage and the lowest odds ratios for live birth. Additionally, according to various combinations of HOMA-β and HOMA-IR, the association of a lower HOMA-β value with the odds ratio of miscarriage was strengthened by a high HOMA-IR value, indicating that although both insulin resistance and β-cell dysfunction were closely associated with miscarriage, the effect of IR overweighed that of β-cell dysfunction. A recent study indicated that women with PCOS and IR might result in a higher risk of miscarriage, but did not impair live birth rate (27). It has been reported that the variation of HOMA-β is hyperbolic in the progression of diabetes and is highly interfered by insulin resistance, which increases the difficulty of determining whether the value of HOMA-β represents the compensation stage or decompensation stage (28). In this study, we found that women with IR has significantly lower live birth rate, which is not in accordance with previous findings. Moreover, although the live birth rate were comparable between the β-cell dysfunction and non-β-cell dysfunction, women with both high HOMA-β and high HOMA-IR values exhibited the lowest odds ratios for live birth after interactive analysis. This result indicates that the negative association between IR and adverse reproductive outcomes seemed to be amplified with more vulnerable β-cell function in women with PCOS.

When compared with women with both low values of HOMA-IR (HOMA-IR< 1.63) and HOMA-β (HOMA-β<82.28), women with both insulin resistance (HOMA-IR>3.72) and β-cell dysfunction (HOMA-β>188.75) have approximately 5.16-fold higher miscarriage rate, and 1.46-fold lower live birth rate. Therefore, from a clinical point of view, it could be hypothesized that after appropriate pretreatment to lower HOMA-IR and HOMA-β, the reproductive outcomes in women with PCOS might have improved significantly. These insights shine a light on the application of a novel therapy for PCOS with insulin-sensitizing drugs. Metformin, an insulin-sensitizing drug, has been widely applied in the pretreatment of women with PCOS undergoing IVF treatment during the past decades (29). Metformin administration could reduce the risk of ovarian hyperstimulation syndrome and miscarriage in women with PCOS, which provides a new idea to improve the reproductive outcomes in PCOS women with insulin resistance and β-cell dysfunction (30).

Several potential mechanisms account for the negative effects of β-cell dysfunction as well as IR on reproductive outcomes in women with PCOS. First, the high insulin level in peripheral blood leads to the abnormal secretion of insulin-like growth factor (IGF-1) and IGF-2. IGF facilitates the implantation of the human embryo in the endometrium during IVF and plays an important role in trophoblast morphogenesis and placental microvasculature (31, 32). Second, studies on PCOS-like rats indicate that IR causes the

activation of ferroptosis in the gravid uterus and placenta. Furthermore, necroptosis and apoptosis might play a role in compensating or coordinating for IR-induced ferroptosis when the gravid uterine and placental dysfunction occur (33). In addition, hyperinsulinemia and IR can lead to increased secretion of reactive oxygen species, which further cause mitochondrial and placental dysfunction after pregnancy, thus increasing the risk of miscarriage and decreasing the live birth rate (34, 35).

To our knowledge, this study was unique in that we included women with PCOS undergoing their first IVF cycle and studied both the independent and combined effects of β-cell dysfunction and insulin resistance on IVF outcomes. The novelty of our study was the independent and joint effect of HOMA-β and HOMA-IR on reproductive effects. Considering that PCOS women with various combinations of β-cell dysfunction and IR might present different odds ratios for miscarriage, early screening and individualized intervention should thus be tailored. Therefore, even in PCOS women with mild hyperglycemia, glucose-lowering interventions before IVF could improve pregnancy outcomes. In addition, the calculation of HOMA-IR and HOMA-β is easy and has been widely acknowledged, which anticipates a highly practical value of our findings in the clinical practice. However, the present study had several limitations and should be taken into consideration. First, this is a retrospective study that looks back for a long span of time in which clinical practice, biochemical measurements, IVF treatments might have changed dramatically. In order to minimize the potential bias, we analyzed the subjects from Q1 to Q4 according to the tertiles of ovarian stimulation date in Table 1. We found that there were no significant differences in subjects from Q1 to Q4 in terms of the grouping of ovarian stimulation date in both Tables. However, prospectively designed multi-centers studies, follow-up of newborns and the effect of medical pretreatment on reproductive outcomes are still needed for further evaluation. Second, glucose clamp has been undeniably recognized as the gold standard for evaluating insulin metabolism. However, they may be perceived as invasive and expensive for use in clinical studies with large samples. Thus, surrogate markers, such as HOMA, have been proposed as alternative markers for insulin sensitivity and secretion, which can be repeatable and reproducible in the same way as gold standards. Although validation studies have indicated tight correlations between the HOMA models and gold-standard methods, the findings should be interpreted carefully. Third, although carefully adjusted for a set of confounders in the analysis, unmeasured confounders, such as dietary factors and physical activity, may affect the results to some extent. In addition, since the subjects in the present study were Chinese women, these conclusions might not be directly applied to populations of other ethnicities.

Conclusions

In summary, our findings indicate that β-cell dysfunction is independently associated with increased miscarriage rate and decreased live birth rate in women with PCOS. Furthermore, it also plays a synergistic role with IR in terms of the reproductive outcomes, while the influence of IR overweighs that of β-cell dysfunction. Therefore, early screening and interventions for β-cell dysfunction and IR in women with PCOS may be extremely helpful for improving conception opportunities.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by The First Affiliated Hospital of Wenzhou Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because all the data were deidentified in this retrospective study.

Author contributions

WH: Conceptualization, Formal Analysis, Methodology, Writing – original draft. CL: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. LD: Data curation, Writing – review & editing. YL: Data curation, Writing – original draft. HZ: Formal Analysis, Software, Writing – original draft. SW: Formal Analysis, Writing – review & editing. HY: Conceptualization, Funding acquisition, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewers YH and JZ shared parent affiliation with the AUTs CL, YL, HZ, HY to the handling editor at time of review.

Publisher's note

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Thyroid function and polycystic ovary syndrome: a Mendelian randomization study

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Background: Multiple evidence suggests that thyroid function is associated with polycystic ovary syndrome (PCOS), but whether thyroid function is causally related to PCOS is unclear. To investigate whether the association reflect causality, a Mendelian randomization (MR) analysis was conducted.

Methods: Single nucleotide polymorphisms (SNPs) involved in this study were acquired from The ThyroidOmics Consortium and the IEU Open Genome-wide association study (GWAS) database, respectively. In forward MR analysis, we included normal free thyroxine (FT4, n=49,269), normal thyroid-stimulating hormone (TSH, n=54,288), hypothyroidism (n=53,423) and hyperthyroidism (n=51,823) as exposure. The outcome was defined as PCOS in a sample size of 16,380,318 individuals. The exposure in the reverse MR analyses was chosen as PCOS, while the outcome consisted of the four phenotypes of thyroid function. The inverse-variance weighted (IVW) method was performed as the major analysis, supplemented by sensitivity analyses.

Results: The occurrence of PCOS was associated with increased risk of hyperthyroidism (IVW, OR=1.08, 95%CI=1.02-1.13, P=0.004). No evidence suggested that other phenotypes of thyroid function were related to PCOS.

Conclusions: Our findings demonstrate a cause-and-effect connection between PCOS and hyperthyroidism. The study established foundation for further investigation for interaction between thyroid function and PCOS.

KEYWORDS

Mendelian randomization, polycystic ovary syndrome, hyperthyroidism, hypothyroidism, free thyroxine, thyroid-stimulating hormone

Abbreviations: PCOS, polycystic ovary syndrome; MR, Mendelian randomization; SNP, single nucleotide polymorphisms; GWAS, genome-wide association study; IVW, inverse variance weighted; HT, Hashimoto's thyroiditis; FT4, free thyroxine; TSH, thyroid-stimulating hormone; IV, instrumental variables; LD, linkage disequilibrium; MREPRESSO, Mendelian Randomization Pleiotropy Residual Sum and Outlier; AIT, autoimmune thyroiditis; AITD, autoimmune thyroid disease; GD, Grave's disease; Th1, type 1 helper; Th2, type 2 helper.

1 Introduction

Polycystic ovary syndrome (PCOS) is one of the most common diseases in women of reproductive age, with an incidence as high as 15% worldwide according to the diagnostic criteria (1). PCOS is defined by 4 clinical indicators of reproductive abnormalities, including imbalances in sex hormones, excessive secretion of androgen, persistent lack of ovulation, and the presence of polycystic ovarian morphology (2). Persistent metabolic disorders will cause a series of diseases, such as infertility and type 2 diabetes (3). Studies have revealed that the susceptibility of PCOS is influenced by interaction of gene-environment, but it is still vital to elucidate the mechanism of pathogenesis of PCOS for improving the prevention and treatment of PCOS patients (4).

Thyroid hormone is crucial for the regulation of female hypothalamic-pituitary gonadal axis, thus the correlation between thyroid function and PCOS have been extensively studied. A prospective study indicated that the incidence of PCOS is higher in patients with Hashimoto's thyroiditis (HT) compared with people without HT (5). The study by Natalia Zeber-Lubecka et al. indicated that more mitochondrial variants were found in PCOS patients with HT compared to patients with HT only, which suggested that mitochondrial DNA genetic variants may play a crucial role in the joint occurrence of PCOS and HT (6). Another animal experiment showed that the number of follicular was lower in rats with hypothyroidism than that in control group and rats with hypothyroidism exhibited lower ovulation rate which were similar to chronic anovulation state in PCOS (7). Likewise, the changes of thyroid hormone in PCOS patients remains a subject of vivid debate. A large cohort study from Denmark confirmed that patients with PCOS had twice the risk of having hyperthyroidism compared to age-matched controls (8). However, multiple studies have shown that compared with hyperthyroidism, hypothyroidism was more common in PCOS patients (9–11). Yet there was also study which demonstrated that the risk of thyroid disease did not differ between patients with PCOS and controls (12). As observational studies are vulnerable to confounding, bias and reverse causality, it is still unclear whether the relationship between thyroid function and PCOS are causal or not.

Given the above, thyroid function is closely associated with PCOS therefore it is necessary to clarify whether the relationship involves causation. Mendelian randomization (MR) analysis, a strategy for investigating causation between different traits, is widely used to explore the casual correlation between an exposure and an outcome (9). In this study, we performed two-sample MR analysis to assess the potentially association between free thyroxine (FT4) as well as thyroid-stimulating hormone (TSH) in normal range, hypothyroidism and hyperthyroidism and PCOS. Furthermore, a reverse MR analysis was also conducted to determine the causal effect of PCOS on thyroid function.

2 Methods

2.1 Study design and data source

The flowchart of two-sample MR analyses in this study is shown in Figure 1. Samples from exposure and outcome are restricted to Europeans to avoid bias caused by population stratification.

Genome-wide association study (GWAS) data for PCOS was obtained from the IEU open GWAS database (ID: finn-b-E4_POCS), which contained 642 cases and 16379676 controls. GWAS data for FT4, TSH, hypothyroidism and hyperthyroidism were derived from the ThyroidOmics Consortium database. Of these, FT4 data included 19 cohorts with 49269 subjects, TSH data included 22 independent cohorts with 54288 subjects, 53423 subjects (3440 cases) for hypothyroidism and 51823 subjects (1850 cases) for hyperthyroidism (13).

Ethical approval is not sought as the datasets in this study are publicly available.

2.2 Genetic variants selection criterion

We chose single-nucleotide polymorphisms (SNPs) as instrumental variables (IV) in this study.

The legitimate IVs should meet the following assumptions: (1) they are closely associated with exposures; (2) they are independent

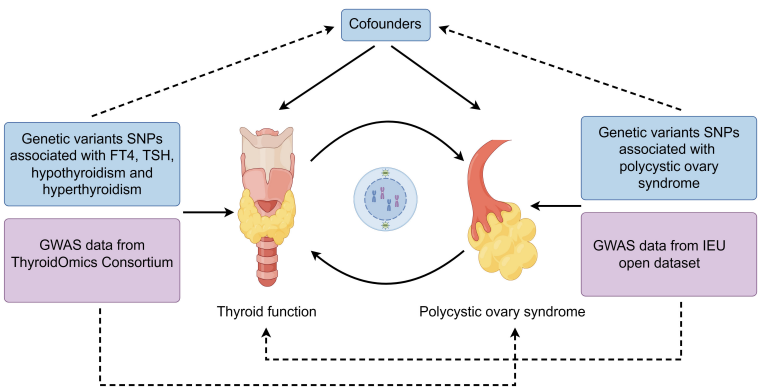

FIGURE 1
Flow chart of overall design in the present study.

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of cofounding factors; (3) they are solely related to outcomes through exposures. In forward MR analysis, the p value significance threshold of SNP was set to be 5×10^{-8}. Next, linkage disequilibrium (LD) pruning was conducted to remove linked SNPs (R^2<0.001, kb=10000). No proxy SNP was used in this MR analysis. To detect the strength of IVs, F-statistics was calculated by as $\beta^2_{exposure}/SE^2_{exposure}$ (14). SNPs with F-statistics smaller than 10 were removed. In addition, the data was harmonized by removing palindromic SNP to avoid accidental bias. In reverse MR analyses, SNPs with $P<5\times10^{-6}$ were considered significant. The following procedures was similar to the above one.

2.3 Statistical analysis

To estimate causal effects, inverse variance weighted method, MR Egger, weighted median, simple mode and weighted mode were used in this study. Among these, IVW method was the primary analysis. Then heterogeneity was evaluated by Cohran Q test. Mendelian Randomization Pleiotropy Residual Sum and Outlier (MRPRESSO) technique was utilized to detect and delete the SNPs with heterogeneity. Besides, we also used MR-Egger intercept and funnel plots to assess horizontal pleiotropy. Sensitivity analysis was carried out based on the leave-one-out method. Furthermore, p-values were corrected by Bonferroni adjustment (p-value/number of exposure). As a result, P 0.05/4 = 0.0125 was defined as significant. All analysis in this study were based on R version 4.2.3. The R package TwoSampleMR was used for all statistical analyses in this investigation.

3 Results

3.1 Thyroid function and PCOS: a causal link

The screening process of SNPs is shown in Supplementary Figure 1. After removing palindromic SNPs and outliers, we obtained 24, 40, 12 and 15 SNPs for FT4, TSH, hypothyroidism and hyperthyroidism, respectively. Supplementary Table 1 contains all SNPs selected as instrumental variables. As shown in Figure 2, IVW indicated that there was no causal association between thyroid function and the risk of PCOS [for FT4: odds ratio (OR) was 1.20 at 95% confidence interval (CI) of 0.81-1.79, P=0.368; for TSH: OR was 0.84 at 95% CI of 0.61-1.16, P=0.301; for hypothyroidism: OR was 1.06 at 95% CI of 0.87-1.28, P=0.559; for hyperthyroidism: OR was 1.09 at 95% CI of 0.93-1.29, P=0.282]. Likewise, similar results were observed in MR-Egger, weighted median, simple mode and weighted mode. The Cochran's Q test and MR Egger regression suggested no evidence of heterogeneity or horizontal pleiotropy (Supplementary Table 2). In addition, leave-one-out analysis and funnel plot and forest plot results are presented in Supplementary Figure 2.

3.2 PCOS and thyroid function: a causal link

In reverse MR analysis, we considered PCOS as an exposure and thyroid function as outcome. As a result of SNP filtering, 15, 8, 15 and

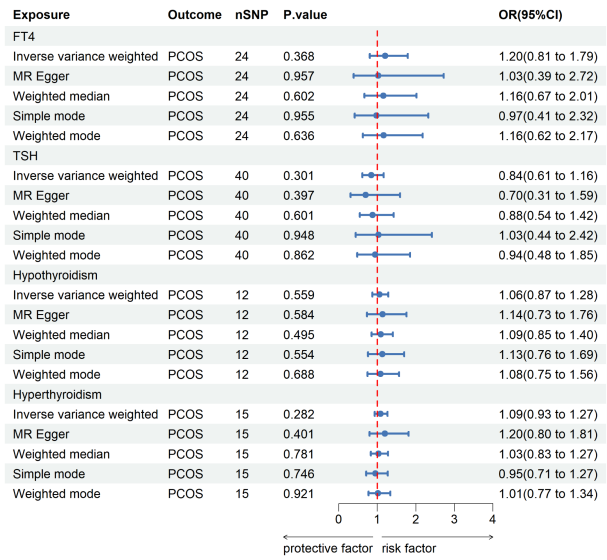

FIGURE 2
MR analysis for the causal effect of thyroid function on PCOS. IVW, inverse variance weighted; SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval; PCOS, polycystic ovary syndrome; TSH, thyroid-stimulating hormone; FT4, free thyroxine.

15 SNPs were included for FT4, TSH, hypothyroidism and hyperthyroidism, respectively, in final analysis (Supplementary Figure 3). Detailed information about SNP include for analysis are listed in Supplementary Table 1. According to Figure 3A, IVW findings suggested that the existence of PCOS was linked to a reduction in TSH levels (β=-0.019, 95%CI=0.035-0.003, P=0.023). Nevertheless, following the application of the Bonferroni correction, this discovery was not statistically significant. Furthermore, it was discovered that the presence of PCOS was linked to a higher likelihood of developing hyperthyroidism (IVW, OR=1.08, 95% CI=1.02-1.13, P=0.004). This association persisted after P value adjustment. The results of MR Egger and weighted median were consistent with IVW results although statistical significances were lost after Bonferroni correction (MR Egger: OR=1.08, 95%CI=1.01-1.16, P=0.044; weighted median: OR==1.07, 95%CI=1.01-1.14, P=0.022) (Figure 3B). No evidence of heterogeneity or horizontal pleiotropy were detected by Cochran Q test and MR Egger regression (Supplementary Table 2). Funnel plots and forest plots were provided in (Supplementary Figures 4A, B). Furthermore, no influential study was reported by leave-one-out analysis (Supplementary Figure S4C).

4 Discussion

Our two-sample MR study provided novel evidence for causal association between thyroid function and PCOS. In our study, we found that the genetic susceptibility pf PCOS was associated with the elevated risk of hyperthyroidism, while the susceptibility of hyperthyroidism was not related to PCOS. Besides, no forward or reverse causation was observed between PCOS and FT4, TSH and hypothyroidism.

The findings of our study were in line with previous study. That study compared the percentage of thyroid disease between patients

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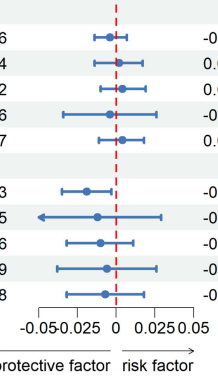
A

Exposure	Outcome	nSNP	P.value	β(95%CI)
PCOS				
Inverse variance weighted	FT4	15	0.506	-0.004(-0.014 to 0.007)
MR Egger	FT4	15	0.844	0.002(-0.014 to 0.017)
Weighted median	FT4	15	0.562	0.004(-0.010 to 0.019)
Simple mode	FT4	15	0.816	-0.004(-0.034 to 0.026)
Weighted mode (NOME)	FT4	15	0.617	0.004(-0.011 to 0.018)
PCOS				
Inverse variance weighted	TSH	8	0.023	-0.019(-0.035 to -0.003)
MR Egger	TSH	8	0.585	-0.012(-0.053 to 0.029)
Weighted median	TSH	8	0.356	-0.010(-0.032 to 0.011)
Simple mode	TSH	8	0.729	-0.006(-0.038 to 0.026)
Weighted mode (NOME)	TSH	8	0.608	-0.007(-0.032 to 0.018)



B

Exposure	Outcome	nSNP	P.value	OR(95%CI)
PCOS				
Inverse variance weighted	Hypothyroidism	15	0.093	1.03(0.99 to 1.07)
MR Egger	Hypothyroidism	15	0.284	1.03(0.98 to 1.09)
Weighted median	Hypothyroidism	15	0.392	1.02(0.97 to 1.08)
Simple mode	Hypothyroidism	15	0.843	0.99(0.92 to 1.07)
Weighted mode	Hypothyroidism	15	0.484	1.02(0.97 to 1.08)
PCOS				
Inverse variance weighted	Hyperthyroisdism	15	0.004	1.08(1.02 to 1.13)
MR Egger	Hyperthyroisdism	15	0.044	1.08(1.01 to 1.16)
Weighted median	Hyperthyroisdism	15	0.022	1.07(1.01 to 1.14)
Simple mode	Hyperthyroisdism	15	0.278	1.06(0.96 to 1.17)
Weighted mode	Hyperthyroisdism	15	0.028	1.07(1.01 to 1.13)

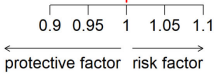


FIGURE 3
MR analyses for the causal effect of PCOS on thyroid function. **(A)**: Casual analyses of PCOS to FT4 and TSH. **(B)**: Casual analyses of PCOS to hypothyroidism and hyperthyroidism. IVW, inverse variance weighted; SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval; PCOS, polycystic ovary syndrome; TSH, thyroid-stimulating hormone; FT4, free thyroxine.

with PCOS (n=18476) and age-matched healthy controls (n=54757). As a result, the percentage of thyrotoxicosis in PCOS patients was markedly higher than that in the control group. The Cox proportional hazard model demonstrated an independent positive association between PCOS and hyperthyroidism (OR=1.7, 95%CI=1.1-2.3) (8). Additional study showed that no significant difference in thyroid disease between PCOA patients and control group. However, FT4 was elevated in PCOS group compared with control group after exclusion of patients with drug medication (12). While the effect of these drug in female hypothalamic-pituitary gonadal axis remains unknown.

However, some studies believe that a tendency towards a significant relation between PCOS and hypothyroidism. Three independent case-control studies from different countries indicated that patients with PCOS displayed a greater incidence of hypothyroidism compared to control groups (9–11). Besides, findings from another cohort study supported the established common mechanism between PCOS and autoimmune thyroiditis (AIT), which is the most common cause of hypothyroidism (15). These results are opposite to our data. Nevertheless, the study population in above results are from Pakistan, Iran, Saudi Arabia and China. So, the contradiction may be attributed to racial differences. Data from a Danish cohort study indicated that the incidence rate for thyrotoxicosis were 1.4 per 1000 patients for PCOS patients versus 0.5 per 1000 patients for patients without PCOS (8), which are consistent with our findings.

In addition, our study also revealed that PCOS had a stronger association with hyperthyroidism compared with hypothyroidism, which is in agreement with a previous study. Uma Sinha et al. found that the incidence of thyrotoxicosis was higher in PCOS patients compared to autoimmune thyroiditis (22.5% vs 2.5%) in a cross-section study in a rural population in India (16). There are two clinical manifestations of autoimmune thyroid disease (AITD): Grave's disease (GD) and HT, both characterized by the presence of circulating antibodies in blood and lymphocytic infiltration in thyroid parenchyma (17). The clinical hallmarkers of HT and GD are hypothyroidism and thyrotoxicosis, respectively. In HT, type 1 helper (Th1) lymphocyte-mediated autoimmunity induces thyroid cell lysis and eventually leads to hypothyroidism (18). While the major mechanism of GD is hyperthyroidism caused by humoral immunity mediated by type 2 helper (Th2) cells (19).

One of the characteristics of PCOS patients is excessive androgen production. It was shown that testosterone intervention given to rats were able to increase the level of FT4 (20). Another study proved that

female mice intervened by testosterone exhibited Th2-biased cytokine profiles, which suggested that androgen was capable of foster Th2-mediated-immune responses (21). This may be one of the mechanisms by which PCOS women are more likely to suffer from hyperthyroidism. In addition to that, a case-control study showed that high level of testosterone was associated with poorer performance of psychomotor speed and visual-spatial abilities in PCOS patients (22). Furthermore, it was reported that the level of testosterone was positively correlated with urine albumin-to-creatinine ratio in PCOS patients, and the follicular fluid extracted from PCOS patients with high level of serum testosterone could induce fibrotic lesion in tubular epithelial cell line (23). This reminds us that more attention should be paid to PCOS patients with higher level of testosterone to minimize complications.

Apart from autoimmune mechanism, thyroid disfunction and PCOS are closely related each other through a series of physiological metabolism. Insulin resistance is an important feature of PCOS patients (24). Study showed that insulin resistance was correlated with FT3 elevation in euthyroid subjects (25). Other study demonstrated that metformin treatment improved insulin resistance, modulated TSH level and reduced thyroid volume (26). However, the mechanism of crosstalk between thyroid function and PCOS is still unknown and needs to be further investigated.

In addition to that, our results could be interpreted from another perspective. Elena Vasyukova et al. examined the serum levels of cytokines in PCOS patients and age-matched females without PCOS. As a result, the serum concentration of CD40 in PCOS patients was significantly higher than that in controls (27). Multiple studies have shown that CD40 was significantly highly expressed in patients with hyperthyroidism and contributed to GD pathogenesis in several pathways (28, 29). On one hand, CD40 could present antigen and deliver costimulatory signals to T cells and induce the activation of T cells and ultimately enhancing the autoimmunity (30). On the other hand, overexpression of CD40 is able to activate downstream cytokines, such as IL6, which increase the production of thyroid-specific autoantibodies and promote hyperthyroidism (31). So, we speculate that overexpression of CD40 is an underlying mechanism that predispose PCOS patients to hyperthyroidism.

We have to admit that there are several limitations of our study. Firstly, due to the limitation of database, the association of other thyroid hormones such as FT3 with PCOS are not discussed in this study. Secondly, no distinction was made according to gender, so the bias caused by gender could not be avoided. Lastly, our study population was limited to European individuals, thus whether the results can be extended to other groups remains to be confirmed.

5 Conclusion

In conclusion, our study demonstrated that some SNPs predisposing to PCOS were associated with the development of hyperthyroidism. Further studies will be required to clarify the underlying mechanism between thyroid function and PCOS through large-scale randomized controlled trials and animal experiments.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

ZZ: Conceptualization, Writing – original draft, Writing – review & editing. YG: Data curation, Writing – review & editing. XP: Data curation, Formal Analysis, Writing – review & editing. WW: Data curation, Software, Writing – review & editing. RW: Software, Visualization, Writing – review & editing. HZ: Funding acquisition, Writing – original draft, Writing – review & editing, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1364157/full#supplementary-material

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Association between mild depressive states in polycystic ovary syndrome and an unhealthy lifestyle

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Objective: Polycystic Ovary Syndrome (PCOS) is a prevalent and frequently encountered gynecological disorder. Its high variability and the complexities associated with its management often lead to psychological stress in affected women, manifesting in symptoms of depression. Embracing a healthy lifestyle is fundamental in PCOS treatment. Consistent adherence to a healthy lifestyle not only aids in improving PCOS symptoms but also plays a role in enhancing mental well-being. However, there is currently limited research examining the extent of depression, its prevalence, and its correlation with lifestyle among individuals with PCOS. Therefore, this study aims to explore the impact of lifestyle factors on the depressive state of individuals with PCOS.

Methods: This cross-sectional study gathered data from 411 individuals with PCOS at a comprehensive hospital in Henan, China. Depression status was assessed using the Hamilton Depression Scale, and demographic information as well as lifestyle habits were simultaneously collected. Univariate and multivariate analyses using logistic regression were conducted to identify risk factors associated with the depressive state in PCOS.

Results: Among the surveyed 411 individuals with PCOS, approximately 49.4% exhibited symptoms of depression, with 83.7% experiencing mild depressive symptoms. A disease duration of 1–3 years, the presence of acne, and unhealthy lifestyle factors such as high-fat diet, staying up late, lack of exercise, and mental stress emerged as significant risk factors for the onset of depressive symptoms.

Conclusion: Depressive symptoms in individuals with PCOS are predominantly mild. The risk of comorbid depression in PCOS is associated with the presence of acne, frequent high-fat diet, regular staying up late, lack of exercise, and mental stress.

KEYWORDS

polycystic ovary syndrome, depressive symptoms, high-fat diet, staying up late, lack of exercise

1 Introduction

Polycystic Ovary Syndrome (PCOS) affects 5–21% of women and is a disorder characterized by reproductive dysfunction, metabolic disturbances, and psychological abnormalities. It exerts a significant impact on the entire lifespan of affected women (1). Compared to healthy women, individuals with PCOS experience a higher incidence of psychological issues and exhibit poorer self-recovery capabilities (2). The latest literature analysis indicates that the clinical prevalence of depression in women with PCOS is around 37% or even higher, surpassing that of healthy individuals by more than four times (3). Postpartum depression (4, 5) and the risk of neurodevelopmental and psychiatric disorders in offspring (6) are also significantly elevated in individuals with PCOS. PCOS, when accompanied by depression, persists throughout a woman's life and proves challenging to cure, imposing a substantial economic and psychological burden on both society and individuals. Therefore, current guidelines both domestically and internationally highlight the importance of conducting depression screening for individuals with PCOS at their initial medical consultations (7), underscoring the growing recognition of the psychological issues associated with this condition. However, there is no standardized treatment protocol, which may be attributed to the lack of clarity in reported levels of depression. Lifestyle adjustments and psychological counseling may contribute to the improvement of mild depressive states in individuals with PCOS, preventing further deterioration. For severe depression, intervention with antidepressant medications may be necessary.

The prevalence of PCOS accompanied by depressive symptoms varies across different countries and regions. The occurrence rate of depression in individuals with PCOS ranges from 37 to 52% (8). In conflict-ridden regions such as Syria and Jordan, the prevalence of depression in women with PCOS is reported to be over 65% or even higher (9). In a cross-sectional survey conducted in Pakistan, 80% of PCOS women screened positive for depression (10). Research in China has found varying rates of depression in individuals with PCOS, with an overall prevalence of 39% (11). In the North China region, the rate is reported to be 38% (12), while in Chengdu, the prevalence reaches as high as 51.8% (13). It is evident that the psychological well-being of individuals with PCOS is a subject of concern. However, the lack of specific descriptions regarding the severity of depression poses a significant challenge for the implementation of subsequent intervention methods. Early identification of depressive symptoms in individuals with PCOS and timely intervention can effectively prevent the onset of depression in these individuals.

PCOS is a highly heterogeneous disorder encompassing reproductive dysfunction, metabolic disturbances, and various clinical manifestations. Current treatments primarily focus on symptomatic relief, with a cornerstone being a healthy lifestyle, which proves to be the most fundamental and effective intervention. It not only aids in improving the psychological state but also becomes challenging to maintain due to the involvement of depression. This not only exacerbates clinical symptoms of PCOS but also intensifies the psychological burden, creating a vicious cycle between the two. Recent research has revealed a significant increase in the risk of binge eating among individuals with PCOS (14). Binge eating exacerbates metabolic disruptions in PCOS, leading to elevated levels of inflammatory factors, disturbances in gut microbiota, and triggering neuroinflammation. Consequently, this worsens the phenotypic expression of PCOS and increases the risk of depression. Prolonged periods of sedentary behavior and lack of physical activity contribute to weight gain and metabolic abnormalities in patients. Therefore, an unhealthy lifestyle may indeed heighten the risk of both PCOS and its associated depressive symptoms.

The First Affiliated Hospital of Henan University of Chinese Medicine is a tertiary comprehensive hospital in Henan, China. With an annual outpatient volume of up to 100,000 in the Obstetrics and Gynecology department, to some extent, it can reflect the prevalence of PCOS accompanied by depression in the local area. This study aims to investigate the prevalence and severity of depression in individuals with PCOS, exploring the relationship between lifestyle, general conditions, and depressive symptoms. A comprehensive understanding of the relationships between research factors contributes to the development of effective intervention strategies. The goal is to enhance the lifestyle of individuals with PCOS, reduce depressive states, and mitigate the occurrence of depression, ultimately improving the quality of life for individuals with PCOS.

2 Materials and methods

2.1 Study design

From January 2023 to June 2023, this cross-sectional study was conducted at the First Affiliated Hospital of Henan University of Chinese Medicine. The study utilized a simple random sampling method, focusing on individuals with PCOS attending the Obstetrics and Gynecology outpatient clinic as the research subjects.

2.2 Sample size calculation

Using the formula for calculating the overall rate in a cross-sectional survey.

$$n = \left(\frac{Z_{1-\alpha/2}}{\delta}\right)^2 \times p \times (1-p)$$

under 95% confidence level (thus $Z_{1-\alpha/2} = 1.96$), we set $p = 0.5$ (the most conservative method), error rate $\delta = 5\%$, and get $N = 384$,15% contingency was taken into account to increase sample size accuracy due to potential non- responses and missing data. The study ultimately completed surveys with 450 cases, ensuring the reliability of the investigation results.

2.3 Data collection

This study examined the basic demographic characteristics of the participants, including age, acne, hirsutism, family medical history, and lifestyle factors such as high-fat diet, exercise habits, and bedtime. Data were collected from patients visiting the outpatient department of the First Affiliated Hospital of Henan University of Chinese

Medicine who met the diagnostic criteria for PCOS. Information was obtained through the administration of a Chinese version of a paper questionnaire and interviews.

PCOS was diagnosed using the 2018 International Evidence-based Guideline criteria (7), which built on the consensus based 2003 Rotterdam criteria (15). This requires the presence of two of the following: (i) clinical/biochemical hyperandrogenism; (ii) ovulatory dysfunction; and (iii) polycystic ovaries on ultrasound. Exclusion of other etiologies.

The Hamilton Depression Scale-17 was employed to evaluate the depression status of individuals with PCOS (16). A score of ≤7 was interpreted as indicative of individuals with PCOS without depression, whereas a score exceeding 7 signified the presence of individuals with PCOS with depression. Based on the total score, the severity of depression can be classified as follows: Mild: 8-17,Moderate: 18-24,Severe: >24.

Considering that the prevalence of smoking and alcohol abuse among Chinese women is relatively low, lifestyle factors primarily focused on in the assessment include high-fat diet consumption, exercise habits, and late-night activities. A high-fat diet is characterized by a dietary pattern where a minimum of 40–45% of the total daily calories are derived from fat. Additionally, as per the Chinese Dietary Guidelines (17), adults are advised to limit their daily intake of cooking oil to 25–30 grams and trans-fatty acids to 2 grams or less. Common sources of high-fat foods encompass snacks, ice cream, animal fats, dark chocolate, butter, and fried foods. The survey questions focus on the frequency of consuming takeout, fried foods, cakes, ice cream, and snacks, specifically querying whether they are consumed more than three times per week, with responses being either yes or no.

Exercise recommendations for individuals with PCOS are derived from the "WHO Guidelines on Physical Activity and Sedentary Behavior 2020" (18) and the "Recommendations from the 2023 International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome" (19). It is suggested that individuals aim for 150–300 min of moderate-intensity exercise or 75–150 min of high-intensity aerobic exercise weekly, including activities like brisk walking, jogging, or cycling. Achieving this goal typically entails exercising for 40 min to 1 h per day, with sessions occurring more than 3 times per week. The survey includes questions regarding whether participants engage in a daily exercise routine of 1 h and whether they exercise more than 3 times a week, with responses being either yes or no.

Among humans, social factors are one of the most significant determinants of sleep duration. Due to the strong demand for social activities, ubiquitous artificial lighting, and the development of communication channels, sleep is often not solely determined by the physiological regulation of the sleep/wake cycle (20). Normally, the optimal time for sleep is between 10 pm and 6 am. Based on the characteristics of social jet lag and chronotype among the Chinese population, with morning types being the majority (21), we define staying up late as going to bed after 11 pm. We inquire about whether individuals go to bed after 11 pm and how often they exceed this time. If it occurs more than 3 times a week, it is considered as "yes"; otherwise, it is "no."

Perceived Stress refers to the cognitive process in which individuals perceive stress from external or internal stimuli. When individuals feel demands, challenges, or stress from the environment or events, they experience perceived stress. This process is subjective, perceptual, and relatively stable, with different individuals perceiving varying degrees of stress from the same event (22). Therefore, we utilize the Chinese Perceived Stress Scale (CPSS) to assess individual stress levels. Individuals rate the frequency of experiencing each item applicable to them over the past month, on a scale from 0 to 4. The total score ranges from 0 to 40. Based on the reference literature (23), we set stress levels between 0 and 13 as no stress, and above 13 as experiencing stress.

Currently, there is no standardized acne scale. We refer to the questionnaire from the literature (24) to determine the presence or absence of acne. We ask the following question: "Do you (or did you) have acne?" Your acne was diagnosed by: A dermatologist/Your general practitioner/Another physician or surgeon/Another healthcare professional (pharmacist, nurse, physiotherapist, midwife, naturopath, homeopathic practitioner, etc.)/Yourself (self-diagnosis). The response options are yes and no.

Hirsutism is assessed using the modified Ferriman-Gallwey (mFG) scoring system, where a score of ≥8 indicates hirsutism, while a score below 8 indicates the absence of hirsutism (25).

Social demographic characteristics include age (<20, 20–35, and > 35), education level (<9, 9–12, >12 years), duration of illness (<1 year, 1–3 years, >3 years), fertility requirements (yes, no), maternal or sibling history of PCOS (yes, no), and family history of mental illness (yes, no). BMI is calculated using participants' height and weight, which are collected by trained researchers using standard anthropometric measurements.

Prior to the commencement of the survey, a review of the questionnaire was conducted. All personnel involved in the survey received training to ensure consistency. The HAMD scale requires the patient to be jointly assessed by two trained evaluators. This assessment usually involves conversation and observation. Following the assessment, the two evaluators independently assign scores to the patient. All participants anonymously filled out the questionnaire with their informed consent. This study received approval from the Ethics Review Committee of the First Affiliated Hospital of Henan University of Chinese Medicine (Approval No: 2023HL-200).

2.4 Statistical analysis

The demographic details and clinical features of individuals with PCOS exhibiting depressive symptoms were outlined using frequency and percentage. The prevalence of depressive symptoms across various subgroups was presented based on their distinct characteristics. Univariate analysis was conducted using a chi-square test. The comparative variables included age, BMI, fertility demand, acne, hirsutism, family history, high-fat diet habits, exercise habits, frequency of staying up late, and mental stress exposure. Depressive symptoms served as the dependent variable, while statistically significant indicators from univariate analysis were considered independent variables. A binary logistic stepwise backward regression approach was utilized to identify potential risk factors. The multifactor regression model employed in this study primarily examined lifestyle factors such as frequent high-fat diet, infrequent exercise, regular staying up late, and mental stress. Statistical analysis was conducted

using IBM SPSS Statistics Version 26.0 (IBM SPSS Inc., Chicago, IL, United States). A significance level of $p < 0.05$ was applied to all analyses. Graphical presentation was prepared using GraphPad Prism software (version 9.0).

3 Results

3.1 Characteristics of the study subjects

A total of 450 individuals with PCOS were initially included in this study, with 411 individuals (91.3%) considered for further analysis. Among the surveyed patients, 68 (16.1%) were in the adolescent PCOS group, and 345 (83.9%) were in the reproductive-age PCOS group. The majority of participants were aged between 20 and 35, approximately 325 individuals (79.0%). Among them, 255 expressed a desire for fertility, accounting for 62.0%. There were 183 participants classified as obese, representing 44.5%, and 145 individuals with acne, constituting 35.3%. Additionally, 225 participants had dense hair growth, making up 54.7%. Furthermore, 28 individuals (4%) reported a family history of PCOS in their mothers or sisters. In terms of lifestyle, 190 participants (46.2%) reported a high-fat diet, 266 (64.7%) frequently stayed up late, 262 (63.7%) engaged in infrequent physical activity, and 356 (86.6%) experienced chronic stress (Table 1).

3.2 Basic clinical feature of individuals with PCOS with depressive symptoms

Among the 411 participants, a total of 203 individuals exhibited symptoms of depression, resulting in a prevalence rate of 49.4%. Among these, 170 individuals (83.7%) experienced mild depressive symptoms, 31 (15.3%) had moderate symptoms, and 2 (1%) were classified as having severe symptoms. In the majority of cases, individuals primarily exhibited slight impairments in mood and social functioning. Specific clinical characteristics are outlined in Table 2 and Supplementary Table S1.

3.3 Univariate analysis

The results of the univariate analysis indicate that there are statistically significant differences ($p < 0.05$) between the PCOS with depression group and individuals with PCOS without depression group in terms of disease duration, high-fat diet, frequent late-night activities, infrequent exercise, mental stress, acne, and a family history of mental disorders. The age, BMI, education duration, fertility requirements, family medical history of PCOS, and hirsutism showed no statistically significant differences between the PCOS with depression group and individuals with PCOS without depression group ($p > 0.05$; Table 3).

TABLE 1 Basic characteristics of survey subjects.

Variable	Total number of people	Percentage (%)	Variable	Total number of people	Percentage (%)
Age (year)			High-fat diet (>3x/week)		
<20	68	16.5	YES	190	46.2
20 ~ 35	325	79.1	NO	221	53.8
>35	18	4.4	Stay up late (>3x/week)		
BMI			YES	266	64.7
<18.5	44	10.7	NO	145	35.3
18.5 ~ 23.9	182	44.3	Frequency of exercise (week)		
≥24	185	45	never	201	48.9
Disease duration (year)			1 ~ 3	61	14.8
<1	52	12.6	>3	149	36.3
1 ~ 3	166	40.3	Mental stress		
>3	193	47.0	YES	356	86.6
Education experience (year)			NO	55	13.4
<9	24	5.8	Acne		
9 ~ 12	48	11.7	YES	145	35.3
>12	339	82.5	NO	266	64.7
Fertility demand			Hairy		
YES	255	62	YES	225	54.7
NO	156	38	NO	186	45.3
Mother or sister has PCOS			Family history of psychological disorder		
YES	28	6.8	YES	26	5.8
NO	383	93.2	NO	385	94.2

3.4 Multivariate analysis

Multivariate analysis revealed that high-fat diet, staying up late, mental stress, infrequent exercise, disease duration, and acne are all factors influencing the comorbidity of depression in individuals with PCOS. Frequent high-fat diet (frequent vs. infrequent: OR,1.873;95%CI,1.148 ~ 3.053), staying up late (frequent vs. infrequent:OR,2.357;95%CI,1.401 ~ 3.964), mental stress (yes vs. no: OR,6.549;95%CI,2.934 ~ 14.619), acne (present vs. absent: OR,1.791;95%CI,1.058 ~ 3.053), disease duration(1 ~ 3 year vs.<1 year: OR,8.258;95%CI,3.381 ~ 20.170), exercise (never vs. >3x/week: OR,7.496;95%CI,4.298 ~ 13.075). These factors are positively correlated with the incidence rate of comorbid depression in PCOS (Table 4; Figure 1).

TABLE 2 Basic clinical feature of depression patients.

Variable	Total number of people	Percentage (%)
No depression	208	50.6
Depression	203	49.4
Mild	170	83.7
Moderate	31	15.3
Severe	2	1

4 Discussion

PCOS is a prevalent reproductive endocrine disorder in women, primarily characterized by disturbances in reproductive function, metabolic irregularities, and psychological issues. In recent years, there has been significant research on reproductive and metabolic issues. However, the mechanisms underlying the comorbidity of depression in PCOS remain unclear. Additionally, the onset of depression in PCOS is often subtle, making it challenging to detect, and many individuals lack self-awareness (26).There is an issue of insufficient attention to the psychological and mental state of individuals with PCOS. Research indicates that the incidence of depression in individuals with PCOS ranges from 34 to 64%, and this rate shows an increasing trend over the years. Among them, reproductive-age women, particularly those between 20 and 35 years old, face a more significant risk of developing mental disorders (27). Reproductive-age women may experience a higher incidence of depression, possibly due to pressures related to childbirth, social and work-related stress. It is crucial to pay particular attention to the psychological well-being of this specific demographic.

In this study, the prevalence of depression in individuals with PCOS was found to be 49.4%, which is generally consistent with previous research. However, a noteworthy discovery in this study was that 83.7% of the individuals exhibited mild depressive symptoms, characterized by emotional distress without significant impact on social and occupational functioning. This suggested an increasing

TABLE 3 Results of univariate analysis (n = 411).

Variable	No depression n(%)	Depression n(%)	p-value	Variable	No depression n(%)	Depression n(%)	p-value
Age			0.112	High-fat diet (>3x/week)			<0.001
<20	42(61.8)	26(38.2)		NO	132(59.7)	89(40.3)	
20 ~ 35	156(48.0)	169(52.0)		YES	76(34.5)	144(65.5)	
>35	10(55.6)	8(44.4)		Stay up late (>3x/week)			<0.001
BMI			0.294	NO	102(70.3)	43(29.7)	
<18.5	23(51.1)	21(48.9)		YES	106(39.8)	160(60.2)	
18.5 ~ 23.9	101(54.9)	83(45.1)		Frequency of exercise (week)			<0.001
≥24	86(47.0)	97(53.0)		never	57(39.6)	144(60.4)	
Disease duration (year)			<0.001	1 ~ 3	41(67.2)	20(32.8)	
<1	41(78.8)	11(21.2)		>3	110(73.8)	39(26.2)	
1 ~ 3	62(37.3)	104(62.7)		Mental stress			<0.001
>3	105(54.4)	88(45.6)		NO	46(82.1)	10(17.9)	
Education experience (year)			0.355	YES	162(45.6)	193(54.4)	
<9	12(50.0)	12(50.0)		Acne			
9 ~ 12	29(60.4)	19(39.6)		NO	149(56.0)	117(44.0)	0.003
>12	167(49.3)	172(50.7)		YES	59(40.7)	86(59.3)	
Fertility demand			0.831	Hairy			0.56
NO	80(51.3)	76(48.7)		NO	97(52.2)	89(47.8)	
YES	128(50.2)	127(49.8)		YES	111(49.3)	114(50.7)	
Mother or sister has PCOS			0.102	Family history of psychological disorder			0.009
NO	198(51.7)	185(48.3)		NO	20(76.9)	6(23.1)	
YES	10(55.6)	8(44.4)		YES	188(48.8)	197(51.2)	

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TABLE 4 Results of multivariate analysis ($n = 411$).

	B	S.E.	Wald	*p*-value	OR	Lower limit	Upper limit
High-fat diet (>3x/week)	0.627	0.249	6.324	0.012	1.873	1.148	3.053
Stay up late (>3x/week)	0.857	0.265	10.447	0.001	2.357	1.401	3.964
Mental stress	1.879	0.410	21.038	0.001	6.549	2.934	14.619
Acne	0.583	0.269	4.709	0.03	1.791	1.058	3.032
Disease duration (year)							
1 ~ 3 vs.<1	2.111	0.456	21.470	0.001	8.258	3.381	20.170
>3vs.<1	0.810	0.432	3.518	0.061	2.248	0.964	5.240
Frequency of exercise (week)							
Never vs. > 3	2.014	0.284	50.377	0.001	7.496	4.298	13.075
1 ~ 3vs. >3	0.502	1.813	1.813	0.178	1.652	0.796	3.432

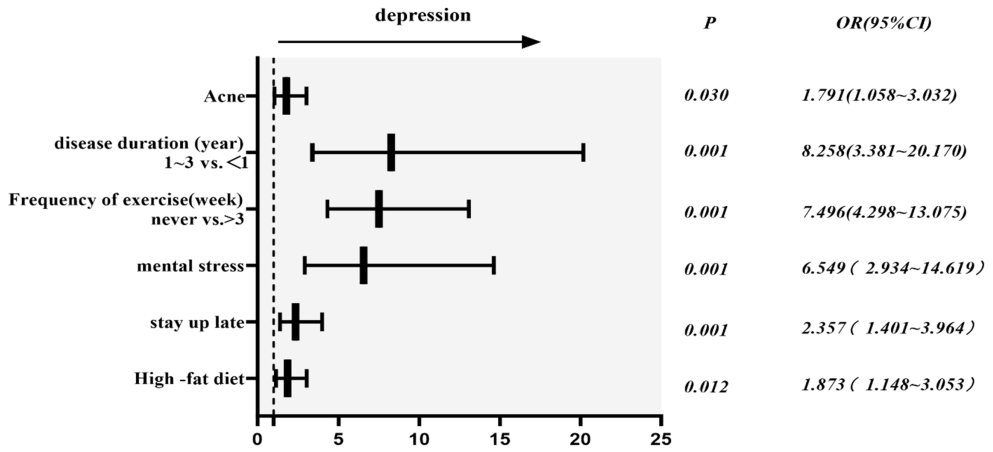

FIGURE 1
OR(95%CI) of risk factors.

awareness of psychological issues in individuals with PCOS, highlighting the possibility of an overestimation of the incidence of clinical depression.

In our investigation, both univariate and multivariate analyses indicated a positive correlation between unhealthy lifestyle habits and the incidence of depression in individuals with PCOS. Individuals with PCOS were influenced by various factors such as menstrual irregularities, infertility, and hormonal disturbances. Most of them exhibit unhealthy lifestyle habits, including lack of physical activity, staying up late, high-fat diet, and mental stress (28), consistent with previous literature reports. The majority of individuals with PCOS often face barriers to physical activity. The reasons may include insufficient awareness, denial of the therapeutic benefits of exercise, poor compliance, lack of time, or a lack of confidence in maintaining a regular exercise routine (29). Clinical studies have found that a combination of exercise and medication can reduce patients' weight, regulate female hormone levels, and decrease the severity of anxiety, depression, and other adverse emotional states (30). Additionally, both continuous and interval aerobic training have shown significant improvements in body image, anxiety, depression, and sexual dysfunction in individuals with PCOS (31). A recent meta-analysis (32) discovered a dose–response correlation between exercise and the likelihood of depression. Even participation in activities below the recommended threshold can yield substantial mental health advantages. Accumulating 2.5 h of brisk walking per week can decrease the risk of depression by 25% compared to those who are completely sedentary, while individuals engaging in half of this amount of activity can still lower their risk of depression by 18%. These connections can be elucidated by multiple mechanisms, such as the activation of acute neuroendocrine and inflammatory response pathways (such as the endocannabinoid system) during physical activity, as well as long-term adaptations, changes in neural brain structure, and factors like improved self-perception, body image, and increased social interaction. Animal experiments indicated that exercise enhances oxidative stress in PCOS rats, leading to changes in body composition and nutritional behavior (33).

Similarly, studies have found that poor sleep quality is positively correlated with depression scores in individuals with PCOS (34). The mental health status is closely related to sleep quality (35), and the occurrence of sleep disorders significantly increases in individuals with PCOS with depression (36). Sleep disorders are considered a risk factor for the onset and development of anxiety and depression (37).

Sleep chronotype significantly influences sleep quality. Evening types, in comparison to morning or intermediate types, often experience a variety of sleep-related issues, including more frequent nightmares, shorter sleep duration, increased use of sleep medication, and poorer overall sleep quality (38). Late chronotype individuals, if they wish to adapt to their own biological rhythm, will experience some misalignment between their daily schedule and the natural light–dark cycle. Meanwhile, the natural light–dark cycle also influences human metabolic regulation. Compared to late chronotype individuals, those with an early chronotype exhibit a reduced risk of depressive symptoms, with an odds ratio of 0.79 (95%CI: 0.77–0.81), and a decreased risk of depressive disorders, with an odds ratio of 0.84 (95% CI: 0.82–0.88). Early chronotype individuals have a lower risk of depression and anxiety compared to late chronotype individuals, and they also experience lower disease severity when such conditions arise (39). Existing research offers substantial empirical evidence elucidating the link between chronotype and mental health (40). For example, a comprehensive population study involving 10,503 Finnish adults revealed a significant correlation between evening chronotype and heightened levels of depressive symptoms or clinical diagnosis of depression (41). In summary, these findings affirm that having an evening chronotype may indeed serve as a risk factor for the onset of depression. This is consistent with the results of our study. Therefore, the management of sleep issues should be considered as part of the overall care.

Cheng et al. (42) found through a lifestyle survey of individuals with PCOS that the main risk factors for emotional disorders include poor sleep quality, late bedtime, and high stress levels. The main protective factors include a light diet and regular exercise. Health management for individuals with PCOS should focus on aspects such as diet, exercise, and sleep. Lifestyle interventions significantly improve patients' glucose and lipid metabolism, as well as their depressive and anxious conditions. They also contribute to the restoration of patients' independent menstrual cycles (43). However, individuals with PCOS face the risk of disrupted eating attitudes and behaviors, which could impact the implementation of lifestyle interventions. Therefore, clinical practitioners should pay attention to the dietary habits of individuals with PCOS, not just focusing on weight loss outcomes (44). A study discovered (45) that a high-fat diet (HFD) not only induces anxiety and anhedonia but also disrupts intracellular cascades related to synaptic plasticity, insulin signaling/glucose homeostasis (including Akt, extracellular signal-regulated kinase (ERK), and P70 S6 K). Furthermore, it leads to heightened corticosterone levels and triggers activation of the innate immune system, resulting in elevated inflammatory cytokines (such as IL-6, IL-1β, and TNFα), thus impacting the onset of depression.

Some studies suggest that the increased risk of depression in individuals with PCOS may be related to the condition itself, such as weight gain, infertility, elevated androgens, insulin resistance, and high cortisol levels (46). While the severity of depressive symptoms increases with BMI in PCOS women, those with underweight PCOS also have a risk of developing depressive disorders. Other studies have found that the level of negative mood inhibition is unrelated to BMI (47). Wang (48) found that although the detection rate of depression in obese individuals with PCOS is higher than in non-obese patients, the difference is not statistically significant. Multiple studies (49–51) have also confirmed that there is no significant correlation between PCOS with depression and BMI. The results of our study indicated that factors such as fertility needs, hirsutism, and genetic factors are not associated with depressive symptoms. This aligns with the findings of Lin (49), who concluded that emotional disorders in PCOS are not significantly correlated with factors such as marital status, fertility, BMI, waist circumference, waist-to-hip ratio, hirsutism, acne, and acanthosis nigricans.

Additionally, mental stress is a significant risk factor for the development of depressive symptoms. While our research findings reveal a wide confidence interval in stress assessment using the Perceived Stress Scale, this could be attributed to significant variations in individual stress perception under similar circumstances. Moreover, our study solely relied on self-report methods, omitting objective indicators like heart rate and blood pressure, which might introduce inherent biases to the findings. However, existing literature extensively discusses the correlation between mental stress and depression in PCOS. Compared to individuals without PCOS, individuals with PCOS exhibit increased levels of depression, anxiety, and perceived stress. Stress may play a role in the association between PCOS, depression, and anxiety (52). The increase in stress affects female reproductive endocrine function, exacerbates metabolic disturbances and reproductive disorders, worsens the clinical phenotype of PCOS, and induces depressive emotions (53). Serum cortisol, as a potential stress marker, has been found to be increased in individuals with PCOS (54). Under stress, abnormal activation of the HPA axis leads to the release of glucocorticoids, triggering the activation of hypothalamic microglial cells that release inflammatory cytokines such as IL-1β and IL-6. This interference with hypothalamic neural signaling contributes to reproductive, metabolic disruptions, and emotional disturbances (55). Stress can elevate the migration of monocytes to the cerebrovascular system, particularly to the nucleus accumbens (NAc), a region associated with the brain's reward center. These monocytes produce Matrix Metalloproteinase-8 (MMP-8), which plays a role in remodeling and regulating the extracellular matrix surrounding neurons in the brain. If MMP-8 leaks from the bloodstream into brain tissue, it can modify the matrix structure, consequently disrupting neuronal function. Mice subjected to this phenomenon display behavioral alterations similar to those observed in humans with depression (56).

In this study, we found that in addition to unhealthy lifestyle habits, acne is also a risk factor for depression in individuals with PCOS. Acne is a common clinical manifestation of hyperandrogenism in individuals with PCOS, which can affect women's body image and potentially lead to psychological burden. Jiskoot (50) found that PCOS is associated with widespread psychological changes, which are related to acne (51). A cross-sectional study conducted in France (24), involving 24,452 participants, revealed that individuals with acne exhibited a greater frequency of depressive symptoms compared to those who had never experienced acne, which is consistent with the findings of this study.

Furthermore, our research revealed that a duration of illness of 1–3 years was also a risk factor for depression among individuals with PCOS. However, there is a scarcity of prospective longitudinal studies examining the mental health of individuals with PCOS. Despite the proven weakening of symptoms associated with certain PCOS characteristics, such as irregular menstruation, acne, and hirsutism, as age increases (57), a longitudinal, population-based study conducted within

the Northern Finland Birth Cohort of 1966 revealed that the prevalence of depression remained unchanged among women reporting menstrual abnormalities and hirsutism at ages 31 and 46 (58). In a large, population-based, prospective cohort study involving black and white women, a 25-year follow-up observation revealed that women with PCOS experienced a heightened burden of depressive symptoms throughout their lifespan compared to their peers. However, the risk of depression gradually decreased as they aged (59). Although our study observed an elevated risk of depression among patients with a disease duration of 1–3 years, it is imperative to acknowledge the relatively wide confidence interval. Given the inherent limitations of a cross-sectional design, definitive evidence regarding the influence of medical history duration on depression status in PCOS patients is lacking. Consequently, the relationship between disease duration and depression status remains inconclusive, underscoring the need for additional research to provide more robust insights.

In conclusion, unhealthy lifestyles significantly influence the prevalence of depressive symptoms, and when multiple behaviors coexist, they often interact, exacerbating depressive symptoms (60). Our study further revealed that unhealthy lifestyles exert a significant influence on the depressive state of individuals with PCOS, underscoring the importance of lifestyle management for individuals with PCOS. This finding aligns with the recommendations outlined in the 2023 International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome (19). The guideline advocates for lifestyle interventions for all individuals diagnosed with PCOS, whether through exercise alone or a combination of diet, exercise, and behavioral strategies. These interventions aim to enhance metabolic health and address psychological concerns. Healthcare professionals should recognize that lifestyle management remains a central focus throughout the lifecycle of individuals with PCOS, emphasizing the importance of supporting healthy lifestyles.

5 Limitations and recommendations

This study investigated the degree of depression in individuals with PCOS and the influencing factors of related lifestyles. The findings highlight the significant prevalence of depressive symptoms in individuals with PCOS. Despite the higher incidence, most individuals experience mild depressive states, with slight impairments in work and social functioning. Early detection and intervention are crucial in preventing the progression of the condition. Depressive symptoms may impact the management and treatment adherence of PCOS. Therefore, efforts should be intensified to screen and address the mental health issues of individuals with PCOS (61). Women diagnosed with PCOS should not only receive standard medical care according to the guidelines but also comprehensive psychosocial and neurocognitive support to enhance their quality of life (62). However, this study has its limitations. Firstly, it is a cross-sectional study, which prevents making causal inferences. Secondly, this study is a single-center investigation predominantly focused on urban patients, limiting its generalizability. Future research should broaden the sample population to include individuals with PCOS from rural areas to provide sufficient information for the development of clinical intervention strategies. Thirdly, it is important to note that the sample size of this study is relatively small, and it solely concentrated on specific lifestyle factors such as high-fat diet, staying up late, and exercise habits. The study did not extensively investigate the specific patterns of each behavior in detail. For instance, concerning dietary habits, it solely focused on high-fat diet without taking into account the consumption of vegetables and fruits or regular eating patterns. Consequently, there might be some biases in the results. Fourthly, it is worth noting that the questionnaire employed a combination of interviews and self-reporting methods, and despite rigorous training, there remains a possibility of biases in the results. Fifthly, we utilized a stress perception assessment solely based on questionnaire forms, without incorporating physiological indicators like changes in blood pressure and heart rate, which could impact the generalizability of the study. However, this study also indicates that individuals with PCOS often experience mild depression, primarily characterized by depressive emotions and minor impairment in social and occupational functioning. Recognizing this can alleviate the psychological burden on patients, prevent unnecessary medical interventions, and prompt healthcare providers to focus on early psychological interventions for individuals with PCOS. Implementing effective measures to support patients in maintaining a healthy lifestyle is essential.

6 Conclusion

At a comprehensive hospital in China, the prevalence of depression among individuals with PCOS is 49.4%, with 83.7% exhibiting mild depressive symptoms. Unhealthy lifestyle factors emerged as the primary risk factors. These findings underscore the importance of heightened awareness among individuals with PCOS regarding the need for lifestyle adjustments. Furthermore, the majority of patients only manifest mild depressive emotions with slight impairment in work and social functions. This study alleviates the psychological burden on individuals with PCOS. Future research should delve into the potential causes of depression in individuals with PCOS and explore preventive measures to hinder the progression of depressive emotions into clinical depression.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Ethics Review Committee of the First Affiliated Hospital of Henan University of Chinese Medicine (Approval No: 2023HL-200). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LL: Investigation, Methodology, Writing – original draft, Writing – review & editing. ZK: Resources, Writing – review & editing. PC: Resources, Supervision, Writing – review & editing. BN: Investigation,

Writing – review & editing. YW: Data curation, Writing – review & editing. LY: Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1361962/full#supplementary-material

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Homeobox regulator Wilms Tumour 1 is displaced by androgen receptor at cis-regulatory elements in the endometrium of PCOS patients

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Decidualisation, the process whereby endometrial stromal cells undergo morphological and functional transformation in preparation for trophoblast invasion, is often disrupted in women with polycystic ovary syndrome (PCOS) resulting in complications with pregnancy and/or infertility. The transcription factor Wilms tumour suppressor 1 (WT1) is a key regulator of the decidualization process, which is reduced in patients with PCOS, a complex condition characterized by increased expression of androgen receptor in endometrial cells and high presence of circulating androgens. Using genome-wide chromatin immunoprecipitation approaches on primary human endometrial stromal cells, we identify key genes regulated by WT1 during decidualization, including homeobox transcription factors which are important for regulating cell differentiation. Furthermore, we found that AR in PCOS patients binds to the same DNA regions as WT1 in samples from healthy endometrium, suggesting dysregulation of genes important to decidualisation pathways in PCOS endometrium due to competitive binding between WT1 and AR. Integrating RNA-seq and H3K4me3 and H3K27ac ChIP-seq metadata with our WT1/AR data, we identified a number of key genes involved in immune response and angiogenesis pathways that are dysregulated in PCOS patients. This is likely due to epigenetic alterations at distal enhancer regions allowing AR to recruit cofactors such as MAGEA11, and demonstrates the consequences of AR disruption of WT1 in PCOS endometrium.

KEYWORDS

WT1, AR, transcription, epigenomics, endometrium, decidualization, polycystic ovary syndrome

Background

Successful pregnancy relies on a delicate interplay of molecular and hormonal signals that transform the endometrial environment, making it suitable for blastocyst implantation and setting the stage for foetal development. Decidualization, the extensive cellular and molecular remodelling of endometrial stromal cells, which transform from fibroblast-like cells into large polygonal cells rich in cytoplasmic glycogen and lipids, is critical to this process (1). The ovarian steroids, in particular progesterone (P4), play key roles in decidualization and blastocyst implantation (2), driving a complex array of molecular events that are mediated by genes including Indian hedgehog (IHH) (3), Wigless-type MMTV integration site family (WNT) 4 (4), forkhead box O1 (FOXO1) (5), homeobox A10 (HOXA10) (6) and the Wilms Tumour suppressor gene (WT1) (7–9). Loss of function in any of these critical components can lead to impaired function and subsequent infertility.

Polycystic ovary syndrome (PCOS) is a highly prevalent disorder accounting for up to 44% of unexplained infertility cases and contributing to 21% of infertility in couples with ovulatory dysfunction. PCOS presents with symptoms including menstrual disturbance, hyperandrogenism and infertility, and is polygenic in nature, involving more than twenty associated genes involved in processes including secretion, molecular function, and extracellular matrix formation (10), though a definitive aetiology is still absent. Hyperandrogenism is at the core of PCOS and evidence from animal models indicates specific roles for testosterone in its pathogenicity. In healthy human endometrial stromal cells (hESCs), androgen receptor (AR) is present at low levels and functionally active in decidualization (11). However, in PCOS patients AR levels are significantly elevated, and in combination with higher levels of circulating androgens results in reproductive abnormalities (12). Spontaneous PCOS-like traits are observed in hyperandrogenic female non-human primate models, and exposure to dihydrotestosterone or early development of testosterone produces PCOS-like traits (13, 14). Furthermore, RNA-seq studies have demonstrated that gene networks involved in AR signalling are disrupted in PCOS patients (12), implying AR-mediated contributions to the pathogenesis of PCOS.

Previously we have demonstrated that stromal cell restricted WT1 is present in the endometrium of healthy individuals during the window of implantation, and accumulates at higher levels during decidualization, but crucially is absent in PCOS patients (7). This loss of WT1 coincides with an increase in the levels of activated AR, as well as MAGEA11, an AR coregulator, in PCOS (15). Using chromatin immunoprecipitation sequencing (ChIP-seq) we describe that in fertile hESCs, WT1 binds to cis-regulatory regions of genes exhibiting differential expression between secretory and proliferative phases in fertile endometrium. In PCOS hESCs exposed to dihydroxy testosterone (DHT), AR binds to DNA locations bound by WT1 in fertile hESCs. Colocalization of AR and WT1 binding sites likely results in dysregulation of these pathways in PCOS.

Results

MET events and WT1 levels are perturbed in endometrial stromal cells isolated from PCOS patients

The transition of endometrial stromal cells from a mesenchymal to an epithelial phenotype (MET) is an essential prerequisite to blastocyst implantation (16, 17). hESCs were isolated from endometrial tissue biopsies and the response to a decidualization stimulus determined by monitoring the levels of prolactin. Decidualization was induced by incubating hESCs in the presence of cAMP (0.5mM) or cAMP (0.5 mM) plus MPA (1µM) for 48 h. *WT1* mRNA levels were significantly ($p < 0.05$) increased in hESCs isolated from fertile donors in response to cAMP +/- MPA, but not in endometrial PCOS cells (Figure 1A). Decidual prolactin (dPRL) levels were determined from samples cultured ex vivo and found to be increased in cells obtained from fertile

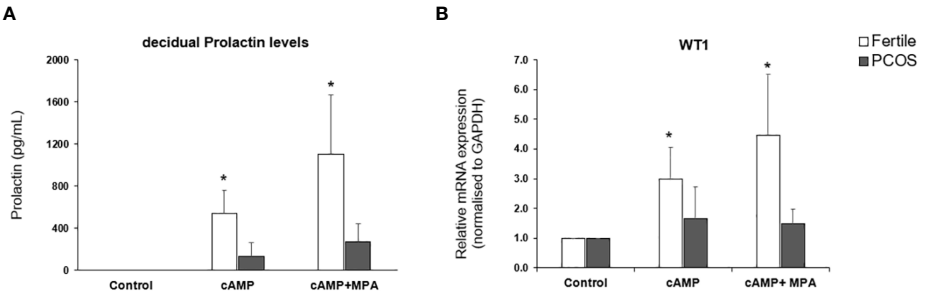

FIGURE 1
In vitro decidualization. Endometrial stromal cells were treated with medium or medium containing cAMP (0.5mM) or cAMP (0.5mM) and MPA (1 x 10-6 M) for 48hrs. Cells were lysed in RLT buffer before storage at -80°C and culture supernatant was collected and stored at -20°C. **(A)** decidual Prolactin levels of supernatant measured by ELISA in PCOS samples (n=10) vs fertile control (n=8). **(B)** Quantitative PCR for gene expression of WT1 mRNA normalised to GAPDH. Data presented as mean ± SD; for fertiles (n=8) and PCOS (n=7). Data was analysed by two-way ANOVA and Dunnett's pairwise multiple comparison test, *p ≤ 0.05.

individuals, whereas there was no response from PCOS derived stromal cells (Figure 1B).

WT1 is recruited across the genome including to the regulatory regions of HOX and FOX genes following stromal cell decidualization

Having established that stromal cells derived from fertile donors were functionally active, the genome-wide targets of WT1 were evaluated to determine how the 'gatekeeper' role that we have previously proposed may manifest itself (7).

We undertook the mapping of genome-wide WT1 DNA-binding (deposited as GSE240055 in the NCBI GEO repository) in hESCs obtained from fertile patients treated with cAMP (0.5 mM). This *de novo* data was analysed and identified 19,417 called peaks (regions bound by WT1) (FDR < 0.05) revealing that WT1 DNA binding occurs across the genome (Figure 2A). DNA binding site motif analysis on genomic sequences contained within the WT1 binding peaks was used to find any consensus binding sites. As expected, MEME-ChIP identified a motif (E-value = 9.1e-47) that mapped to the WT1 motif (E-value = 5.35e-6) (18) (Figure 2B). Interestingly a second highly significant motif (E-value = 1.5e-81) was identified that mapped to the ANDR (AR) motif (E-value = 3.76e-2) (Figure 2D). The observation that AR binding sites are found in the immediate proximity of a high proportion of WT1 binding sites suggested that the two transcription factors could colocalise or that competitive exclusion of WT by AR could occur.

A more in-depth analysis identified 34% of peaks located within gene promoter regions close to the transcription start site (TSS, Figure 2C), with the majority of WT1 binding (29% of peaks) occurring within 1kb of the TSS suggesting that WT1 plays a role in transcription regulation through interactions with the core transcription machinery that assembles at these proximal promoter locations. WT1 was also seen to bind extensively within gene bodies, primarily within intron 1 (13% of peaks) and other introns (17% of peaks) suggesting a potential role in intron retention (19). There was also extensive WT1 binding at distal intergenic regions (23% of peaks, as defined by ChIPseeker) suggesting WT1 may function at enhancer sites.

WT1 binding was found to occur in the promoter regions of a large number of FOX genes (20 from 21 FOX genes, P-value < 0.001, Figure 2E and Supplementary Table S1) and homeobox (HOX) genes (32 from 34 HOX genes, P-value < 0.001, Figure 2F, Supplementary Table S1). HOX genes are essential for endometrial development and endometrial receptivity (20), and loss of FOX gene regulation has been linked PCOS (21), which suggests that the 'gate keeper' function of WT1 could be in the regulation of forkhead and homeobox genes during decidualization. Unsurprisingly therefore, over-representation analysis (ORA) (22) revealed 'activation of HOX genes during differentiation' (FDR < 0.001) as a major process, as well as 'Estrogen dependent gene expression' (FDR < 0.001), and Gene Ontology (GO) (23) biological process pathways revealed 'Wnt signalling pathway' (FDR < 0.001), 'angiogenesis' (FDR< 0.01) and 'myeloid leukocyte cytokine production' (FDR < 0.1) as significantly enriched (Figure 2G). During decidualization, endometrial stromal cells undergo morphological and functional changes, becoming mesenchymal, to allow implantation of the embryo (17, 24). Progression into the secretory phase of the menstrual cycle is governed by increases in the expression of estrogen (25). Wnt signalling is implicated in a number of implantation and decidualization events during mammalian pregnancy, and aberrant Wnt signalling negatively effects these processes (26, 27). Angiogenesis occurs during the secretory phase of the menstrual cycle, forming new blood vessels to supply nutrients in the case of a potential implantation event (28, 29). Many studies have shown the importance of interactions between endometrial cells including hESCs and leukocytes to regulate the processes associated with decidualization (30–32) including vascular remodelling of the decidua and angiogenesis (33).

The promoter regions of a large number (5,341) of DNA coding regions for mRNA, miRNAs and lncRNAs were bound by WT1 in our hESC decidualization model. We sought to determine if this corresponded to differential gene regulation using comparative RNA sequence data analysis. We accessed publicly available transcriptomic data RNA-seq (NCBI GEO repository accession GEO86491) from endometrium samples obtained from fertile patients and identified genes that were differentially expressed during decidualization (34). Comparing differentially expressed genes (DEGs) with WT1 gene targets determined that 598 were significantly upregulated and 836 significantly downregulated during decidualization (FDR < 0.05) (Figure 2H, Supplementary Table S2). These genes included several WT1 HOX genes targets, which displayed significant differential expression between proliferative and secretory phases. HOXB2, HOXB4, HOXB5 and HOXB7 were significantly up-regulated and HOXC8, HOXD8, HOXD9, HOXD10 and HOXD11 were significantly down-regulated in the secretory phase compared with the proliferative phase, and FOXO3 was downregulated in the secretory phase (Figure 2I, Supplementary Table S1). Consistent with our analysis, HOXD10 and HOXD11 have previously been found to be downregulated in the proliferative phase of the menstrual cycle (20). Additionally artificial induction of decidualization in mice elicits an increase in FOXO3 expression that is more pronounced at the implantation site in mouse uteri (35).

AR is primarily located in enhancer regions in the genome of PCOS stromal cells

We determined the genome-wide localisation of AR (deposited as GSE240055 in the NCBI GEO repository) in hESC isolated from PCOS patients treated with DHT (15) to recapitulate events driven by hyperandrogenemia *in vivo*. Here we identified 12,017 significant AR peaks (FDR < 0.05%), of which only 5% were localised in gene promoter regions (Figures 3A, C), whereas 36% of peaks were located in distal intergenic (potential enhancer) regions and 51% were located in the first (16%) or other (35%) introns (Figure 3A). This suggests that the regulation of gene expression by AR in PCOS hESCs in response to elevated androgen levels is primarily via binding at putative enhancer sites, and that there could also be a role in

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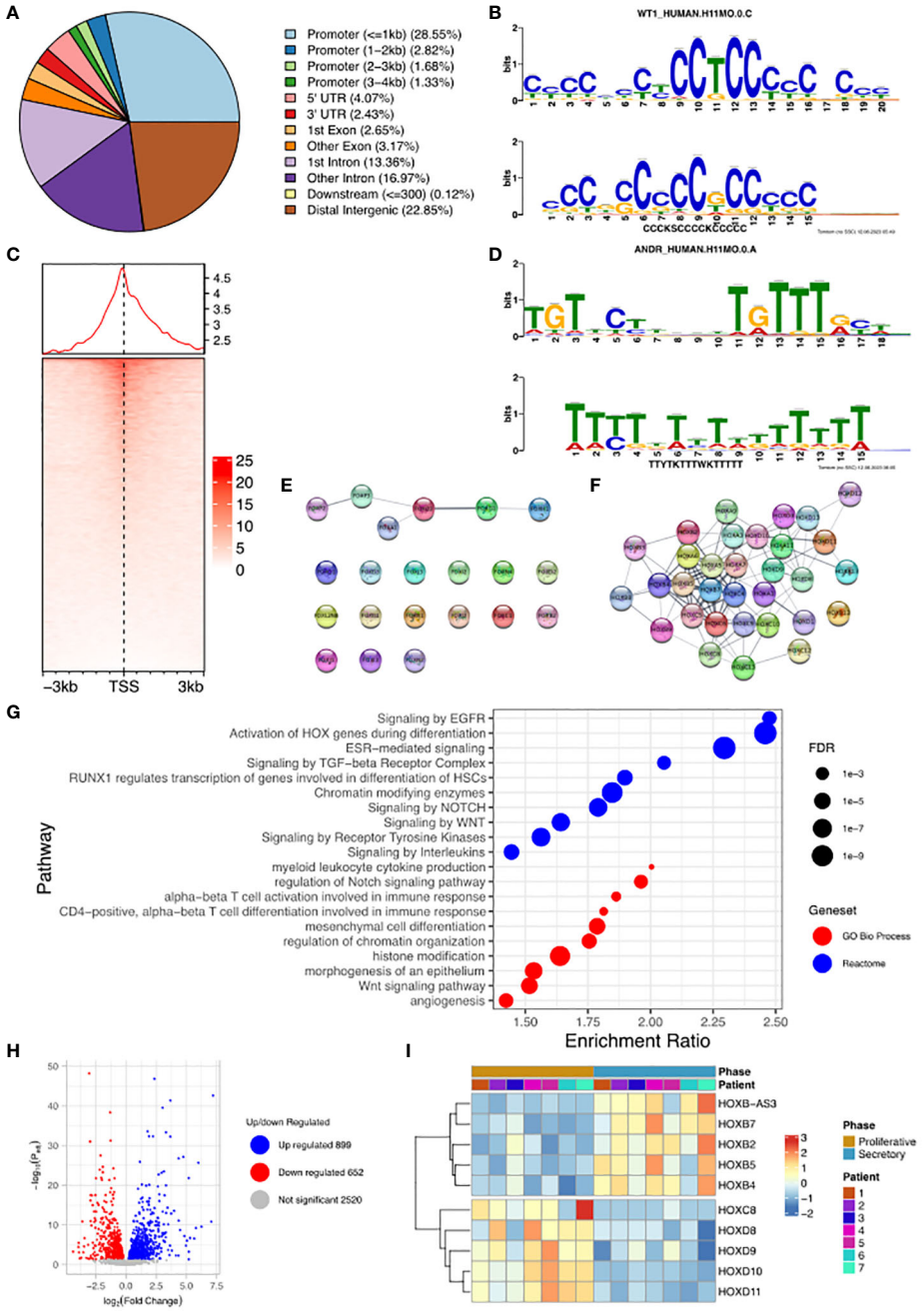

FIGURE 2
Analysis of WT1 binding in cAMP treated human endometrial stromal cells. **(A)** Percentage of WT1 peaks located in genomic feature types, **(B)** Binding MOTIF identified in WT1 binding regions which maps to WT1 binding motif (E = 5.35e-6), **(C)** Occupancy of WT1 binding peaks ±3kb around gene TSS's, **(D)** Binding MOTIF identified in WT1 binding regions which maps to AR binding motif (E = 3.76e-2), **(E)** Network diagram of FOX genes with WT1 peaks in their promoter region, with edges indicating STRING DB protein-protein interactions, **(F)** Network diagram of HOX genes with WT1 peaks in their promoter region with edges indicating STRING DB protein-protein interactions, **(G)** ORA analysis results identifying significantly upregulated pathways for genes with WT1 peaks in their promoter regions (4kb upstream and 1kb downstream of TSS) from GO biological process, GO Cellular Component and Reactome gene sets, **(H)** Differential expression of mRNA in hESC, comparing proliferative phase vs secretory phase for genes with WT1 peaks in promoter regions from publicly available RNA-seq data (GSE86491), **(I)** Expression level of HOX genes with WT1 peaks in promoter regions exhibit significant differential expression between proliferative and secretory phase of menstrual cycle for 7 patients. WT1 ChiP-seq data deposited as GSE240055 in the NCBI GEO repository. RNA-seq expression data retrieved from NCBI GEO repository, accession GSE86491.

intron retention (19) due to the other major proportion of AR binding being in introns. MOTIF analysis of DNA sequences within AR peaks identified a motif (E=2.7e-268) which mapped to both androgen receptor motif ANDR (E = 1.26e-2) (Figure 3B) and progesterone receptor motif PRGR (1.28e-6), and we have previously shown that the progesterone pathway is active in ovulatory PCOS patients (7).

The motif with second highest enrichment (E = 5.1e-89) mapped to the consensus binding site for JUN proteins (E = 2.89e-08) (Figure 3D), components of the AP-1 transcription

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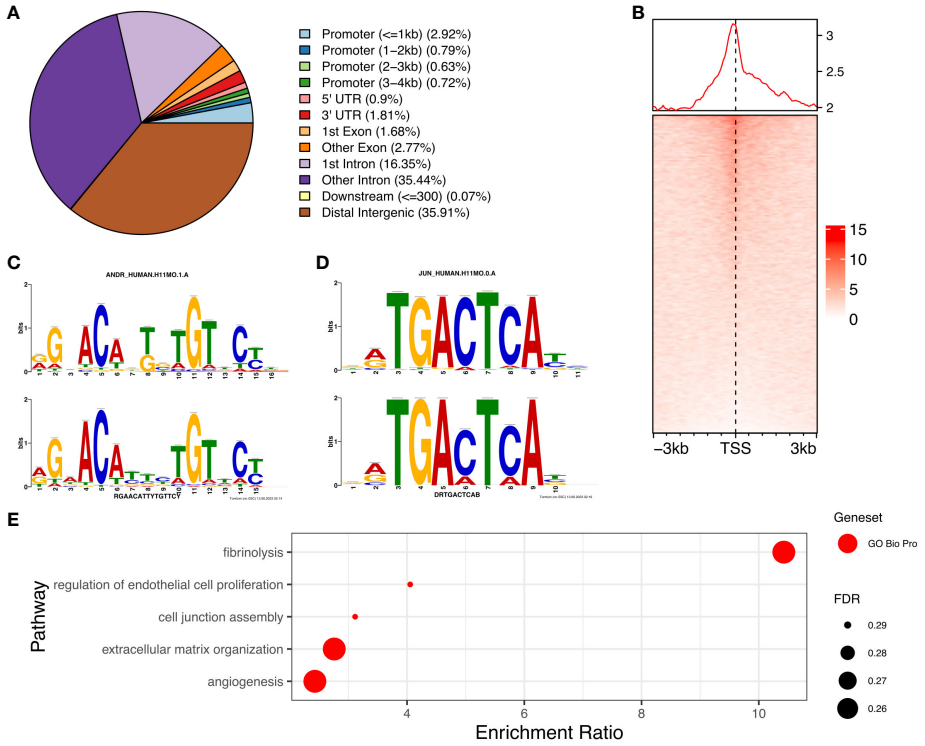

FIGURE 3
Analysis of AR binding in DHT treated human endometrial stromal cells. **(A)** Percentage of AR peaks located in genomic feature types, **(B)** Occupancy of AR binding peaks ±3kb around gene TSS, **(C)** Binding MOTIF identified in AR binding regions which maps to AR (E = 1.26e-2) and PRGR (E = 1.28e-2) binding motifs, **(D)** Binding MOTIF identified in AR binding regions which maps to JUN binding motif (E = 2.89e-8), **(E)** ORA analysis to identify significantly upregulated pathways for genes with AR peaks in their promoter regions (4kb upstream and 1kb downstream of TSS) from GO biological process, GO Cellular Component gene sets. AR ChiP-seq data deposited as GSE240055 in the NCBI GEO repository.

activator complex that are known enhance AR activity and stimulate cellular proliferation in prostate cancer cells (36). ORA analysis of genes with AR peaks in promoter regions using the GO biological processes gene set, identified enrichment of 'regulation of endothelial cell proliferation', 'extracellular matrix organization' and 'angiogenesis' (FDR < 0.3) (Figure 3E), pathways known to be associated with the decidualization process (25). Comparing genes with AR peaks in their promoter regions with DEGs between proliferative and secretory phases revealed 83 genes which are up-regulated and 66 genes which are down-regulated, suggesting a specific AR mediated gene set profile that is dysregulated in PCOS (Supplementary Figure S1).

WT1 and AR binding sites are co-located

The discovery of an enriched AR motif in the WT1 peak set suggested that WT1 and AR binding sites could be co-located across the genome (Figure 4A, Supplementary Figure S2). We therefore measured the distance between each AR peak to the nearest WT1 peak (Figure 4B). We found that 7.0% of AR ChIP peaks directly overlapped with WT1 peaks (P < 2.2e-16) suggesting that the two transcription factors could mutually exclude one another from binding. Merging the WT1 and AR peak sets and keeping only overlapping WT1/AR peaks identified in 826 merged peaks (including multiple overlaps i.e. two WT1 peaks overlapping a single AR peak were considered as a single merged peak). Only 13% of merged peaks were localised in gene promoter regions, corresponding to 106 peaks and 100 genes (considering promoters may contain multiple peaks) (Figure 4C, Supplementary Table S3) including 15 lncRNAs and 10 miRNAs (Supplementary Table S4). The remaining 741 (87%) of WT1/AR merged peaks were located outside gene promoter regions (Figure 4D), with 39% located in distal intergenic (potential enhancer) regions (Figure 4D).

ORA analysis of shared WT1/AR targets revealed enrichment of GO biological function, including 'blood vessel endothelial cell proliferation involved in sprouting angiogenesis', 'regulation of cytokine production involved in immune response', 'regulation of Notch signalling pathway' and 'positive regulation of angiogenesis' (Figure 4E). Examination of RNA levels for DEGs with WT1/AR merged peaks in promoter regions using the Sigurgeirsson data set revealed 20 significantly up-regulated and 15 significantly down-regulated DEGs (Figure 4F) (34). FKBP5, which had the greatest increase in differential expression, has been shown to regulate decidualization through Ser473 dephosphorylation of AKT (37), and its dysregulation in rats is associated with aberrant PGR-targeted gene expression (38).

STRING database analysis (39) was then used to identify protein/protein interactions of significantly differentially expressed candidate

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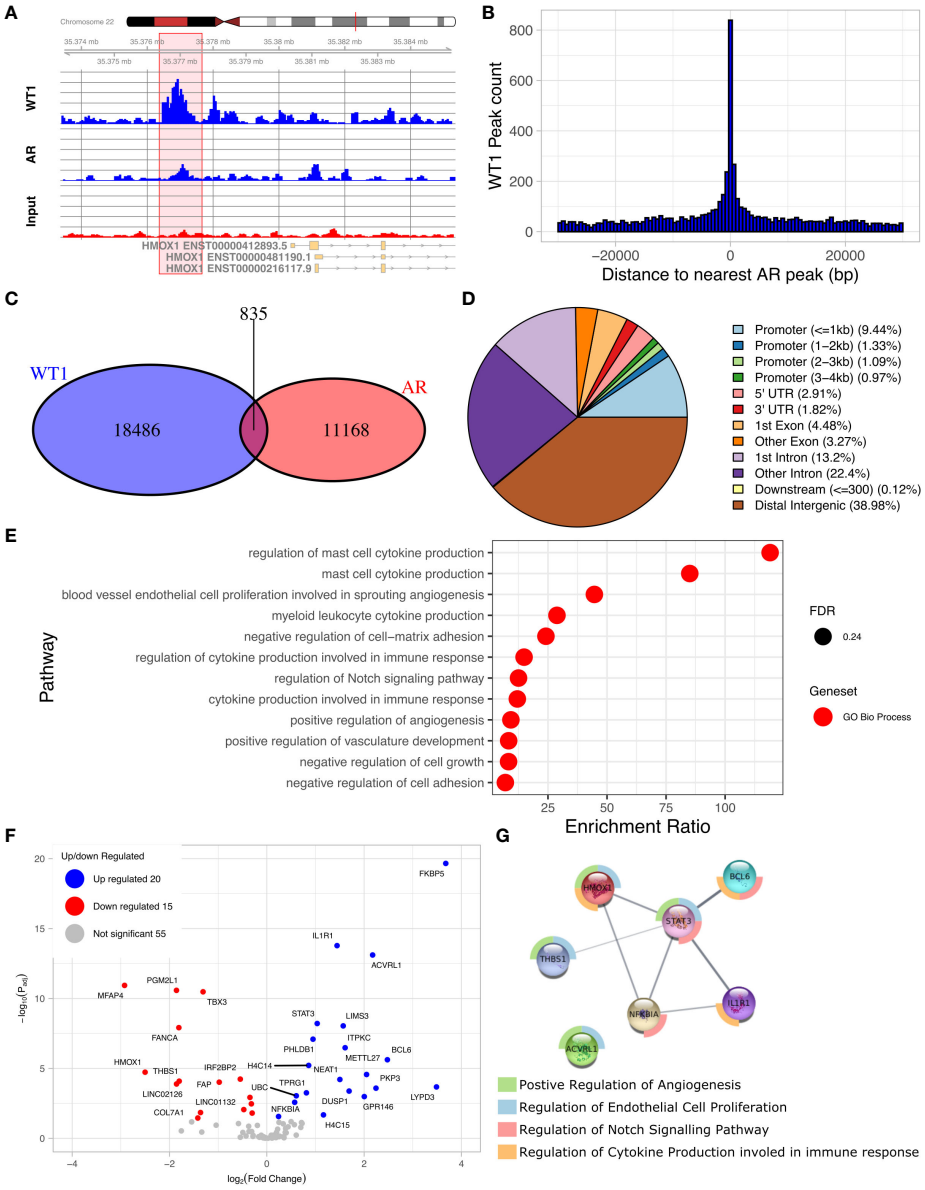

FIGURE 4
Overlaps of WT1 and AR ChIP-peaks in hESC treated with cAMP and DHT respectively. **(A)** Genome browser view showing co-located WT1 and AR ChIP peaks in the promoter region of HMOX1 gene, **(B)** Distance between WT1 peaks to the nearest AR peak, **(C)** Venn diagram showing number of WT1 peaks co-located with AR peaks, **(D)** Percentage of merged WT1/AR peaks located in genomic feature types, **(E)** ORA analysis to identify significantly upregulated pathways for genes with merged WT1/AR peaks in their promoter regions (4kb upstream and 1kb downstream of TSS) from GO biological process, GO Cellular Component and Reactome gene sets, **(F)** Differentially expressed genes (DEG) with merged WT1/AR peaks in promoter regions between proliferative and secretory phase of menstrual cycle, **(G)** Protein/protein interactions of selected DEGs with WT1/AR peaks in promoter regions between proliferative and secretory phase of the menstrual cycle and selected pathways which they are involved in. Outer ring colours indicate pathway. WT1 and AR ChIP-seq data deposited as GSE240055 in the NCBI GEO repository.

WT1/AR target genes, and using networks visualization we revealed that several of these DEGs were present in the GO biological function pathways identified above (Figures 4E, G). This detailed network analysis identified interactions between proteins encoded by STAT3, BCL6, ILR1, HMOX1, dysregulation of which has been implicated in diseases of the female reproductive system (see discussion). Together this reinforces the likely importance of these proteins in the decidualization process, that their dysregulation, due to aberrant AR/WT1 binding in PCOS patients, may affect implantation success.

Finally, we considered the involvement of candidate miRNA and lncRNA WT1/AR targets (Supplementary Table S4) in biological processes and identified pathways upregulated by miRNAs to again include 'Estrogen signalling pathway' (P = 0.0015), 'endometrial cancer' (P = 0.0012), 'Wnt signalling pathway' (P = 0.0195), 'Focal adhesion' (P = 0.0002), 'Adherens junction' (P = 1.11e-16) and 'FOXO signalling pathway' (P = 0.008) (Supplementary Figure S3). Gene products targeted by lncRNAs with merged WT1/AR peaks include HOXA9, IGF1R, WT1, BCL6, FOXO1 and HOXA16 (Supplementary Figure S4).

Co-Location of WT1 and AR corresponds with H3K4me3 and H3K27ac histone modifications in cis-regulatory elements

The proposed functional interplay between WT1 and AR occurs predominantly at distal intergenic regions, and therefore suggests a link to certain epigenetic marks that occur at gene enhancer sites. Enhancer regions are often located many 1000s of bp from gene TSS and are involved in the regulation of these genes through direct interactions with the core promoter via enhancer regions looping over to allow proteins recruited to that site to interact with the core transcription machinery (40). The histone marks H3K4me3 and H3K27ac are purported indicators of promoters and active enhancers respectively (41), with both marks enriched in regions of open chromatin, resulting in active gene transcription. To enable comparative meta-analysis between WT1 and AR binding events and the active histone marks, we exploited publicly available H3K4me3 and H3K27ac ChIP-seq data sets from endometrial stromal cells treated with cAMP and MPA to induce decidualization (GSE61793) (42). Of the 6,676 WT1 peaks located in gene promoter regions, 5,180 were co-located with H3K4me3 peaks (77.6%, P-value < 0.001 one-sided Fishers exact test) (see Figure 5A, Supplementary Table S5) indicating that WT1 binding is associated with active gene transcription in decidualization. Performing the same analysis on merged WT1/AR peaks, we found 59 were co-located with H3K4me3 peaks (63.2%, P-value < 0.001 one-sided Fishers exact test), (Figure 5A, Supplementary Table S5), further substantiating that any competitive binding between WT1 and AR in PCOS patients is likely to result in aberrant regulation of genes that are actively transcribed during decidualization. Performing ORA analysis on genes with both H3K4me3 and WT1 peaks within promoter regions identified a similar set of pathways as the previous analysis for genes with WT1 peaks in promoter regions. In contrast only 16.1% of WT1 peaks were located in H3K27ac enriched distal intergenic regions (P-value < 0.001 one-sided Fishers exact test) (Figure 5B, Supplementary Table S5), indicating that WT1 plays a limited function in regulating decidualization via these putative enhancers. From the WT1/AR merged peaks, 165 occupied H3K27ac enriched regions (22.9%, P-value < 0.001 one-sided Fishers exact test) (Figure 5B, Supplementary Table S4), implying that competitive binding of AR in WT1 binding regions affects putative enhancer activity in PCOS

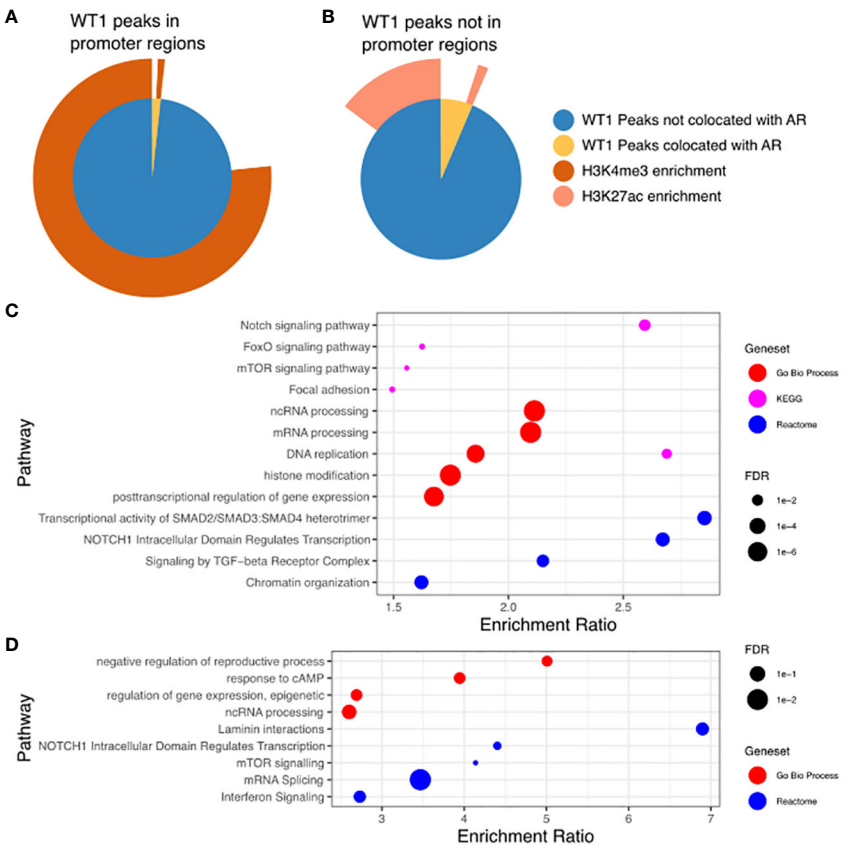

FIGURE 5
(A) WT1 peaks in gene promoter regions co-located with AR peaks and in H3K4me3 enriched regions. Inner pie chart shows peaks co-located with AR, outer ring shows WT1 and WT1/AR peaks in H3K4me3 enriched regions. **(B)** WT1 peaks outside of gene promoter regions co-located with AR peaks and in H3K27ac enriched regions. Inner pie chart shows peaks co-located with AR, outer ring shows WT1 and WT1/AR peaks in H3K27ac enriched regions. **(C)** Pathways enriched for genes regulated by enhancers containing WT1 peaks (gene/enhancer regulation annotations from GeneHancer database), **(D)** Pathways enriched for genes regulated by enhancers containing merged WT1/AR peaks (gene/enhancer regulation annotations from GeneHancer database). H3K27ac and H3K4me3 ChIP-seq data for decidualisation hESC from healthy human endometrium retrieved from NCBI GEO repository, accession GSE61793.

patients, resulting in aberrant gene regulation. Finally, we explored the GeneHancer database (43) to understand the biological functions regulated by WT1 and WT1/AR binding in distal intergenic regions and determined that 1,157 WT1 occupied locations (of the 4,437 sites identified in distal intergenic regions) were at defined enhancer locations known to regulate 4,117 unique genes (GeneHancer interaction score > 10), and potentially revealing 3,280 novel enhancer sites. Known enhancer sites corresponded to regulator regions for 'Notch signalling' (FDR < 0.01) and 'FoxO signalling' (FDR < 0.1) identified using pathway analysis (44) (Figure 5C), supporting direct ChiP-seq and comparative RNA-seq meta-analysis. Applying the same analysis to WT1/AR occupied distal intergenic regions, we identified a unique set of 153 annotated enhancers locations (from the 322 overlapping sites identified in distal intergenic regions), regulating 565 genes (GeneHancer interaction score > 10) that identified enriched pathways include 'response to cAMP' (P < 0.05) and 'interferon signalling' (P <0.001) (Figure 5D).

Discussion

WT1 is normally expressed in the endometrial stromal cells during the window of implantation, where it appears to have a central role as a 'gatekeeper' in the process of decidualization (7, 45–47). To understand the underlying nature of this gatekeeper effect we conducted genome-wide mapping of WT1 binding in primary hESCs treated to stimulate effective decidualization. Our analysis revealed that indeed WT1 regulates the expression of cascades of genes important in correct endometrial function, and that this regulation occurs through both proximal and distal control of gene expression, often associated with epigenetic modifications localised to WT1 binding. RNA-seq data revealed that a significant proportion of putative WT1 target genes are differentially expressed between the secretory and proliferative phases of the menstrual cycle, providing strong evidence that WT1 plays an important role in implantation and decidualization, prerequisites to successful implantation. Furthermore, comparison with H3K4me3 and H3K27ac ChIP-seq datasets highlighted that WT1 binds in regions of open chromatin, in both promoter and enhancer regions in hESCs during decidualization, supporting the hypothesis that WT1 is a central regulator of gene expression during the window of implantation.

WT1 was recruited to 32 of the 34 promoter sites of HOX genes in hESC, suggesting that WT1 is an important determinant in the regulation of the HOX gene family during decidualization. HOXC8, and HOXD8, 9, 10 and 11 were downregulated in secretory phase, and conversely HOXB2, 4, 5, 7 were upregulated, demonstrating the functional consequences of WT1 binding to the regulatory regions of these genes. HOX genes are essential regulators of morphogenesis which is essential for endometrial development and endometrial receptivity (20). Interestingly HOXC10, HOXC11, HOXD10 and HOXD11, considered functionally redundant paralogs of HOXA10 and HOXA11 in development pathways, have been shown to perform distinct functions from their paralogs during decidualization, and are downregulated in the proliferative phase of the menstrual cycle (20). WT1 binding was observed in 20 of the 21 FOX gene promoter regions. The FOXA proteins are pioneer factors facilitating the opening of chromatin landscapes and subsequent promotion of tissue-specific transcription factor recruitment thus regulating cell specification and cell identity (48). FOXA1 and FOXA2 have been shown to control recruitment of glucocorticoid receptor in uterine cells (49), and regulation of implantation and endometrial remodelling (50). Knockdown experiments in mouse uterine stromal cells suggest that FOXO3 plays a role in regulating decidualisation factors (MMP9, MMP2, BMP2) and apoptosis-related factors (PARP, Bax, Bcl-2, Fas). In vivo knockdown of FOXO3 in mice has been associated with dysregulated apoptosis and reduced embryo numbers during early pregnancy (35). Our observations suggest that during hESC decidualization these pioneer factors are themselves governed by WT1.

Crosstalk between WT1 and AR regulated pathways in PCOS patients

The loss of WT1 in PCOS patients coincides with an increase in the levels of activated AR (7, 15). Here we began to decipher how AR could dysregulate processes in PCOS patients that are regulated by WT1 in fertile women. We identified 106 promoter and 322 enhancers regions that contained overlapping WT1/AR peaks, and infer this is indicative of a competitive transcription factor binding between AR and WT1 within cis-regulatory regions in PCOS patients, governed by the relative abundance of AR within the cells. We identified a small number of putative WT1/AR regulated genes which exhibit significant differential expression between secretory and proliferative phases including FKBP5, which has the highest $\log_2$ fold-change. FKBP5 has been shown to regulate decidualization through Ser473 dephosphorylation of AKT (37), and its dysregulation in rats is associated with aberrant PR-targeted gene expression (38), which coincides with the recruitment to AR to PR sites in PCOS.

Network analysis of WT/AR targets identified interactions between STAT3, BCL6, ILR1, HMOX1, NFKBIA and THBS1; dysregulation of STAT3, BCL6, ILR1 and HMOX1 has been implicated in diseases of the female reproductive system, again demonstrating the validity of our approach. Ablation of STAT3 in murine models results in dysregulation of uterine epithelial remodelling, resulting in aberrant embryonic attachment (51), infertility (52) and implantation failure (53). BCL6 is involved in endometrial cell differentiation, migration, and invasion of trophoblastic cells (54). IL1R1 imbalance in ectopic endometrial tissue of women suffering from / with endometriosis results in heightened sensitivity to IL1 stimulation, which affects their ability to develop into host tissues for implantation (55). HMOX1 dysregulation has been implicated in PCOS (56), endometriosis (57), and *in-vitro* is down-regulated in endometrial stromal fibroblasts obtained from healthy patients in late secretory phase (58). Finally, PCOS patients exhibit significant differences in their immune cell population distribution and associated cytokine production, and display defective vascular remodelling of the endometrium (59). Our results indicate that WT1/AR crosstalk

may affect genes that regulate immune-related and vascular remodelling-related pathways, resulting in reduced fertility in PCOS patients.

Conclusion

The role of WT1 as a key regulator of decidualization is well known, however regulatory mechanisms have previously remained elusive. Here we show that WT1 regulates a large network of genes that are important for the functional and morphological changes in hESC cells. These genes include HOX and FOX transcription factors, which are known to regulate cellular differentiation.

Raised expression of circulating androgens and increased AR in hESC are key indicators of PCOS. Furthermore, WT1 expression in PCOS hESCs is reduced. Our study has shown a significant overlap between AR binding sites in PCOS hESCs and WT1 binding sites in fertile hESCs, revealing a mechanism which may result in irregularities in decidualization leading to symptoms commonly associated with PCOS such as infertility. Our results provide a rationale for development of strategies designed to re-introduce WT1 levels and/or reduce AR levels in endometrium of PCOS patients to re-establish regular decidual processes.

Methods

Stromal cell isolation

Primary hESCs were isolated from endometrial biopsies as previously described from patients of proven fertility and infertile patients diagnosed with anovulatory PCOS (47). Confluent hESC cells were washed twice in PBS and maintained in DMEM/F12 medium supplemented with 10% charcoal stripped media for 24 h prior to the start of the experiment. To induce decidualization, cells cultured in charcoal stripped media were treated with cAMP (0.5 mM), or cAMP (0.5 mM) and medroxyprogesterone acetate (MPA (1 µM) for 48 h. Decidualization was evaluated by analysing changes in cell morphology, and by measuring the secreted levels decidual PRL (dPRL) in the cell culture media, or with DHT (10^{-8} M), or DHT (10^{-8} M) + cAMP (0.5 mM) for 48 h to simulate PCOS conditions.

Measurement of secreted PRL in culture media by ELISA

Concentrations of secreted dPRL in cell culture media were measured using commercial ELISA kits according to the manufacturer's instructions (DY682; R&D systems). Measurements were performed in triplicate.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) was carried out as described previously following treatment with cAMP (0.5 mM) for fertile controls (15, 60). Following treatment, hESC cells were fixed using 1% formaldehyde solution (Sigma®), quenched with 2.5M Glycine (Sigma®) and centrifuged following Active Motif's Epigenetic Services ChIP Cell Fixation Protocol instructions. The pellet was then sent to Active Motif for sequencing. An anti-WT1 antibody (ab89901 Abcam, UK) and anti-androgen Receptor antibody (ab9474, Abcam, UK) was used to probe for WT1 and AR-target region enrichment respectively.

Analysis of ChIP-seq data

Fastq files were received from Active Motif®. Adaptor sequences were removed by Active Motif®. Prior to genome mapping, reads with more than 5 bases with Phred score less than 30 or reads containing undefined bases were removed (61). The sequencing quality of the remaining reads was determined using FASTQC (version 0.11.05, https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) (Supplementary Figure S5). Trimmed sequencing reads were then mapped against the reference genome (hg38, GRCh38) using Bowtie 2 (version 2.2.9, http://bowtie-bio.sourceforge.net/bowtie2/index.shtml) with default parameters (62). Normalized strand coefficient (NSC) and relative strand correlation (RSC), indicators of ChIP-seq experiment signal to noise ratio, were assessed using phantompeakqualtools (63) (Supplementary Figure S6). SAM files were converted to BAM files, sorted and indexed using SAMTools (64). Reads mapping to DAC consensus excluded regions were removed (65). Peak calling for WT1 and AR enrichment above the input control using MACS software (1.4.2) (66) with p-value cut-off 1e-5 (66).

Fastq files of H3K4me3 and H3K27ac ChIP-seq samples on human endometrial tissue samples treated with cAMP were downloaded from the NCBI Geo repository from dataset GSE61793 using the NCBI SRA-toolkit. Quality control and mapping to the genome were performed in the same way as with transcription factor ChIP-seq samples. Peak calling of H3K4me3 samples was performed using MACS2 software, with broad peak option, and qvalue cut-offs of 5e-2 and 1e-1 for narrow peak and broad peak regions respectively. Peak calling for H3K27ac samples was performed using epic2 (version 0.041, https://github.com/biocore-ntnu/epic2) with FDR cut-off of 0.05 (67). Bigwig files were generated from BAM files using Deeptools (version 3.3.1) (68).

P-values of overlapping peaks from WT1, AR and histone mark datasets were calculated using one-sided Fisher's exact test. For each pairwise comparison between peak sets, the genome was divided into equal length bins of 654 bp long (corresponding to the mean length of WT1 and AR peaks). A two-by-two contingency table was constructed with upper left containing the number of bins without any peaks, lower left containing the number of bins exclusively containing peaks from the first peak set, the upper right bin containing the number of bins exclusively containing peaks from the second peak set and the lower right cell containing the number of bins with peaks from both peak set 1 and peak set 1. P-values were calculated from the contingency table using the fisher.test R function (69).

RNA-seq analysis

RNA-seq raw count data, containing sequencing results from seven paired healthy endometrial tissue samples taken in proliferative and secretory phases of the menstrual cycle were downloaded from the NCBI GEO repository GSE86491 (34). Differential analysis was performed comparing mRNA expression in secretory to proliferative phases using DESeq2 (70).

Data analysis

Data analysis was performed using R (version 3.6.3) (69). Overlapping peak between samples were evaluated and merged peak sets were generated using the findOverlapsOfPeaks function in the ChIPPeakAnno (71) Bioconductor R package. Pairs of peaks defined as overlapping contained at least one common genomic coordinate. Peaks were assigned to genomic regions using the ChIPPeakAnno function assignChromosomeRegion. Genes were associated with peaks using the findOverlaps function in the GenomicRanges R Bioconductor package (72), to determine genes within 5000bp of peaks. Gene overlaps were assessed using the intersect function in R. Gene coordinates were obtained from EnsDb.Hsapiens.v86 R Bioconductor package (73). Enrichment heatmaps were created using the EnrichedHeatmap Bioconductor package (74). Genome browser plots were created using GViz R package (75). Venn diagrams were created using the ChIPPeakAnno function makeVennDiagram for overlapping peaks and the R VennDiagram package for overlapping genes. All other graphics were created using ggplot2 (76) in R. Motif analysis was performed using the MEME Suite MEME-ChIP web application (http://meme-suite.org/tools/meme-chip) (77). Multiple Expectation maximization for Motif Elicitation (MEME) (version 5.1.1) and Discriminative Regular Expression Motif Elicitation (DREME) software's (version 5.1.1) were used for motif discovery, with threshold of $E \leq 0.05$. HOCOMOCO Human (v11 CORE) input motif set was used. Over-representation analysis was performed using WebGestaltR R package (78). The 'BH' FDR method was used, with an FDR threshold of 0.05. ORA analysis was performed for GO Biological process, GO cellular component (23), and Reactome (79) gene sets. Network analysis to identify protein/protein interactions was performed using the STRING DB plugin (39) for Cytoscape (80). Enhancer locations and enhancer/gene regulatory relationships were obtained from the GeneHancer database (43). Genes and pathways associated with miRNAs were identified using DIANA-mirPath v.3 web application (81). Genes and pathways associated with lncRNAs were identified using LncSEA web application (82).

Gene expression analysis

Gene expression analysis was performed according to the MIQE guidelines (83). Total RNA was isolated from cells following lysis in RLT buffer using the RNeasy Mini kit (Qiagen, Manchester, UK), according to the manufacturer's instructions. Reverse transcription of 1 µg mRNA was performed in a 20 µl reaction volume using the High Capacity cDNA reverse transcription kit (Thermo Fisher), according to the manufacturer's instructions. Quantitative PCR primers were designed using the Primer-BLAST primer design software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and validated by BLAST analysis against the *Homo sapiens* (taxid:9606) Refseq mRNA database. Primers and *GAPDH* reference gene primers were obtained from Sigma–Aldrich: *WT1* forward, 5'-CTATTCGCAATCAGGGTTACAG-3', reverse, 5'-CATGCTTGAATGAGTGGTTGG-3'; *GAPDH* forward, 5'-GTCC ACTGGCGTCTTCAC-3', reverse, 5'-CTTGAGGCTGTTGTC ATACTT-3'. Quantitative PCR was performed in a 10 µl reaction volume comprising 1 × iTaq Universal SYBR Green Supermix (Bio-Rad) with primers added in nuclease-free water to a final concentration of 0.4 mM and 2 µl of cDNA. Thermal cycling parameters were as follows: one cycle of 95°C for 5 min, followed by 40 cycles of 95°C for 10 s and 60°C for 60 s. The relative quantification method was employed to quantify target gene mRNA within samples (84). To generate standard curves, total RNA extracted from cells was reverse transcribed to cDNA, as described. Ten-fold serial dilutions of this reference cDNA were prepared (neat to 1×10^{-3}) in nuclease-free water (Qiagen). For each sample, target and reference gene mRNA abundance was determined from the appropriate standard curve (quantification cycle, Cq). Changes in mRNA abundance between samples were then determined from the ratio of the target gene Cq to the reference gene Cq.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 22 with biological replicate as the experimental unit. Initially the data were tested for homogeneity, and log or square root transformed if appropriate. Parametric data were analysed by analysis of variance (ANOVA) using Dunnett's pairwise multiple comparison t-test for individual group comparisons. Data are presented as mean with standard deviation (SD), $p < 0.05$ was considered statistically significant, and the number of independent experiments is stated in the figure legends.

Statistical analysis of enrichment overlapping gene or peak sets was performed in R using the phyper function (which performs one-sided Fisher's exact test).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, GSE240055.

Ethics statement

Ethical approval for the collection of biopsy samples from fertile and PCOS patients used in this study was obtained from the HRA NHS Research Ethics Committee Wales REC 6 (LREC reference 05/WMW02/103 and 12/WA/0298). Written, informed consent was obtained from all patients prior to enrolment into the study. The studies were conducted in accordance with the local legislation and institutional requirements.

Author contributions

DJ: Formal analysis, Methodology, Writing – original draft, Writing – review & editing, Data curation, Investigation, Software. MQ: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. LL: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. NA: Writing – review & editing. GH: Data curation, Writing – original draft. NJ: Writing – review & editing, Data curation, Formal analysis. KY: Data curation, Resources, Writing – review & editing. AB: Resources, Writing – review & editing. LM: Conceptualization, Formal analysis, Resources, Writing – review & editing. LF: Conceptualization, Methodology, Supervision, Writing – review & editing. DG: Conceptualization, Methodology, Supervision, Writing – review & editing. RC: Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1368494/full#supplementary-material

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Live birth rate per fresh embryo transfer and cumulative live birth rate in patients with PCOS under the POSEIDON classification: a retrospective study

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Background: Ovarian stimulation (OS) for *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) in women with PCOS often results in multiple follicular development, yet some individuals experience poor or suboptimal responses. Limited data exist regarding the impact of poor/suboptimal ovarian response on pregnancy outcomes in women with PCOS.

Objectives: The aim of this study was to evaluate whether the live birth rate (LBR) per fresh embryo transfer and cumulative live birth rate (CLBR) per aspiration cycle differ in women with PCOS defined by the Patient-Oriented Strategy Encompassing IndividualizeD Oocyte Number (POSEIDON) criteria.

Methods: A retrospective study involving 2,377 women with PCOS who underwent their first IVF/ICSI cycle at Sun Yat-sen Memorial Hospital from January 2011 to December 2020 was used. Patients were categorized into four groups based on age, antral follicle count, and the number of oocytes retrieved, according to the POSEIDON criteria. The LBR and CLBR were compared among these groups. Logistic regression analysis was performed to assess whether the POSEIDON criteria served as independent risk factors and identify factors associated with POSEIDON.

Results: For patients <35 years old, there was no significant difference in the clinical pregnancy rate between POSEIDON and non-POSEIDON patients, whereas POSEIDON patients exhibited lower rates of implantation and live birth. POSEIDON Group 1a displayed lower rates of implantation, clinical pregnancy, and live birth. However, no significant differences were observed in the rates of clinical pregnancy and live birth between POSEIDON Group 1b and non-POSEIDON groups. For patients ≥35 years old, there were no significant differences in the rates of implantation, clinical pregnancy, and live birth between POSEIDON and non-POSEIDON patients. CLBRs were significantly lower in

POSEIDON Groups 1 and 2, compared with the non-POSEIDON groups. The levels of body mass index (BMI), follicle-stimulating hormone (FSH), and antral follicle count (AFC) were associated with POSEIDON hypo-response. POSEIDON was found to be associated with lower CLBR, but not LBR per fresh embryo transfer.

Conclusions: In patients with PCOS, an unexpected suboptimal response can achieve a fair LBR per fresh embryo transfer. However, CLBR per aspirated cycle in POSEIDON patients was lower than that of normal responders. BMI, basal FSH level, and AFC were independent factors associated with POSEIDON. Our study provides data for decision-making in women with PCOS after an unexpected poor/suboptimal response to OS.

KEYWORDS

polycystic ovarian syndrome, POSEIDON criteria, hypo-response, live birth rate, cumulative live birth rate

Highlights

In patients with PCOS, an unexpected suboptimal response can achieve a fair LBR per fresh embryo transfer. However, CLBR per aspirated cycle in POSEIDON patients was lower than that of normal responders.

Introduction

Polycystic ovarian syndrome (PCOS) is a heterogeneous endocrine disorder, which is characterized by ovulatory dysfunction, hyperandrogenism, and polycystic ovarian morphology (PCOM). Various factors including genetic, epigenetic, and environmental factors can influence PCOS (1–3). The prevalence of infertility in women with PCOS varies between 70% and 80% (4). *In vitro* fertilization (IVF) is the third-line treatment for infertility of women with PCOS (5). Challenges to IVF treatment in patients with PCOS include ovarian hyperstimulation syndrome (OHSS), impaired endothelial tolerance (6), altered oocyte competence (7), and increased risk of adverse pregnancy outcomes (8, 9). Adoption of an optimal ovarian stimulation (OS) protocol to overcome OHSS challenges is highly important (10–12). The starting dose of gonadotropins (Gn) is often calculated individually based on the patient's age, body mass index (BMI), and ovarian reserve (13). Previous studies have shown that the heterogeneity of PCOS disease affects ovarian response to Gn stimulation. Hyperandrogenism or excessively high anti-Mullerian hormone (AMH) require higher Gn doses (14, 15). These make it difficult to standardize optimal OS regimens. Patients and physicians encounter decision-making challenges when unexpected poor/suboptimal ovarian response is detected. This decreases the patient's expectation of success and increases the risk of cancelled oocyte retrieval and repeated IVF treatment.

The Patient-Oriented Strategy Encompassing IndividualizeD Oocyte Number (POSEIDON) criteria have refined the concept of poor ovarian response (POR) and allowed for the identification of infertile women with a low probability of pregnancy during IVF (16). Using this classification system, patients are grouped into four categories according to their age, ovarian reserve [antral follicle count (AFC) and/or AMH], and the number of oocytes retrieved from previous standard ovarian stimulation cycles: Group 1 includes patients who are under 35 years of age with adequate ovarian reserve; Group 2 comprises patients who are 35 years or older with adequate ovarian reserve; Group 3 consists of patients under 35 years with poor ovarian reserve; Group 4 includes patients who are 35 years or older with poor ovarian reserve (16).

Not all women with PCOS are high or normal responders to OS. Although not a high proportion, some women with PCOS fulfill the POSEIDON group, particularly for Groups 1 and 2. However, there are limited data available regarding the pregnancy outcomes of women with PCOS classified as poor responders (17, 18). There is no study to address LBR per fresh embryo transfer and CLBR per aspiration cycle for women with PCOS categorized as poor responders in fertility treatments.

This study aimed to assess LBR per fresh embryo transfer and CLBR per aspiration cycle, in women with PCOS categorized under POSEIDON and non-POSEIDON groups. Additionally, we wanted to investigate the potential variables associated with the occurrence of POSEIDON.

Materials and methods

Study population

This is a single-center retrospective study, which analyzes the records of women with PCOS who received IVF/intracytoplasmic

sperm injection (ICSI) treatment at Sun Yat-sen Memorial Hospital. The study included patients aged between 20 and 46 years old, who underwent first cycle from January 2011 to December 2020. Inclusion criteria were (i) PCOS diagnosis according to the Rotterdam criteria; (ii) treatment with a standard ovarian stimulation protocol [GnRH antagonist (GnRH-ant) protocol or long GnRH agonist (GnRH-a) protocol]; and (iii) oocyte retrieval and delivery of a live birth after fresh or frozen–thawed embryo transfer (ET/FET), or no live birth delivery after transferring all embryos (19). Exclusion criteria were patients undergoing preimplantation genetic testing, those having ovarian operation history, or those who were lost to follow-up. The study was approved by the Sun Yat-sen Memorial Hospital ethical review board (SYSKY-2023–200-02). The anonymity of the data waived the need for informed consent.

Clinical procedures

All participants received treatment with standard ovarian stimulation protocol using either the GnRH-a or the GnRH-ant protocol. Individualized doses of recombinant FSH or highly purified FSH were used based on ovarian reserve, age, and body weight, then doses were adjusted according to serum estradiol levels and follicular development. Oocyte maturation was induced via the administration of hCG, GnRH-a, or dual triggers. The retrieved oocytes were subjected to IVF or ICSI, and cultured reaching either Day 3 or the blastocyst stage. Embryo transfer was performed under the guidance of ultrasound and the remaining embryos were vitrified. Patients at risk of OHSS, with a premature progesterone elevation (i.e. >1.5 ng/mL), with endometrial polyps, and with a thin endometrium or intrauterine fluid used an embryo freezing strategy. Frozen embryo transfer was conducted in a natural cycle, an ovulation induction cycle, or a hormone replacement treatment cycle. Clinical pregnancy was confirmed by ultrasound. Luteal phase support was continued until 10–12 weeks of gestation for pregnant patients. Live birth delivery data were obtained by telephone interview.

Patient classification

Women with PCOS were classified into four groups, retrospectively according to the POSEIDON criteria as described below. AFC was used as the biomarker for classification since it was available in all patients, whereas AMH levels were only available in 60.29% of cases (n = 1,433). In this study, AFC ranged from 12 to 80, hence, all women with PCOS exhibited either high or normal ovarian reserve, implying that no POSEIDON Group 3 or POSEIDON Group 4 patients were identified. Patients who did not meet the POSEIDON criteria were categorized as a separate group, termed “non-POSEIDON”. The classification criteria were as follows:

I. POSEIDON Group 1 (Group 1): Comprising patients aged <35 years with AFC ≥5. Subgroup 1a included patients with <4 oocytes retrieved, and Subgroup 1b included patients with 4–9 oocytes retrieved.
II. POSEIDON Group 2 (Group 2): Comprising patients aged ≥35 years with AFC ≥5. Subgroup 2a included patients with <4 oocytes retrieved, and Subgroup 2b included patients with 4–9 oocytes retrieved.
III. Non-POSEIDON (Groups 3 and Group 4): Comprising patients with AFC ≥5 and >9 oocytes retrieved. Group 3 included patients aged <35 years old, and Group 4 included patients ≥35 years old.

Main outcome measures

The main aim of this study was to determine the LBR per fresh embryo transfer. The CLBR per aspiration cycle with at least one live birth was also calculated. The criteria for live birth were defined according to the guidelines established by the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) (19). A single or multiple delivery was recorded as one delivery.

Statistical analysis

Continuous data were tested using the Shapiro–Wilk method. All the continuous data were non-normally distribution and presented as median and 25%–75% interquartile range, which were analyzed using the Mann-Whitney U-test. Categorical data were described by the percentages and were analyzed using Pearson chi-square. A p-value of <0.05 was considered as a statistically significant difference. Logistic regression analysis was performed to identify clinically significant variables associated with POSEIDON, and to assess whether the POSEIDON criteria served as independent risk factors for affecting LBR and CLBR. The results were reported as odds ratio (OR) with 95% confidence interval (CI). Patient covariates did not include AMH due to a high frequency of missing values. Data analysis was performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA).

Results

This study included a total of 2,377 patients with PCOS, of whom 547 patients met the POSEIDON criteria. The POSEIDON patients were stratified into one of the low-prognosis groups: Group 1a (n = 44), Group 1b (n = 430), Group 2a (n = 6), and Group 2b (n = 67). Non-POSEIDON patients were further divided into two groups based on age: <35 years old (n = 1,618) and ≥35 years old (n = 212) (Supplementary Figure 1). Supplementary Figure 2 displays the distribution of patients in different groups across years. Patient characteristics, treatment parameters, fertilization rate, embryo development, and pregnancy outcomes were compared between the POSEIDON and non-POSEIDON groups within the same age stratum.

Baseline information of patients

The baseline characteristics of women with PCOS included in our study are displayed in Table 1. Among patients <35 years old, no significant difference was found in age, infertility type, duration of infertility duration, and basal LH level between POSEIDON Group 1 and the non-POSEIDON groups. However, POSEIDON Group 1 had a higher BMI and basal FSH level, as well as lower AMH and AFC compared to the non-POSEIDON groups. Among patients ≥35 years old, there were no significant differences in baseline characteristics between POSEIDON Group 2 and the non-POSEIDON groups.

Treatment characteristics

Table 2 presents the treatment characteristics. The initial Gn dose was similar between POSEIDON and non-POSEIDON groups. However, POSEIDON Group 1 had longer stimulation duration and higher total Gn dose compared to the non-POSEIDON groups. The proportion of ovarian stimulation protocols, including GnRH-a and GnRH-ant, was similar between the POSEIDON and non-POSEIDON groups.

Fertilization rate and embryo development

Fertilization rate and embryo development are presented in Table 3. The POSEIDON patients had an over 2-fold lower number of oocytes retrieved than their non-POSEIDON counterparts. The number of usable embryo and top embryo was lower in the POSEIDON groups than in the non-POSEIDON groups.

Pregnancy outcomes

Pregnancy outcomes are displayed in Table 4. The rate of fresh embryo transfer cancellation was significantly higher and more frozen embryo transfers were conducted in the non-POSEIDON groups compared with their POSEIDON counterparts. For patients <35 years old, the rates of implantation and live birth per fresh transfer were significantly lower in POSEIDON patients, whereas the clinical pregnancy rate had no significant difference. Supplementary Table 1 displays the pregnancy outcomes for Group 1a and Group 1b, which showed no significant differences of clinical pregnancy rate and live birth rate between POSEIDON Group 1b and the non-POSEIDON groups (60.1% vs. 63.2%, p = 0.276; 50.0% vs. 55.4%, p = 0.070). For patients ≥35 years old, there were no significant differences in the rates of implantation, clinical pregnancy, and live birth per fresh transfer between POSEIDON and non-POSEIDON patients, as well as Group 2a and Group 2b (Supplementary Table 2). For FET cycles, LBR per FET was significantly lower in Group 1 compared with the non-POSEIDON groups, but Group 2 did not reach statistical difference. The CLBRs in both age groups were lower in the POSEIDON groups compared to the non-POSEIDON groups (<35 years old: 56.5% vs. 81.0%, $p < 0.001$; ≥35 years old: 50.7% vs. 74.1%, $p < 0.001$). POSEIDON suboptimal responders (Group 1b: 58.6%; Group 2b: 52.2%) had higher CLBR than poor responders (Group 1a: 36.4%; Group 2a: 33.3%). Fresh embryo transfer was the most common conception mode to achieve a live birth delivery for POSEIDON patients in both age groups (75.7% for Group 1, 91.9% for Group 2).

Logistic regression analysis

Tables 5–7 showed the OR and adjusted OR (aOR) with their 95% CI for POSEIDON hypo-response, LBR per fresh embryo transfer, and CLBR per oocyte aspiration in patients with PCOS. BMI, basal FSH level, and AFC were significantly associated with POSEIDON hypo-response after adjusting for confounders (BMI: aOR 1.085, $p < 0.001$; basal FSH: aOR 1.095, $p = 0.001$; and AFC: aOR 0.988, $p = 0.033$). As for LBR, POSEIDON Group 1a was associated with a significantly lower LBR, even after adjusting for important confounders such as age, BMI, and the type of infertility. However, POSEIDON Group 1b, POSEIDON Group 2a, and POSEIDON Group 2b had no significant association with LBR. As for CLBR, both POSEIDON Group 1 and POSEIDON Group 2 were significantly associated with lower CLBR even after adjusting for important confounders (Group 1: OR 0.308, 95% CI 0.246–0.384, $p < 0.001$; and Group 2: OR 0.337, 95% CI 0.188–0.606, $p < 0.001$).

Discussion

Ovarian stimulation is an important first step in IVF treatments. OS aims to obtain a sufficient number of oocytes in one cycle to maximize the patient's pregnancy outcome. When considering the fresh embryo transfer LBR, optimal outcomes are achieved when retrieving between 6 and 15 oocytes (20, 21). When considering the cumulative LBR, studies have generally reported a positive trend where more oocytes result in a higher cumulative LBR (22, 23). However, when more oocytes are retrieved, the incidence of OHSS in the stimulation cycle increases considerably. Optimal OS strategies balance efficacy and safety. Following the ESHRE guidelines for OS, the Gn dose should not exceed 150 IU for high responders and should be initiated at a lower dose for those at risk (24). However, a low Gn dose leads to a risk of increased cancellation rate and decreased oocyte yield (25). In our study, starting doses below 150 IU accounted for 56% of cycles, and 23% had a poor or suboptimal response to OS. Among them, approximately 90% of patients exhibit a suboptimal response, with less than 10% of patients exhibiting a poor response. The mechanism why ovaries respond differently to Gn stimulation in women with PCOS is unclear. The heterogeneity of PCOS disease may be a possible explanation. The phenotype group (presence of both hyperandrogenism and chronic anovulation) tended to have higher doses of used Gn than the other PCOS phenotypes (14). Insulin resistance, a critical aspect of pathophysiology in patients with PCOS (26), may be associated with reduced ovarian sensitivity to exogenous Gn (27). More Gn were needed to obtain adequate

TABLE 1 Baseline characteristics of women with PCOS stratified according to the POSEIDON criteria and age.

	<35 years old					≥35 years old				
	POSEIDON			Non-POSEIDON	*p*1	POSEIDON			Non-POSEIDON	*p*2
	1a (*n* = 44)	1b (*n* = 430)	1 (*n* = 474)	3 (*n* = 1618)		2a (*n* = 6)	2b (*n* = 67)	2 (*n* = 73)	4 (*n* = 212)	
Female age (years)	29 (26, 31)	30 (27, 31)	29 (27, 31)	29 (27, 31)	0.086	37 (36, 37)	36 (35, 38)	36 (35, 38)	36 (35, 37)	0.728
BMI (kg/m^2)	23 (21, 26)	22 (20, 25)	22 (20, 25)	21.5 (19.5, 23.5)	<0.001	23 (22, 26)	24 (21, 27)	23.8 (21.5, 26.7)	23.6 (21.1, 25.9)	0.223
Infertility type % (*n*)										
Primary	65.9 (29/44)	61.2 (263/430)	61.6 (292/474)	60.3 (976/1,619)	0.631	33.3 (2/6)	47.8 (32/67)	46.6 (34/73)	38.7 (82/212)	0.270
Secondary	34.1 (15/44)	38.8 (167/430)	38.4 (182/478)	39.7 (643/1,619)		66.7 (4/6)	52.2 (35/67)	53.4 (39/73)	61.3 (130/212)	
Infertility duration (years)	4 (2, 6)	4 (2, 6)	4 (2,6)	4 (2, 5)	0.064	5 (2, 9)	6 (3, 8)	6 (3,9)	5 (3, 8)	0.813
Basal FSH (U/L)	7.1 (6.0, 8.4)	7.1 (6.1, 8.2)	7.1 (6.1, 8.1)	6.8 (5.8, 9.1)	<0.001	6.7 (5.4, 7.4)	6.7 (5.7, 7.8)	6.8 (5.7, 7.6)	6.7 (5.7, 7.5)	0.510
Basal LH (U/L)	7.0 (3.9, 10.5)	6.5 (4.5, 10.0)	6.6 (4.4, 9.9)	6 (4.2, 9.6)	0.769	6.0 (4.5, 13.8)	6.5 (4.0, 9.0)	6.0 (3.8, 9.8)	5.8 (4.1, 9.2)	0.788
AFC (*n*)	28 (24, 35)	27 (24, 33)	28 (24, 33)	29 (24, 35)	0.008	25 (24, 45)	27 (24, 31)	26 (24, 30)	28 (24, 33)	0.157
AMH (ng/mL)[#]	7.5 (4.9, 9.4)	8.0 (5.8, 11.5)	7.8 (5.8, 11)	9.2 (6.2, 12.7)	0.001	11.4 (2.3, 17.8)	6.1 (4.0, 8.9)	6.1 (4.0,9.2)	7.2 (5.1, 10.0)	0.086

Values are median and interquartile range and percentage. BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AFC, antral follicle count; AMH, anti-Müllerian hormone. p1, p-value between Group 1 (combined 1a+1b) and younger non-POSEIDON; p2, p-value between Group 2 (combined 2a+2b) and older non-POSEIDON. [#] based on data of 1,312 patients.

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TABLE 2 Treatment characteristics of women with PCOS stratified according to the POSEIDON criteria and age.

	<35 years old					≥35 years old				
	POSEIDON			Non-POSEID-ON	*p*1	POSEIDON			Non-POSEID-ON	*p*2
	1a (*n* = 44)	1b (*n* = 430)	1 (*n* = 474)	3 (*n* = 1618)		2a (*n* = 6)	2b (*n* = 67)	2 (*n* = 73)	4 (*n* = 212)	
Total Gn dose (IU)	1,706 (1,303, 2,381)	1,588 (1,200, 2,100)	1,600 (1,200, 2,100)	1,350 (1,100, 1,763)	<0.001	1,781 (1,462, 2,475)	1,800 (1,431, 2,438)	1,800 (1,463, 2,400)	1,700 (1,350, 2,400)	0.291
Initial Gn dose (IU)	113 (112, 150)	113 (100, 150)	112.5 (100, 150)	113 (100, 150)	0.990	150 (112, 175)	150 (112, 150)	150 (113, 150)	150 (113, 150)	0.642
Stimulation duration (days)	13 (10, 15)	11 (9, 13)	11 (9, 13)	10 (9, 12)	<0.001	11 (9, 15)	11 (9, 14)	11 (9, 14)	11 (9, 12)	0.176
Fertilization mode % (*n*)										
IVF	81.8 (36/44)	74.9 (322/430)	75.5 (358/474)	69.8 (1,129/1,618)	0.021	66.7 (4/6)	71.6 (48/67)	71.2 (52/73)	70.3 (149/212)	0.865
ICSI	18.2 (8/44)	11.6 (50/430)	12.2 (58/474)	13.1 (212/1,618)		33.3 (2/6)	10.4 (7/67)	12.3 (9/73)	10.8 (23/212)	
Half-ICSI	0	13.5 (58/430)	12.2 (58/474)	17.1 (277/1,618)		0	17.9 (12/67)	16.4 (12/73)	18.9 (40/212)	
Protocol % (*n*)										
GnRH-a	43.2 (19/44)	44.4 (191/430)	44.3 (210/474)	49.1 (795/1,618)	0.064	33.3 (2/6)	50.7 (34/67)	49.3 (36/73)	59.0 (125/212)	0.172
GnRH-ant	56.8 (25/44)	55.6 (239/430)	55.7 (264/474)	50.9 (823/1,618)		66.7 (4/6)	49.3 (33/67)	50.7 (37/73)	41.0 (87/212)	

Values are median and interquartile range and percentage. BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AFC, antral follicle count; AMH, anti-Müllerian hormone. p1, p-value between Group 1 (combined 1a+1b) and younger non-POSEIDON; p2, p-value between Group 2 (combined 2a+2b) and older non-POSEIDON.

oocytes in patients with PCOS with a higher BMI (28). High levels of AMH are highly correlated with PCOM and are a high-risk factor for developing OHSS. However, it has also been found that excessive AMH causes the ovaries to be resistant to Gn stimulation, requiring a higher Gn dose (15, 29). It was hypothesized that high concentrations of AMH would inhibit the action of FSH and thus be detrimental to the folliculogenesis process. Genetic variants in Gn and receptors may be another reason for differences in ovarian sensitivity. Polymorphisms of FSHR, as well as the variant of the β subunit of LH, have been associated with sensitivity to Gn (30, 31). In our study, we found that BMI, basal FSH level, and AFC may be potential influencing factors for POR in patients with PCOS. Further research is needed on the causes, risk factors, and predictors of unintended POR after OS in women with PCOS.

The POSEIDON classification system is anticipated to improve counseling and management of low-prognosis patients undergoing ART. Based on our limited search, this is the first study to focus on pregnancy outcomes in women with PCOS who have a poor/suboptimal response to OS. This study revealed that patients with PCOS categorized under the POSEIDON suboptimal response (Groups 1b and 2b) exhibited no statistically significant differences in the LBR per fresh embryo transfer compared to the non-POSEIDON groups (50.0% vs. 55.4%, $p = 0.079$; 51.6% vs. 57.4%, $p = 0.450$). However, patients meeting the POSEIDON poor response (Group 1a) had lower LBR per fresh embryo transfer when compared with the non-POSEIDON groups (37.8% vs. 55.4%, $p = 0.035$). Given that Group 2a included only four cases for fresh embryo transfer, the results of the statistical test comparing LBR to the non-POSEIDON groups were less meaningful due to the large standard error caused by the small sample size (25% vs. 57.4%, $p = 0.198$). This indicated that unexpected suboptimal response could achieve fair LBR per fresh embryo transfer in women with PCOS. However, all the POSEIDON groups (Groups 1a, 1b, 2a, and 2b) had lower CLBRs per aspirated IVF/ICSI cycle than normal responders. The CLBRs varied across different POSEIDON subgroups, with rates of 36.4%, 58.6%, 33.3%, and 52.2% for Group 1a, Group 1b, Group 2a, and Group 2b, respectively.

In our study, we found that in patients with PCOS, there were no statistically significant differences in the LBR per fresh embryo transfer between POSEIDON Group 1b and Group 2b compared with the non-POSEIDON groups. This indicated that only the quantity of oocytes was decreased, and not the quality, in unexpected suboptimal responders in women with PCOS. Our findings were consistent with

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TABLE 3 Fertilization rate and embryo development of women with PCOS stratified according to the POSEIDON criteria and age.

	<35 years old					≥35 years old				
	POSEIDON			Non-POSEIDON	p1	POSEIDON			Non-POSEIDON	p2
	1a (*n* = 44)	1b (*n* = 430)	1 (*n* = 474)	3 (*n* = 1618)		2a (*n* = 6)	2b (*n* = 67)	2 (*n* = 73)	4 (*n* = 212)	
Oocytes retrieved (*n*)	2.5 (2, 3)	7 (6, 9)	7 (5, 8)	17 (13, 22)	<0.001	2 (1.75, 3)	7 (6, 9)	7 (5, 8)	16 (13, 20)	<0.001
2PN zygote rate % (*n*)	88.3 (76/86)*	81.4 (1,942/2,386)*	81.6 (2,018/2,472)	78.9 (17,609/22,312)	0.002	80.0 (8/10)	73.2 (262/358)*	73.4 (270/368)	81.2 (2,230/2,747)	0.001
Useable embryo (*n*)	2 (1, 2)	3 (2, 5)	3 (2, 5)	7 (4, 10)	<0.001	1.5 (0, 2.25)	3 (2, 4)	3 (2, 4)	7 (4, 9)	<0.001
Useable embryo rate % (*n*)	88.2 (67/76)*	81.6 (1,585/1,942)*	81.9 (1,652/2,018)	59.5 (12,699/21,352)	<0.001	100 (8/8)	85.5 (224/262)*	85.9 (232/270)	67.4 (1,502/2,230)	<0.001
Top embryo (*n*)	0 (0, 1)	1 (0, 2)	1 (0, 2)	2 (0, 5)	<0.001	0.5 (0, 1.25)	1 (0, 1.5)	1 (0, 1)	2 (0, 4)	<0.001
Top embryo rate % (*n*)	41.8 (28/67)	42.7 (677/1,585)*	42.7 (705/1,652)	47.6 (6,040/12,696)	<0.001	62.5 (5/8)	38.4 (86/224)*	39.2 (91/232)	47.3 (711/1,502)	0.023

p1, p-value between Group 1 (combined 1a+1b) and younger non-POSEIDON; p2, p-value between Group 2 (combined 2a+2b) and older non-POSEIDON. *Compared with its non-POSEIDON counterpart in same age stratum and p<0.05.

TABLE 4 Pregnancy outcomes of women with PCOS stratified according to the POSEIDON criteria.

	<35 years old					≥35 years old				
	POSEIDON			Non-POSEIDON	p1	POSEIDON			Non-POSEIDON	p2
	1a (*n* = 44)	1b (*n* = 430)	1 (*n* = 474)	3 (*n* = 1618)		2a (*n* = 6)	2b (*n* = 67)	2 (*n* = 73)	4 (*n* = 212)	
	1a (*n* = 44)	1b (*n* = 430)	1 (*n* = 474)	3 (*n* = 1618)		2a (*n* = 6)	2b (*n* = 67)	2 (*n* = 73)	4 (*n* = 212)	
ET cancel rate % (*n*)	15.9 (7/44)*	12.1 (52/430)*	12.4 (50/474)	38.1 (617/1,618)	<0.001	33.3 (2/6)	4.5 (3/67)*	6.7 (5/75)	35.8 (76/212)	<0.001
Type of transfer										
Fresh	77.3 (34/44)	63.7 (274/430)	65.0 (308/474)	37.5 (607/1,618)	<0.001	50 (3/6)	77.6 (52/67)	75.3 (55/73)	42.0 (89/212)	<0.001
FET	2.3 (1/44)	10.2 (44/430)	9.5 (45/474)	39.5 (639/1,618)		0	3.0 (2/67)	2.7 (2/73)	34.9 (74/212)	
Fresh + FET	6.8 (3/44)	24.2 (104/430)	22.6 (107/474)	22.4 (362/1,618)		16.7 (1/6)	17.9 (12/67)	17.8 (13/73)	22.2 (47/212)	
No transfer	13.6 (6/44)	1.9 (8/430)	3.0 (14/474)	0.6 (10/1,618)		33.3 (2/6)	1.5 (1/67)	4.1 (3/73)	0.9 (2/212)	
Fresh cycles (*n* = 1,620)	*n* = 37	*n* = 378	*n* = 415	*n* = 1001		*n* = 4	*n* = 64	*n* = 68	*n* = 136	
Embryo transferred (*n*)	2 (1,2)*	2 (2,2)*	2 (2,2)	2 (0,2)	0.682	1 (0,1.3)*	2 (2,3)*	2 (2,2)	2 (0,2)	0.242
Blastocyte transferred (%)	0*	2.1 (8/378)*	1.9 (8/415)	10.5 (105/1,001)	<0.001	0	6.3 (4/64)*	5.9 (4/68)	15.4 (21/136)	0.068

(Continued)

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TABLE 4 Continued

	<35 years old					≥35 years old				
	POSEIDON			Non-POSEIDON	*p*1	POSEIDON			Non-POSEIDON	*p*2
	1a (*n* = 44)	1b (*n* = 430)	1 (*n* = 474)	3 (*n* = 1618)		2a (*n* = 6)	2b (*n* = 67)	2 (*n* = 73)	4 (*n* = 212)	
Type of transfer										
Implantation rate % (*n*)	33.9 (21/62)*	42.8 (307/717)*	42.1 (239/411)	47.6 (879/1,847)	0.01	20.0 (1/5)	37.3 (53/142)	36.7 (54/147)	43.7 (124/284)	0.181
Clinical pregnancy rate % (*n*)	43.2 (16/37)*	60.1 (227/378)	58.6 (243/415)	63.2 (633/1,001)	0.105	25.0 (1/4)	62.5 (40/64)	60.3 (41/68)	67.6 (92/136)	0.35
LBR per transfer fresh % (*n*)	37.8 (14/37)*	50.0 (189/378)	48.9 (203/415)	55.4 (555/1,001)	0.026	25.0 (1/4)	51.6 (33/64)	40.0 (34/68)	57.4 (78/136)	0.371
FET cycles (*n* = 1,898)	*n* = 4	*n* = 188	*n* = 192	*n* = 1490		*n* = 1	*n* = 15	*n* = 16	*n* = 200	
LBR per transfer FET % (*n*)	50.0 (2/4)	33.5 (63/188)*	33.9 (65/192)	50.7 (755/1,490)	<0.001	100.0 (1/1)	13.3 (2/15)*	20.0 (3/15)	39.5 (79/200)	0.115
CLBR % (*n*)	36.4 (16/44)*	58.6 (252/430)*	56.5 (268/474)	81.0 (1,310/1,618)	<0.001	33.3 (2/6)	52.2 (35/67)*	50.7 (37/73)	74.1 (157/212)	<0.001
Conception mode % (*n*)										
IVF/ICSI fresh % (*n*)	87.5 (14/16)*	75.0 (189/252)*	75.7 (203/268)	42.4 (555/1,310)	<0.001	50 (1/2)	94.3 (33/35)*	91.9 (34/37)	49.7 (78/157)	<0.001
IVF/ICSI FET % (*n*)	12.5 (2/16)	25.0 (63/252)	24.3 (65/268)	57.6 (755/1,310)		50 (1/2)	5.7 (2/35)	8.1 (3/37)	50.3 (79/157)	

CLBR, cumulative live birth rate: cumulative delivery rate from one aspiration IVF/ICSI cycle. p1, p-value between Group 1 (combined 1a+1b) and younger non-POSEIDON; p2, p-value between Group 2 (combined 2a+2b) and older non-POSEIDON. *Compared with its non-POSEIDON counterpart in same age stratum and p<0.05.

other studies. Chinta et al. reported no significant differences in the LBR per fresh embryo transfer between POSEIDON Groups 1 and 2 and the non-POSEIDON groups (32). A study of 2,455 cycles found that the LBR after fresh transfer was highest in patients with 6–15 oocytes retrieved and reduced in patients with 0–5 or >15 oocytes retrieved (20). For women with PCOS <35 years old, it was found that the proportion of live births per fresh embryo transfer was similar for those who had less than 6 oocytes retrieved, and those who had 7–16 oocytes retrieved (33). These findings suggested that fair LBR per fresh embryo transfer can still be achieved in unexpected suboptimal responders. Moreover, the lower embryo transfer cancellation rate in POSEIDON groups may result in a shorter time to live birth, providing valuable information for counseling patients with unexpected suboptimal ovarian response in PCOS.

Here, we also found that LBR per FET in POSEIDON Group 1 was significantly lower than the non-POSEIDON groups paired with

TABLE 5 Odds ratio for POSEIDON hypo-response of women with PCOS.

Indicators	OR (95% CI)	*p*-value	Adjusted OR (95% CI)	Adjusted *p*-value
Age (years)	1.023 (0.998, 1.050)	0.076	1.009 (0.983, 1.035)	0.518
BMI (kg/m^2)	1.079 (1.049, 1.111)	<0.001	1.085 (1.053, 1.118)	<0.001
Infertility type				
Primary	1.076 (0.886, 1.307)	0.458	–	–
Secondary	1.000			
Basal FSH (U/L)	1.078 (1.025, 1.134)	0.004	1.095 (1.039, 1.153)	0.001
Basal LH (U/L)	0.994 (0.977, 1.011)	0601	–	–
AFC (*n*)	0.985 (0.974, 0.996)	0.009	0.988 (0.976, 0.999)	0.033

OR, odds ratio.

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TABLE 6 Odds ratio for LBR from one aspiration IVF/ICSI cycle according to POSEIDON groups.

	Patient category	Unadjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
<35 years old	**Non-POSEIDON**	**Reference**	–	–	–
	POSEIDON Group 1a	0.494 (0.249, 0.981)	0.044	0.491 (0.246, 0.977)	0.043
	POSEIDON Group 1b	0.834 (0.659, 1.057)	0.133	0.829 (0.654, 1.050)	0.120
	POSEIDON Group 1 (a + b combined)	0.798 (0.635, 1.003)	0.053	0.793 (0.630, 0.998)	0.048
≥35 years old	**Non-POSEIDON**	**Reference**	–	–	–
	POSEIDON Group 2a	0.296 (0.030, 2.920)	0.297	0.278 (0.028, 2.815)	0.279
	POSEIDON Group 2b	0.616 (0.339, 1.116)	0.110	0.581 (0.310, 1.089)	0.090
	POSEIDON Group 2 (a + b combined)	0.593 (0.330, 1.064)	0.080	0.558 (0.301, 1.035)	0.064

Age, BMI, and infertility type were adjusted in the multivariate analysis. OR, odds ratio.

age (33.9% vs. 50.8%, $p < 0.001$). LBR per FET in POSEIDON Group 2 was also lower than its counterparts, though there was no statistical difference (20.0% vs. 39.5%, $p = 0.115$). This observation is expected, given that fewer oocytes were picked up and fewer good embryos were obtained in POSEIDON patients. In the FET cycle, available embryos were transferred after being selected for fresh embryo transfer. Therefore, the optimal number of oocytes is a critical factor in maximum CLBR for women with PCOS. The CLBR was significantly lower in POSEIDON Groups 1 and 2 compared with the non-POSEIDON groups paired with age (56.5% vs. 81.0%, $p < 0.001$; 50.7% vs. 74.1%, $p < 0.001$), according to our study. A retrospective study that included 18,455 cycles also showed lower CLBR in POSEIDON Groups 1 and 2 compared to the non-POSEIDON patients, which is consistent with our finding in women with PCOS (34). A real-world study of 9,073 patients, including 212 patients from Group 1a, 1,785 patients from Group 1b, 293 patients from Group 2a, 1,275 patients from Group 2b, and 4,640 non-POSEIDON patients, revealed that CLBR differed across the POSEIDON groups (Group 1a: 27.8%, Group 1b: 47.8%, Group 2a:14%, and Group 2b: 30.5%) and was lower than the non-POSEIDON groups (50.6%) (35). In our study, the CLBRs were 36.4%, 58.6%, 33.3%, and 52.2% in Group 1a, Group 1b, Group 2a, and Group 2b, respectively. These rates seemed higher compared to other studies and could be explained by the high ovarian reserve of women with PCOS. The CLBR after including frozen embryo transfer cycles increased with oocyte number (20). Similarly, in women with PCOS, high CLBR can be obtained with 15 or more oocytes retrieved (33). However, a higher number of oocytes retrieved does not necessarily lead to a better prognosis for patients with PCOS, but a higher surplus embryo rate. A retrospective study in women with PCOS found that CLBR could reach up to 81.91% when 10–15 oocytes were retrieved, but when the number of oocytes were ≥16, a higher risk of OHSS was achieved but not a higher CLBR (36).

The management of POR in women with PCOS is varied: cancellation of the cycle, switching to intrauterine insemination (if the tubal obstruction is not present), dual stimulation, switching from IVF to IVM as a salvage strategy (37), or transvaginal ovarian drilling followed by controlled ovarian stimulation from the next day onwards (38). Our study provides new data that when the expected number of oocytes is more than four, the live birth rate of a fresh transfer is not affected. When fewer than four oocytes are obtained, the live birth rate decreases but is still cost-effective considering the

TABLE 7 Odds ratio for CLBR from one aspiration IVF/ICSI cycle according to POSEIDON groups.

	Patient category	Unadjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
<35 years old	**Non-POSEIDON**	**Reference**	–	–	–
	POSEIDON Group 1a	0.134 (0.072, 0.251)	<0.001	0.136 (0.072, 0.256)	<0.001
	POSEIDON Group 1b	0.333 (0.265, 0.418)	<0.001	0.333 (0.264, 0.420)	<0.001
	POSEIDON Group 1 (a + b combined)	0.306 (0.245, 0.381)	<0.001	0.308 (0.246, 0.384)	<0.001
≥35 years old	**Non-POSEIDON**	**Reference**	–	–	–
	POSEIDON Group 2a	0.175 (0.031, 0.983)	0.048	0.155 (0.027, 0.896)	0.035
	POSEIDON Group 2b	0.383 (0.217, 0.677)	0.001	0.365 (0.199, 0.669)	<0.001
	POSEIDON Group 2 (a + b combined)	0.360 (0.207, 0.625)	<0.001	0.337 (0.188, 0.606)	<0.001

Age, BMI, and infertility type were adjusted in the multivariate analysis. OR, odds ratio.

time and economic costs of retrieving the oocytes again. Our study can be useful for the counseling decision-making process.

Our study was the first to investigate LBR per fresh embryo transfer and CLBR per aspiration IVF/ICSI cycle in women with PCOS under the POSEIDON classification. However, a limitation of our study was the small number of cases in POSEIDON Group 2a, an issue that happened for observational data, which were too low to draw any definitive conclusions. Additionally, we were unable to calculate CLBR after all frozen embryos were transplanted, because preserved embryos were common in most women with PCOS. Therefore, we calculated the CLBR per aspiration cycle with at least one live birth after embryo transfer or did not get a live birth delivery after transferring all embryos. Further studies are needed to compare CLBR of women with PCOS and women without PCOS who were identified with POSEIDON. Another limitation was the high frequency of missing values of AMH for analysis. We have not analyzed PCOS phenotypes, although only specific PCOS phenotypes may fall into POSEIDON criteria. We hope that, in the future, we will gather more comprehensive baseline data on women with PCOS and conduct analyses of LBR across different phenotypes. All IVF data were derived from a single center, whereas the pregnancies were followed in different centers. The risk of pregnancy complications is high in patients with PCOS, and specialized monitoring could influence the data. This potentially limits the broad applicability of our results. Therefore, our findings necessitate further validation in a large and diverse population.

In this study, we found that in patients with PCOS, the unexpected suboptimal response could achieve fair LBR per fresh embryo transfer; however, the CLBR per aspirated cycle in POSEIDON patients was lower than that of normal responders. BMI, basal FSH level, and AFC were identified as independent factors associated with unexpected poor/suboptimal response to standard ovarian stimulation, while POSEIDON classification was an independent risk factor for CLBR. Therefore, the unexpected suboptimal response could achieve fair LBR per fresh embryo transfer, which can be useful information for counseling patients with unexpected suboptimal ovarian response in PCOS. Efforts should be made to recognize POSEIDON risk factors and obtain appropriate oocyte retrieval number to maximize IVF/ICSI outcomes for women with PCOS.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Sun Yat-sen Memorial Hospital ethical review board (SYSKY-2023-200-02). The studies were conducted in accordance with the local legislation and institutional requirements. The anonymity of the retrospective data waived the need for informed consent.

Author contributions

LJ: Writing – original draft, Formal analysis. YS: Writing – original draft, Formal analysis. PP: Writing – review & editing, Data curation. LL: Writing – review & editing, Data curation. DY: Writing – review & editing, Data curation. JH: Writing – review & editing, Methodology, Conceptualization. YL: Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1348771/full#supplementary-material.

SUPPLEMENTARY FIGURE 1
Flow diagram showing total patient breakdown. POSEIDON Group 1 (Group 1): Age <35 years and AFC ≥5. Subgroup 1a included patients with fewer than four oocytes retrieved, and Subgroup 1b included patients with four to nine oocytes retrieved. POSEIDON Group 2 (Group 2): Age ≥35 years and AFC ≥5. Subgroup 2a included patients with fewer than four oocytes retrieved, and Subgroup 2b included patients with four to nine oocytes retrieved. Non-POSEIDON (Group 3 and Group 4): Patients with AFC ≥5 and >9 oocytes retrieved. Group 3 included patients aged <35 years, and Group 4 included patients aged 35 years and older.

SUPPLEMENTARY FIGURE 2
Bar chart showing the distribution of patients in each POSEIDON group in different years from 2011 to 2020.

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Genetic insights of blood lipid metabolites on polycystic ovary syndrome risk: a bidirectional two-sample Mendelian randomization study

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Background: Pathologically, metabolic disorder plays a crucial role in polycystic ovarian syndrome (PCOS). However, there is no conclusive evidence lipid metabolite levels to PCOS risk.

Methods: In this study, genome-wide association study (GWAS) genetic data for 122 lipid metabolites were used to assign instrumental variables (IVs). PCOS GWAS were derived from a large-scale meta-analysis of 10,074 PCOS cases and 103,164 controls. An inverse variance weighted (IVW) analysis was the primary methodology used for Mendelian randomization (MR). For sensitivity analyses, Cochran Q test, MR-Egger intercept, MR-PRESSO, leave-one-out analysis,and Steiger test were performed. Furthermore, we conducted replication analysis, meta-analysis, and metabolic pathway analysis. Lastly, reverse MR analysis was used to determine whether the onset of PCOS affected lipid metabolites.

Results: This study detected the blood lipid metabolites and potential metabolic pathways that have a genetic association with PCOS onset. After IVW, sensitivity analyses, replication and meta-analysis, two pathogenic lipid metabolites of PCOS were finally identified: Hexadecanedioate (OR=1.85,95%CI=1.27–2.70, *P*=0.001) and Dihomo-linolenate (OR=2.45,95%CI=1.30–4.59, *P*=0.005). Besides, It was found that PCOS may be mediated by unsaturated fatty acid biosynthesis and primary bile acid biosynthesis metabolic pathways. Reverse MR analysis showed the causal association between PCOS and 2-tetradecenoyl carnitine at the genetic level (OR=1.025, 95% CI=1.003–1.048, *P*=0.026).

Conclusion: Genetic evidence suggests a causal relationship between hexadecanedioate and dihomo-linolenate and the risk of PCOS. These compounds could potentially serve as metabolic biomarkers for screening PCOS and selecting drug targets. The identification of these metabolic pathways is valuable in guiding the exploration of the pathological mechanisms of PCOS, although further studies are necessary for confirmation.

KEYWORDS

Mendelian randomization, lipid metabolites, polycystic ovarian syndrome, genetics, meta-analysis, metabolic pathway analysis

1 Introduction

Globally, PCOS affects 2.2% to 26% of women, making it the most prevalent reproductive and endocrine disorder (1). The clinical manifestations are varied, such as menstrual disorders, infertility, hirsutism, acne, glucose and lipid metabolism disorder (2). Moreover, it can increase the risk of cardiovascular disease, diabetes, gynecological tumors and other diseases (3, 4). PCOS is a multisystem disease that affects women from adolescence to menopause, imposing a heavy economic burden on patients and posing threats to their physical and mental well-being. The delayed detection and inadequate management of PCOS have generated discontent among women worldwide (5). Therefore, exploring the key targets for the pathogenesis and treatment of PCOS has increasingly become a public health issue that requires urgent attention.

Metabolites, which are the outcomes of genetic and environmental influences on organisms, exhibit sensitivity to physiological and pathological variations within the body (6). Metabolomics play an imperative role identifying pertinent biomarkers for disease diagnosis and treatment. Numerous clinical and basic studies in recent years have reported the effects of lipid metabolism on PCOS. Lipid metabolism disorders and PCOS can form a detrimental cycle, which can be responsible for the main pathological features of PCOS, including insulin resistance and hyperandrogenism (7).Moreover, certain lipid metabolites' aberrant changes are implicated in the chronic low-grade inflammation process of PCOS, consequently influencing oocyte maturation and exacerbating ovulation disorders (8, 9). Yu et al. (10) reported that the blood lipid metabolites of PCOS differed significantly from those of healthy controls. They also observed alterations in related pathway profiles, such as fatty acid degradation and ether lipid metabolism. Additional, Buszewska-Forajta et al. (11) found a deterioration of lipid metabolism in PCOS patients, with higher sphingolipids and lower fatty acids. These studies are, however, mostly cross-sectional or observational, rendering their conclusions susceptible to confounding factors. Currently, there is no detailed and in-depth study exploring whether blood lipid metabolites are causally associated with PCOS.

Genetic causality can be determined by Mendelian randomization (MR), an emerging and effective scientific technique. The genetic variants or single nucleotide polymorphism (SNPs) may be used as an useful instrument to assess causal link between exposure and outcome (12). By circumventing reverse causality and confounding factors encountered in traditional epidemiological studies, MR can provide scientifically reliable results for elucidating disease pathogenesis (13). Therefore, it has gained widespread adoption for investigating PCOS pathology (14). In this study, we utilized extensive GWAS data to explore the genetic relationship between blood lipid metabolites and PCOS through bidirectional two-sample MR. Our findings aim to provide innovative insights directed towards the prevention and treatment of PCOS.

2 Methods

2.1 Study design

In our study, we utilized validated genetic variants from publicly available GWAS data as instrumental variables (IVs) to investigate their causal relationship with outcome by replacing exposure. Blood lipid metabolites were preliminarily considered as "exposure", while PCOS was considered the "outcome". Our study design adhered to the three fundamental assumptions of MR outlined by Bowden et al. (15): (1) The IVs should exhibit a strong connection with the blood lipid metabolites; (2) IVs should not be influenced by any confounding factors between blood lipid metabolites and PCOS; (3) IVs must solely impact PCOS through blood lipid metabolites. These assumptions ensure that the genetic variants randomly assigned during meiosis are strongly linked to lipid metabolites,

Abbreviations: PCOS, polycystic ovarian syndrome; GWAS, genome-wide association studies; IVs, Instrumental variables; IVW, inverse variance weighted; MR, Mendelian randomization; SNPs, single nucleotide polymorphisms; NIH, National Institutes of Health; LD, linkage disequilibrium; WM, weighted median; OATP, organic anion transporting polypeptide; PUFA, polyunsaturated fatty acid; LDSC, linkage disequilibrium score regression.

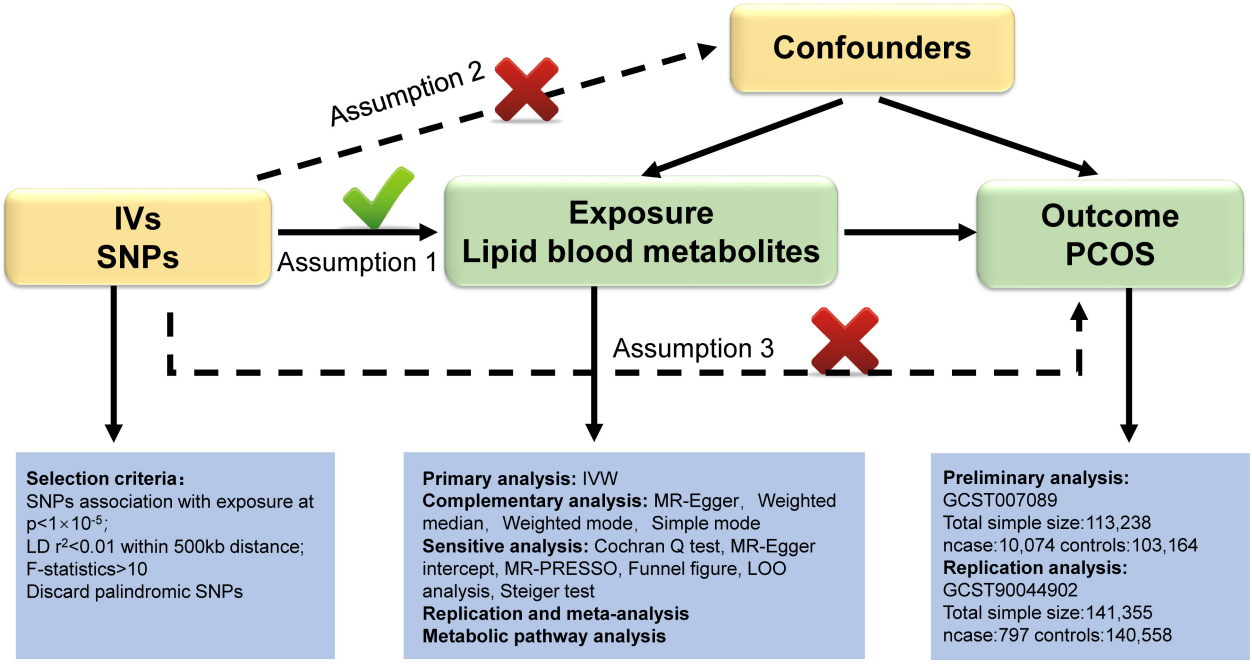

FIGURE 1
Overview of this Mendelian randomization (MR) analysis. Abbreviations: IV, instrumental variables; SNPs, single nucleotide polymorphisms; IVW, inverse variance weighted; LD, linkage disequilibrium; LOO analysis, leave- one- out analysis; MR- PRESSO, MR- Pleiotropy Residual sum and outlier.

unaffected by confounding factors or reverse causality, thereby ensuring the validity of causal inferences. Figure 1 illustrates the three hypotheses and the research methodologies employed. We established stringent criteria for IV selection to fulfill hypothesis 1. Primary and supplementary analyses in MR were used to systematically assess the causal effects of lipid metabolites on PCOS. A series of sensitivity analyses were performed to confirm that hypotheses 2 and 3 were not violated. Following preliminary analysis, we utilized another PCOS GWAS dataset (Number: GCST90044902) to conduct replication and meta-analysis for further screening of target lipid metabolites. Metabolic pathway analysis and reverse analysis were also performed to make findings.

2.2 GWAS data for 122 blood lipid metabolites and PCOS

Blood lipid metabolite GWAS data were identified by Shin et al. using genome-wide association scans and high-throughput metabolic measurements. They assessed the genetic relevance of 122 lipid metabolites in 7824 individuals of European ancestry using nearly half a million SNPs (16). Access to the metabolomics GWAS server was made (http://metabolomics.helmholtz-muenchen.de/gwas).

PCOS GWAS stem from a comprehensive meta-analysis conducted by Day et al. that utilized Rotterdam criteria, National Institutes of Health (NIH) criteria, or self-reported criteria to diagnose PCOS. 10,074 patients and 103,164 controls of European descent were enrolled in this study (17). The data accessing code is GCST007089 and access is available via the GWAS catalog (https://www.ebi.ac.uk/gwas).

It is important to highlight that weak instrument bias resulting from sample overlap can influence the observational association between exposure and outcome, potentially leading to inflated type I error rates for causal effects (18). In our study, we selected samples from different datasets to mitigate the bias caused by overlapping samples.

2.3 Instrumental variables selection

A rigorous set of criteria was set to screen for excellent IVs related to exposure. 1) We extracted IVs with significance thresholds below 1×10^{-5} in GWAS data of exposure, which are considered to be highly correlated with exposure. Our linkage disequilibrium (LD) was set at 0.01 within 500 KB to ensure independence of IVs.2) F-statistic was used to quantify the strength of genetic variation for individual SNP. IVs with F-statistic <10 were removed to reduce the possibility of weak instrument bias. $F = (n-k-1)\ R^2/k\ (1-R^2)$ (n represents the sample size, k represents the number of IVs included, and R^2 represents the instrumental variable explaining the degree of exposure) (19).3) IVs were extracted from outcome GWAS data, and the alleles of exposure- and outcome-SNPs were harmonized. We then eliminated palindromic SNPs with intermediate effect allele frequencies (EAF > 0.42).

2.4 Primary analysis and sensitivity analysis

When all included SNPs are valid instrumental variables, the IVW method can provide accurate and unbiased estimates of causality (20).So IVW method was considered as the primary method to evaluate the causal relationship between lipid metabolites and PCOS (P <0.05 was considered significant). Additionally, we employed the weighted median (WM), MR-Egger, simple mode, and weighted mode methods as supplementary analyses. The WM grants robust outcomes for causal analyses when less than half of the SNPs are deemed invalid (21). MR-Egger can access the horizontal pleiotropy of all invalid IVs (22). Weighted mode and simple mode were also employed to address bias resulting from a limited number of IVs that do not meet MR's causal inference criteria (23). Significance estimates provided by the primary analysis were considered to be significant if they were in the same direction as those provided by the supplementary analysis. Five methods are helpful to achieve the comprehensive evaluation of causal effects.

A series of sensitivity analyses were conducted to verify that the findings did not violate the core MR assumptions and to enhance the reliability of causal effects. Cochran's Q statistic was conducted to quantify the heterogeneity among IVs. The egger-intercept method can assess whether instrumental variables are related to confounding factors and test whether causal effects are biased by horizontal pleiotropy (20, 24) (P >0.05 indicated no significant heterogeneity and horizontal pleiotropy). Furthermore, the MR-PRESSO method was utilized to identify and correct for any significant outliers and to address the limited statistical power of the Egger-intercept method in order to reduce pleiotropic bias (25). Leave-one-out sensitivity analysis involved systematically excluding individual SNPs one by one to assess the impact on effect estimates and to ensure that the causal inference was not heavily reliant on a single SNP, thus ensuring the robustness of the overall causal conclusions. Finally, We also performed the Steiger method to verify that the selected SNPs explained greater variability in the exposure than in the outcome(P <0.05), which is important to avoid reverse causality bias in the study (26).

2.5 Replication and meta-analysis

To further validate the identification of lipid metabolites based on the mentioned criteria, we conducted a repeat IVW analysis using data from another GWAS in the GWAS catalog involving 141,355 individuals of European ancestry (GCST90044902) (27). The results from both the initial and replication analyses were then combined in a meta-analysis to strengthen the evidence supporting the genetic association of protective and pathogenic lipid metabolites with PCOS.

2.6 Metabolic pathway analysis

The KEGG database metabolic pathway analysis was conducted using MetaboAnalyst 5.0 (https://www.metaboanalyste.ca/) (28) to explore the potential pathogenesis of lipid metabolites affecting the pathology of PCOS. P<0.1 was considered statistically significant (29).

2.7 Reverse MR analysis

In order to in-depth and comprehensive analysis of blood lipid metabolites and PCOS genetic causality, we regarded PCOS as "exposed", and the identified blood lipid metabolites as "outcome". Similarly, we set the significance threshold at 1×10^{-5} and LD r^2<0.01 within 500KB to screen for IVs strongly associated with PCOS. Palindromic SNPs were removed and weak IVs interference was excluded. Then, we applied IVW methods to investigate whether the PCOS onset contributes to genetic changes in lipid metabolites. Results were robustly tested using sensitivity analysis.

3 Results

3.1 IVs selection

Following meticulous selection of SNPs strongly associated with every lipid metabolite, SNPs for caprate (10:0) and butyrylcarnitine were not found in harmonizing with the PCOS GWAS. Consequently, MR of these two metabolites were abandoned. Details of the corresponding SNPs for the remaining 120 lipid metabolites for MR analysis are provided in Supplementary Table S2. These SNPs contains no palindrome SNPs and F statistic > 10, be deemed to be valid IVs.

3.2 Primary analysis and sensitivity analysis

Nine lipid metabolites were preliminarily identified to be associated with PCOS using the IVW method (IVW P<0.05) (Supplementary Table S3). The direction of the odds ratio (OR>1 or OR<1) indicates the positive or negative correlation trend of the causal effect. Further analysis revealed inconsistent OR values among the five methods, leading us to conclude that the causal relationship between dehydroisoandrosterone sulfate (DHEA-S), 2-linoleoylglycerophosphocholine, and PCOS may be a false positive. Therefore, after preliminary analysis, we identified 2-tetradecenoyl carnitine(IVW OR=0.55, 95% CI=0.32–0.95, P=0.032) and 7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca) (IVW OR=0.16, 95% CI=0.04–0.69, P=0.014)had causal relationship with the decreased risk of PCOS. While the remaining 5 metabolites had causal relationship with the increased risk of PCOS: hexanoylcarnitine (IVW OR=2.85,95%CI=1.25–6.50,P=0.013), 3-dehydrocarnitine (IVW OR=3.53,95%CI=1.23–10.10,P=0.019), 1-arachidonoylglycerophosphoethanolamine(IVW OR=3.97,95% CI=1.61–9.76,P=0.033), hexadecanedioate(IVW OR=1.92, 95% CI=1.10–3.37, P=0.022) and dihomo-linolenate (20:3n3 or n6) (IVW OR=3.17, 95% CI=1.18- 8.49, P=0.022) (Figure 2). Scatter plots show the MR effects of the seven metabolites estimated by the different methods with PCOS (Supplementary Figure S1). The results of a series of sensitivity analyses ensured the robustness of causal effects. These seven metabolites did not appear to be linked to PCOS by horizontal pleiotropy or heterogeneity according to Cochran's Q statistics and MR-Egger (all P>0.05) (Table 1). The results of MR-PRESSO did not support the presence of outlying SNPs (all P>0.05)

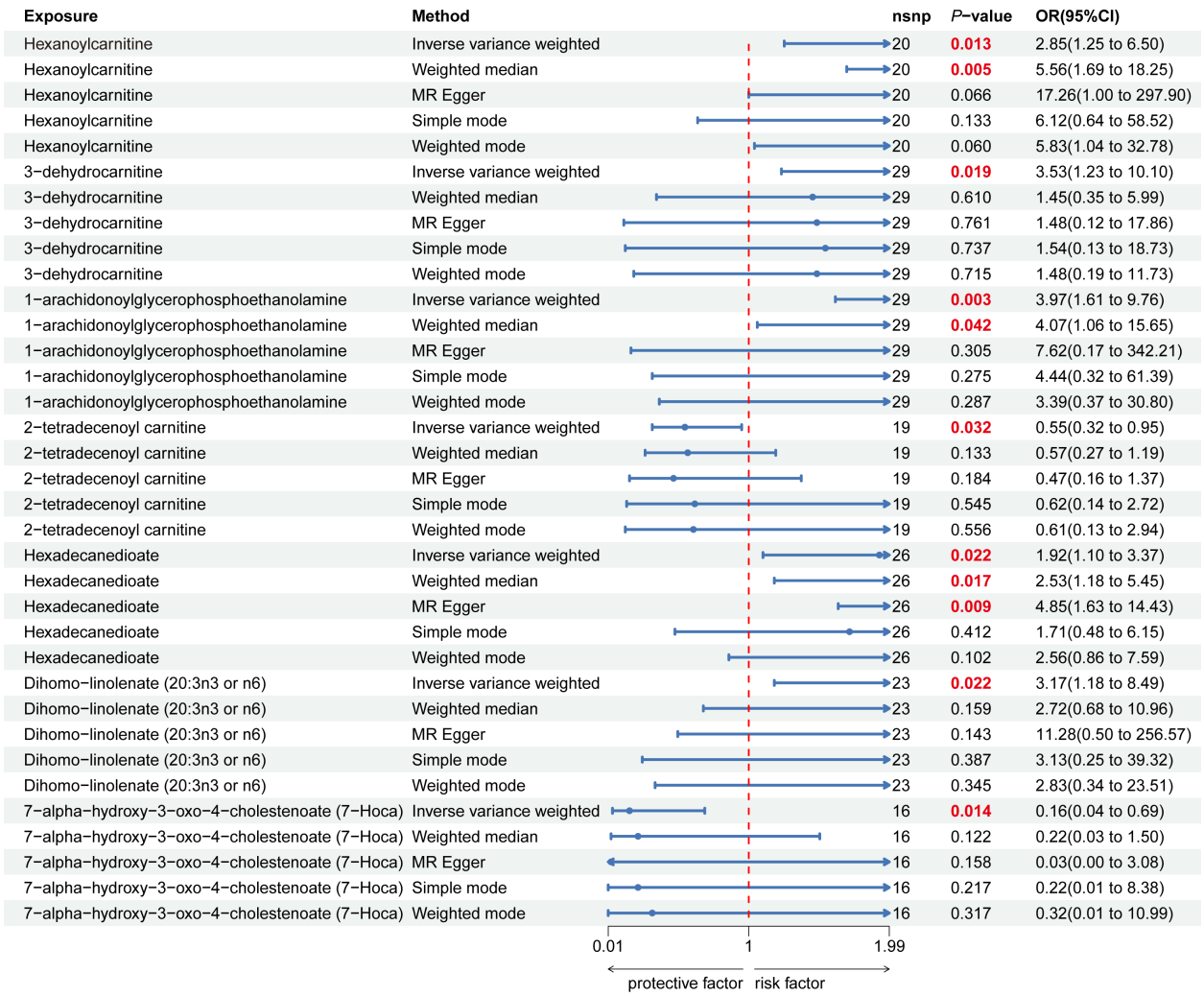

FIGURE 2
Forest plot of the causal relationship between 7 lipid metabolites and PCOS in the result of primary and supplementary analyses The blue dots represent the estimates of five methods, and the blue bars represent the 95% confidence intervals of estimates. The OR > 1 indicates increased risk while< 1 indicates decreased risk.

TABLE 1 The results of heterogeneity, horizontal pleiotropy and MR-PRESSO of the 7 lipid metabolites and PCOS in the MR analysis.

Exposure	Outcome	Pleiotropy test		Heterogeneity test		MR-PRESSO	
		Intercept	p-value	Q	Q_ p-value	Sd	Global Test p-value
Hexanoylcarnitine	PCOS	-0.04	0.21	15.82	0.67	0.384	0.72
3-dehydrocarnitine	PCOS	0.02	0.46	33.80	0.21	0.536	0.23
1-arachidonoylglycerophosphoethanolamine	PCOS	-0.01	0.73	28.20	0.45	0.460	0.50
2-tetradecenoyl carnitine	PCOS	0.01	0.73	12.94	0.79	0.234	0.82
Hexadecanedioate	PCOS	0.02	0.07	31.37	0.18	0.286	0.18
Dihomo-linolenate (20:3n3 or n6)	PCOS	-0.02	0.41	21.50	0.49	0.497	0.47
7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	PCOS	0.03	0.45	11.69	0.70	0.650	0.67

MR, Mendelian randomization; Q, heterogeneity statistic Q.

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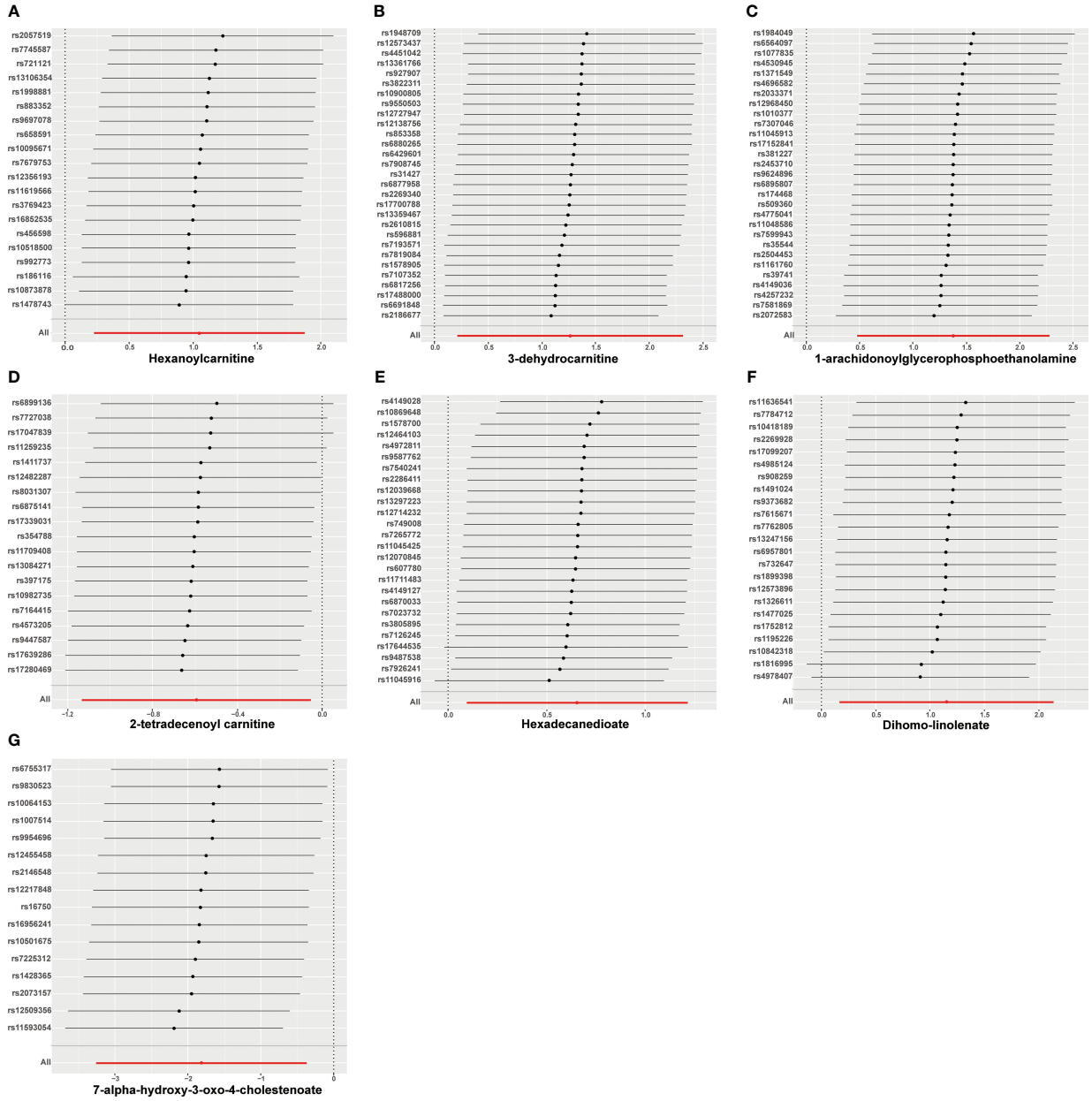

FIGURE 3
Leave-one-out analysis of causal estimates of exposure (Specific lipid metabolites) on polycystic ovarian syndrome(PCOS). Calculate the MR results of the remaining IVs after removing the IVs one by one. **(A)**:*Hexanoylcamitine;* **(B)**: *3-dehydrocarnitine*; **(C)**: *1-arachidonoylglycerophosphoethanolamine*; **(D)**: *2-tetradecenoyl carnitine*; **(E)**: *Hexadecanedioate*; **(F)**: *Dihomo-linolenate*; **(G)**: *7-alpha-hydroxy-3-oxo-4-cholestenoate*;.

(Table 1). The distribution of SNPs for each metabolite is presented using funnel plots. IVW showed that SNPs were distributed roughly symmetrically, indicating that the results of the MR analysis were not biased by outlying SNPs (Supplementary Figure S2). The plot of leave-one-out analysis showed that when one snp was removed, the overall causal effect of the remaining SNPs on the outcome did not deviate substantially, supporting that individual SNPs were not responsible for biasing estimates of the MR total effects (Figure 3). The Steiger test revealed that the results of lipid metabolites and PCOS causality were not interfered by reverse causality effect (all $P<0.05$) (Supplementary Table S4).

3.3 Replication and meta-analysis

Based on the PCOS GWAS data from the FinnGen database, we used 7 identified lipid metabolites as "exposures" to conduct replication analysis and meta-analysis of their causal relationship with PCOS. Common effect model and random effect model ultimately confirmed a strong causal association between two lipid metabolites and PCOS (Supplementary Table S5). Specifically, Hexadecanedioate (OR=1.85,95%CI=1.27–2.70, $P=0.001$) and dihomo-linolenate (20:3n3 or n6) (OR=2.45,95%CI=1.30–4.59, $P=0.005$) were found to be pathogenic lipid metabolites in PCOS

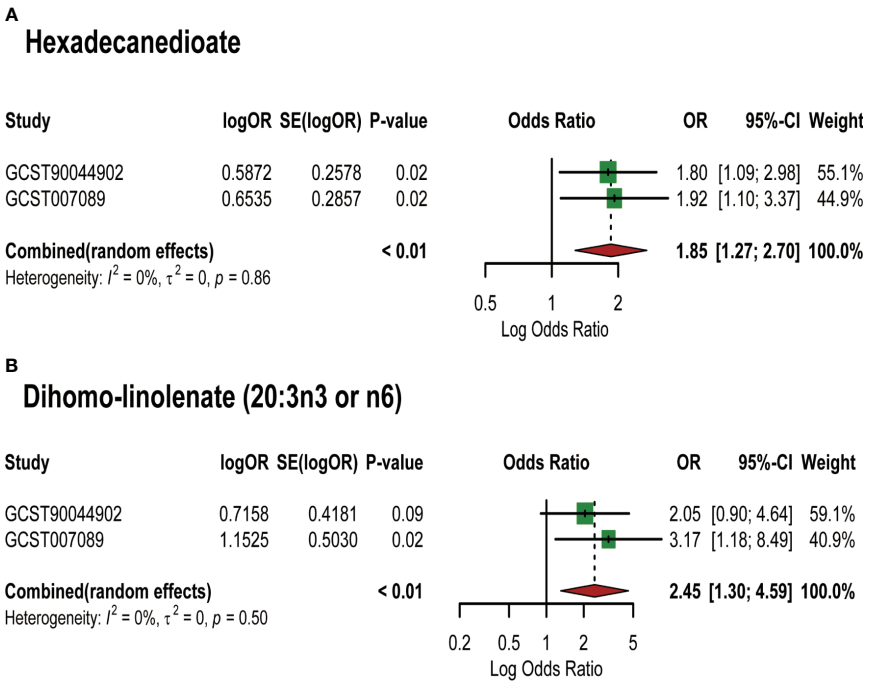

FIGURE 4
Meta-analysis of the causal associations between lipid metabolites and PCOS. GCST007089: GWAS for PCOS used in the primary analysis; GCST90044902: GWAS for PCOS used in the replication analysis. **(A)** Meta-analysis results of MR analysis of hexadecanedioate and different PCOS GWAS; **(B)** Meta-analysis results of MR analysis of dihomo-linolenate (20:3n3 or n6) and different PCOS GWAS. Abbreviations: SE: Standard Error; 95%- CI: 95% confidence interval; OR: odds ratio.

(Figure 4). However, the meta-analysis did not reveal a significant causal relationship between the remaining five metabolites and PCOS onset, such as 3-dehydrocarnitine and 2-tetradecenoyl carnitine (*P* value>0.05). This may be explained by the limited sample size of the PCOS GWAS data used in the replication analysis and the difference in the two PCOS GWAS samples.

3.4 Metabolic pathway analysis

The selected 7 lipid metabolites were used to explore their possible metabolic pathways involved in PCOS pathology. Despite not identifying any metabolic pathways at the 0.05 significance level, the researcher adjusted the significance threshold to P<0.1 to consider findings as statistically significant (29). The results highlighted two significant metabolic pathways: biosynthesis of unsaturated fatty acids (*P*=0.068) and primary bile acid biosynthesis (*P*=0.086), which are believed to contribute to the development and progression of PCOS.

3.5 Reverse MR analysis

According to the reverse MR analysis, we identified the genetic association between PCOS and 2-tetradecenoyl carnitine (IVW OR=1.025, 95% CI=1.003–1.048, *P*=0.026) (Supplementary Table S6). Heterogeneity and horizontal pleiotropy were not significant in the sensitivity analysis (*P*>0.05).

4 Discussion

The polycystic ovary syndrome is an endocrine and metabolic disorder with genetic heterogeneity that affects reproductive health (30).Metabolic disorders, particularly lipid metabolism disorders, are prominent clinical manifestations and key mechanisms of PCOS (31). Recent research has highlighted the significance of identifying differential blood metabolites in understanding the pathogenesis of PCOS (32). However, the specific blood lipid metabolites that may have protective or pathogenic effects on the development of PCOS remain unclear. GWAS from publicly available sources were used in this study to provide the first comprehensive and in-depth exploration of the gene-level link between blood lipid metabolites and PCOS. Through IVW and sensitivity analysis, we identified 7 lipid metabolites that are causally relate to PCOS. Subsequent replication and meta-analysis further confirmed the link between hexadecanedioate, dihomo-linolenate and the increased risk of PCOS, ensuring the robustness of the findings. Additionally, we identified two metabolic pathways that may contribute to PCOS' biological mechanisms. These findings provide ideas for diagnosing and treating PCOS with specific lipid metabolites.

Hexadecanedioate, a long-chain dicarboxylic acid, is synthesized via the ω-oxidation pathway (33). Raji's early studies linked hexadecanedioate to increased all-cause mortality in women and highlighted its negative impact on women's health (34). However, there is a current lack of studies investigating the role of hexadecanedioate in the development of PCOS. Hexadecanedioate is

recognized as a natural substrate for the organic anion-transporting polypeptide (OATP) (35). Francisca et al. (36) observed elevated OATP levels in women with PCOS compared to those without. OATP regulates the uptake of dehydroepiandrosterone sulfate (37),which may be involved in the metabolic process of hyperandrogenism in PCOS. This provides a possible explanation for the mechanism of hexadecanedioate as a pathogenic lipid metabolite in PCOS. Among the 26 SNPs identified as instrumental variables for hexadecanedioate, rs11045916(β=0.0605, SE=0.0069, *P*=1.424E-18) (Supplementary Table S2) (Supplementary Figure S3) showed the strongest association. However, the specific role of this genetic variant in PCOS pathogenesis remains unclear.

Zhang et al. (38) reported an increase in dihomo-linolenate levels in plasma phospholipids in individuals with PCOS. Our study also confirmed the relationship between dihomo-linolenate and PCOS, but the exact mechanism by which dihomo-linolenate contributes to PCOS pathogenesis is still unknown. In a recent review, dihomo-linolenate and its derivatives were discussed as possible mediators of inflammation (39). It can be converted to arachidonic acid, which exhibits proinflammatory properties and may contribute to the chronic inflammatory state observed in PCOS. A cross-sectional study revealed that higher linolenic acid was link to glucose and lipid metabolism disorders, as well as increased insulin resistance (40). However, more research is necessary to determine if linolenic acid directly increases the risk of PCOS by aggravating insulin resistance. Additionally, in the dihomo-linolenate GWAS data, we identified a causal relationship between rs4978407, rs10842318, rs1816995, and PCOS (Supplementary Figure S3). These genetic loci should be further investigated as potential key factors in the pathogenesis of PCOS. Inverse MR analysis revealed interesting results, indicating a decrease in 2-tetradecenoyl carnitine levels with the onset of PCOS. Despite the negative results in the meta-analysis for 2-tetradecenoyl carnitine, this lipid metabolite remains significant. The statistical significance of bidirectional causal studies suggests that it could be a promising target for PCOS treatment.

In the pathways analysis, it has been reported that the biosynthesis of unsaturated fatty acids is crucial to the metabolic pathway of lipid metabolites that affect the risk of PCOS. Polyunsaturated fatty acid metabolism has been found to improve sex hormone disorders, reduce oxidative stress, and reduce inflammation in PCOS, according to a meta-analysis (41). Ma et al.'s animal experiment confirmed that polyunsaturated fatty acids can enhance oocyte quality in PCOS mice by reducing oxidative stress level and improving spindle abnormalities (42). The lipid metabolites identified in our study may serve as important targets in the biosynthetic pathway of unsaturated fatty acids that affect PCOS. We also found that the primary bile acid biosynthesis pathway is of substantial significance. Bile acids contribute greatly to cholesterol metabolism and are known to be important endocrine regulators (43). This pathway has been associated with metabolic diseases such as diabetes mellitus and non-alcoholic fatty liver disease (44, 45). Yu et al. (46) investigated the bile acid profiles in PCOS and controls, and discovered that the bile acid anabolic pathways were crucial for glucose metabolism disorder in PCOS. The next phase of research in this field should focus on the exploration of the downstream molecules in this pathway and the underlying mechanisms involved in PCOS pathology.

Our study has certain advantages. Firstly, we utilized the most comprehensive published GWAS, covering large populations to ensure the objectivity of our results. Secondly, after strict screening effective IVs, rational MR and sensitivity analyses were used to thoroughly evaluate the causal effects to avoid reverse causality and confounding, and to ensure the accuracy and robustness of the results. Thirdly, additional GWAS data used for replicate analysis and meta-analysis were further validated, supporting the reliability of causal inference of certain metabolites with PCOS. In summary, genetic associations identified at the level of genetic variation will provide a metabolomics perspective for screening and identifying significantly altered metabolites that influence PCOS pathogenesis. This is expected to provide new markers for predicting those at increased risk of PCOS. Besides, Studies have confirmed that metabolic interventions by diet, exercise and lipid-lowering drugs are important treatments for PCOS, which can change the blood metabolites of PCOS (47). Our findings may provide preliminary evidence for the mechanistic targets of metabolic interventions in the treatment of PCOS, providing valuable insights for the design of future clinical studies.

However, some limitations need to be considered. Firstly, Due to the insufficient IVs, we relaxed the p-value threshold ($P<1\times10^{-5}$) for screening SNPs related to lipid metabolites. This is considered reasonable threshold in some studies (48, 49). This would lead to weak IVs bias that should be considered, although the F-statistics > 10. Secondly, although we used sensitivity analyses to exclude horizontal pleiotropy, we don't have the strict screening and eliminate potential confounding factors related IVs. Besides, our study may be biased by associations of unknown confounders with IVs. Thirdly, age and weight are recognized as significant factors affecting the onset and treatment of PCOS. However, individual patient details were not available from publicly available GWAS databases, so subgroup analyses for age, weight, and underlying diseases could not be performed in the MR study. Differences in lipid metabolites in different phenotypes of PCOS may cause an overall causal effect bias. Finally, exposure and outcome GWAS data consisted of individuals of European ancestry, which while reducing ethnic heterogeneity, but restrict the applicability of our findings to other ethnic groups. This demographic bias may bias the findings and findings should be validated in different ethnic groups.

5 Conclusion

Our study confirmed the robustness of the causal effect of hexadecanedioate and dihomo-linolenate on PCOS risk at the genetic level. Blood lipid metabolites may potentially regulate the progression of PCOS by interfering with the biosynthesis of unsaturated fatty acids and primary bile acid biosynthesis pathways. The findings of MR provide a reference direction for the study of the pathogenesis of PCOS mediated by metabolomics. Clinical and mechanism studies in the future are needed to confirm the significance of the identified metabolites as clinical biomarkers

for modulating PCOS risk and their potential target roles in treatment.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

Ethical approval was not required for the studies involving humans in accordance with the local legislation and institutional requirements because this study applied publicly accessible GWAS data. Informed consent for studies included in the analyses was provided in the original publications.

Author contributions

XW: Methodology, Writing – original draft. HH: Conceptualization, Data curation, Writing – original draft. XS: Writing – review & editing. XN: Writing – review & editing. RZ: Writing – review & editing. JJ: Funding acquisition, Writing – original draft. HZ: Funding acquisition, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1391826/full#supplementary-material

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Metabolic characteristics of different phenotypes in reproductive-aged women with polycystic ovary syndrome

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Objective: Polycystic ovary syndrome (PCOS) is an endocrine metabolic disorder in reproductive-aged women. The study was designed to investigate the metabolic characteristics of different phenotypes in women with PCOS of reproductive age.

Methods: A total of 442 women with PCOS were recruited in this cross-sectional study. According to different phenotypes, all women were divided into three groups: the chronic ovulatory dysfunction and hyperandrogenism group (OD-HA group, n = 138), the chronic ovulatory dysfunction and polycystic ovarian morphology group (OD-PCOM group, n = 161), and the hyperandrogenism and polycystic ovarian morphology group (HA-PCOM group, n = 143). The metabolic risk factors and prevalence rates of metabolic disorders among the three groups were compared.

Results: The body mass index (BMI), waist circumference, and waist-to-hip ratio (WHR) of women from the OD-HA group and HA-PCOM group were significantly higher than those of women from the OD-PCOM group ($p < 0.05$). The serum insulin concentration and homeostasis model assessment of insulin resistance (HOMA IR) at 2 h and 3 h after oral glucose powder in women from the OD-HA group and HA-PCOM group were significantly higher than those from the OD-PCOM group ($p < 0.05$). The serum total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) in women from the OD-HA group and HA-PCOM group were significantly higher than those in women from the OD-PCOM group ($p < 0.05$). The prevalence rates of impaired glucose tolerance (IGT), type 2 diabetes mellitus (T2DM), insulin resistance (IR), metabolic syndrome (MS), nonalcoholic fatty liver disease (NAFLD), and dyslipidemia of women with PCOS were 17.9%, 3.6%, 58.4%, 29.4%, 46.6%, and 43.4%, respectively. The prevalence rates of IGT, IR, MS, NAFLD, and dyslipidemia of women in the OD-HA group and HA-PCOM group were significantly higher than those of women in the OD-PCOM group ($p < 0.05$). T concentration (>1.67 nmol/L) and Ferriman–Gallwey (F–G) score (>3) significantly increased the risk of metabolic disorders in women with PCOS ($p < 0.05$).

Conclusion: The phenotypes of OD-HA and HA-PCOM in women with PCOS were vulnerable to metabolic disorders compared to OD-PCOM. Thus, the metabolic disorders in women with PCOS especially those with the HA phenotype should be paid more attention in order to reduce long-term complications.

KEYWORDS

metabolic characteristics, different phenotypes, polycystic ovary syndrome, hyperandrogenism, insulin resistance, metabolic syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a lifelong metabolic disorder that will likely influence women's health from adolescence to after menopause (1). The epidemiological investigations showed that the incidence of PCOS in women of reproductive age is 5%–15% according to different diagnostic criteria (2, 3). Although the exact etiology and pathogenesis of PCOS are unclear up to now, its effects on the health of women are well known. Women with PCOS have adverse effects on their health in pregnancy, and the disease also affects the health of their offspring. PCOS is an important risk factor in the development of gestational diabetes mellitus (4). A recent study by Risal et al. reported that daughters of mothers with PCOS have a fivefold increased risk for PCOS (5). Another recent systematic review and meta-analysis by Gunning et al. showed that normal weight children of women with PCOS were prone to developing cardiovascular metabolic disorders in early childhood (6). Furthermore, studies have proven that PCOS is associated with insulin resistance (IR), hyperinsulinemia (HI), type 2 diabetes mellitus (T2DM), metabolic syndrome (MS), and an increased risk of endometrial carcinoma, even in those with normal weight (7, 8). Additionally, women with PCOS are prone to atherogenic dyslipidemia, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis, and increased risk factors for cardiovascular disease (9–11).

The diagnostic criteria have been proposed by different organizations over the past several decades. In 1990, the diagnostic criteria were carried out at a National Institutes of Health (NIH) conference, which required the combination of chronic oligo/anovulation and clinical or biochemical evidence of hyperandrogenism (HA), after the exclusion of related disease (12). Subsequently, the Rotterdam European Society of Human Reproduction and the Embryology/American Society for Reproduction Medicine (ESHRE/ASRM) Consensus Workshop group proposed the addition of ultrasound characteristics for polycystic ovarian morphology (PCOM) to the NIH criteria in 2003, with a statement that women with any two of these three criteria could be diagnosed with PCOS (13). The diagnostic criteria expanded the diagnosis of PCOS, and broadened the complexity of PCOS phenotypes compared with the NIH definition, and resulted in an increased number of women with PCOS. Afterwards, the Androgen Excess Society (AES) proposed new diagnostic criteria and declared that HA is the necessary condition for diagnosis of PCOS (14). However, the Rotterdam criterion is the most widely used diagnostic criteria until now.

PCOS is a heterogeneous disease with diverse clinical manifestations, including menstrual irregularities, HA, infertility, and metabolic disorders. Different reports on the prevalence rate of metabolic disorders and its related long-term implications in women with PCOS are due to different diagnostic criteria. Therefore, this study aims to investigate the metabolic characteristics of different phenotypes in women with PCOS of reproductive age.

Materials and methods

Study design and participants

Figure 1 indicates the flowchart of study participation. A total of 544 women in the First Affiliated Hospital of Xi'an Jiaotong University from January 2018 to March 2022 were initially recruited in this cross-sectional study. Among them, 181 women presented chronic ovulatory dysfunction (OD) and HA, and 43 women were excluded due to hypothalamic amenorrhea, hyperprolactinemia, abnormal thyroid function, premature ovarian insufficiency (POI), premature ovarian failure (POF), or congenital adrenal hyperplasia (CAH). In the end, 138 women were selected as part of the OD-HA group. At the same time, ultrasound scanning was performed to detect PCOM among 363 women with only OD or HA; 161 women were selected as part of the OD-PCOM group, while 143 women were selected as part of the HA-PCOM group. A total of 59 women were excluded because they only have OD or HA. The diagnostic criteria of PCOS in this study were the Rotterdam criteria: (a) chronic OD, (b) clinical manifestations or biochemical evidence of HA, and (c) PCOM: the presence of at least 12 antral follicles measuring 2–9 mm in diameter in unilateral ovary or bilateral ovaries, and (or) an increased ovarian volume (≥10 mL). PCOS could be diagnosed when any two of these three criteria were

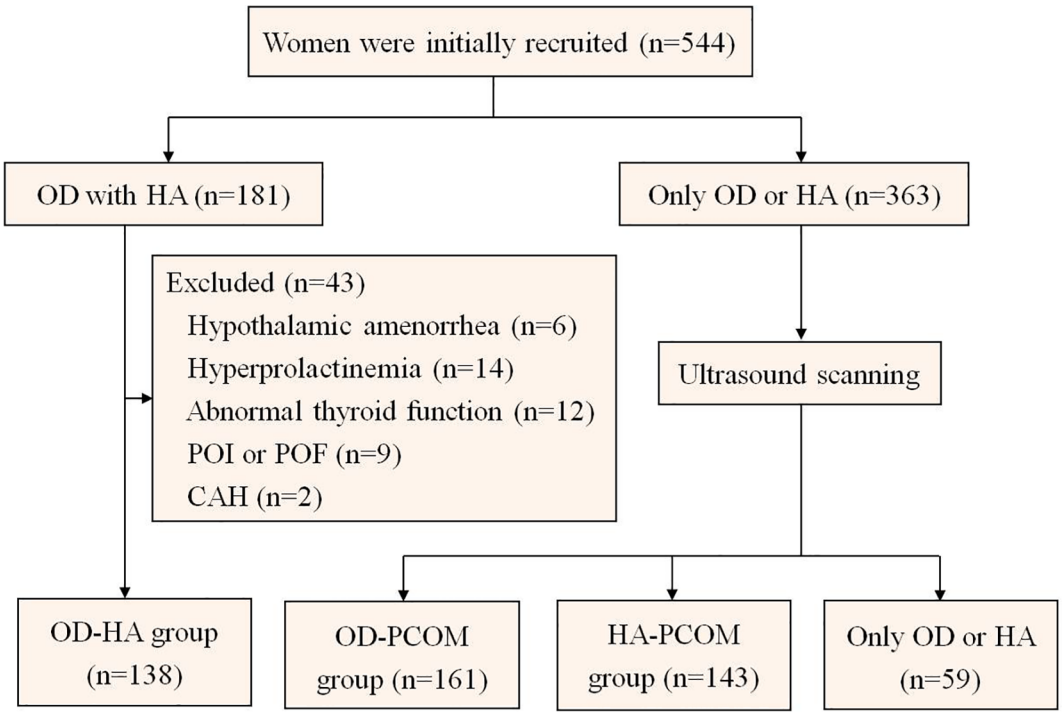

FIGURE 1
Flowchart of study participation. (OD, ovulatory dysfunction; HA, hyperandrogenism; POI, premature ovarian insufficiency; POF, premature ovarian failure; CAH, congenital adrenal hyperplasia).

presented (13). Laboratory evidence was defined as an abnormally increased testosterone level (>1.67 nmol/L). Hirsutism was regarded as the clinical manifestations of HA, which was defined as a modified Ferriman–Gallwey score of more than 3 at the time of physical examination. Participants were excluded if they had autoimmune disease or received treatment with hormone drugs in the past 6 months. In addition, participants who received drugs to treat metabolic disorders and HA were also excluded from this study, such as metformin, insulin, statins, combined oral contraceptive, and spironolactone. All participants gave written informed consent according to procedures granted by the Ethics Committee of The First Affiliated Hospital of Medical College of Xi'an Jiaotong University.

Sample size was calculated considering the prevalence rates of metabolic disorders in women with PCOS. According to the method reported in previous literatures, sample size was calculated with the following parameters: probability of type 1 error (α) of 0.05 and type 2 error (β) of 0.20 (power = 80%), the difference between two means to be detected was 0.52, and expected background standard deviation was 1. Based on this, 120 participants were needed in each group. Considering a loss to follow-up rate of 5%–10%, each group eventually needed at least 132 participants.

Detection of indicators

The basal concentrations of serum sex hormone on 2–4 days of menses, and anti-Müllerian hormone (AMH) concentration were tested in the clinical laboratory of our hospital using the chemiluminescence method. Women took the oral glucose tolerance test (OGTT) and insulin release test (IRT) after fasting for 12 h. Venous blood from the elbow was extracted in the morning on an empty stomach and 1 h, 2 h, and 3 h after taking 75 g of glucose powder to determine the blood sugar and insulin concentrations. Homeostasis model assessment of insulin resistance (HOMA IR) was used to evaluate the degree of IR. The calculation method was as follows: HOMA IR = blood sugar (mmol/L) × insulin (mIU/L)/22.5. At the same time, serum lipid concentrations were detected, including total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C).

Outcome measures

The primary outcome was the prevalence rates of metabolic disorders, including impaired glucose tolerance (IGT), T2DM, IR, MS, NAFLD, and dyslipidemia. IGT was defined as fasting blood glucose < 7.0 mmol/L and 7.8 mmol/L ≤ blood glucose 2 h after oral glucose powder < 11.1 mmol/L. T2DM was defined as fasting glucose ≥ 7.0 mmol/L or blood glucose 2 h after oral glucose powder ≥ 11.1 mmol/L (15). IR was defined as HOMA-IR > 3.0 (16). The diagnostic criteria for MS in this study were proposed by the Chinese Diabetes Society, which were more suitable for Chinese people (15) (Table 1). Dyslipidemia was defined as presenting any one of the following four criteria: TC ≥ 6.2 mmol/L, TG ≥ 2.3 mmol/L, LDL-C ≥ 4.1 mmol/L, and HDL-C < 1.0 mmol/L (17).

TABLE 1 Diagnostic criteria of MS recommended by the Chinese Diabetes Society (the diagnosis of MS must meet at least three out of the following four criteria).

Criteria	Parameters
Waist circumference	≥85 cm for female patients
Hyperglycemia	Fasting glucose ≥ 6.1 mmol/L or glucose ≥ 7.8 mmol/L 2 h after glucose load and (or) diabetes has been diagnosed and treated
Hypertension	Systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg, and (or) treatment of previously diagnosed hypertension
Hypertriglyceridemia	Fasting TG ≥ 1.70 mmol/L or fasting HDL-C < 1.04 mmol/L

The secondary outcomes were as follows: (a) the results of general metabolic parameters, including blood pressure, body mass index (BMI), waist circumference, hip circumference, and waist-to-hip ratio (WHR); (b) the results of OGTT, IRT, and blood lipid; and (c) the effects of HA on metabolic disorders.

Statistical analysis

The data in this study were analyzed using SPSS version 20.0. The Kolmogorov–Smirnov test was used to check the normal distribution prior to statistical tests. For normally distributed variables, the continuous variables were presented as mean ± standard deviation and were analyzed by Student's *t*-test, whereas the Mann–Whitney *U*-test was used to analyze non-normally distributed data. The chi-square test was used to analyze enumeration data, which were expressed as number and percentage (%). To further explore the effect of HA on metabolic disorders in women with PCOS, the multivariate logistic regression analysis was used, and the BMI, waist circumference, and WHR were adjusted. Adjusted odds ratios (OR) with 95% confidence intervals as relative effect estimates were calculated. *p*-values <0.05 were considered statistically significant.

Results

Baseline characteristics of the three groups

Table 2 shows the baseline characteristics of women among the three groups. The Ferriman–Gallwey score and serum testosterone (T) concentration were significantly lower in women from the OD-PCOM group compared with women in the OD-HA group and HA-PCOM group ($p < 0.05$). However, no significant difference was found when comparing other baseline characteristics among the three groups ($p > 0.05$).

General metabolic parameters

The data in Table 3 demonstrate the general metabolic parameters among the three groups. The BMI, waist circumference, and WHR in women from the OD-HA group and HA-PCOM group were significantly higher than those of women in the OD-PCOM group ($p < 0.05$), but the above parameters had no statistical difference between the OD-HA group and HA-PCOM group ($p > 0.05$). Moreover, no significant difference was found when comparing blood pressure and hip circumference among the three groups ($p > 0.05$).

Oral glucose tolerance test, insulin release, and blood lipid

Figure 2 reveals the data of OGTT, IRT, and blood lipid among the three groups. The serum insulin concentration and HOMA IR at 2 h and 3 h after oral glucose powder in women from the OD-HA group and HA-PCOM group were significantly higher compared with the OD-PCOM group ($p < 0.05$). Furthermore, the serum TC, TG, and LDL-C in women from the OD-HA group and HA-PCOM group were significantly higher compared with those of the OD-PCOM group ($p < 0.05$). However, no significant difference was found when comparing the above parameters between women in the OD-HA group and HA-PCOM group ($p > 0.05$), and no significant difference was found when comparing blood sugar concentrations among the three groups ($p > 0.05$).

Prevalence rates of metabolic disorders

The prevalence rates of IGT, T2DM, IR, MS, NAFLD, and dyslipidemia of women with PCOS were 17.9%, 3.6%, 58.4%, 29.4%, 46.6%, and 43.4%, respectively. The data in Figure 3 demonstrate the prevalence rates of metabolic disorders among the three groups. The prevalence rates of IGT, IR, MS, NAFLD, and dyslipidemia of women in the OD-HA group and HA-PCOM group were significantly higher compared with women in the OD-PCOM group (22.5% and 20.3% vs. 11.8%, 66.7% and 60.8% vs. 49.1%, 35.5% and 32.2% vs. 21.7%, 52.9% and 49.7% vs. 37.9%, 50.7% and 46.2% vs. 34.8%, respectively) ($p < 0.05$). However, no significant difference was found when comparing the above parameters between women in the OD-HA group and HA-PCOM group (22.5% vs. 20.3%, 66.7% vs. 60.8%, 35.5% vs. 32.2%, 52.9% vs. 49.7%, and 50.7% vs. 46.2%, respectively) ($p > 0.05$). No significant difference was found when comparing the prevalence rate of T2DM among the three groups (5.1%, 2.5%, and 3.5%, respectively) ($p > 0.05$).

Effect of HA on metabolic disorders

To further explore the effect of HA on metabolic disorders in women with PCOS, the BMI, waist circumference, and WHR were adjusted in the multivariate logistic regression analysis. Table 4 shows the effect of HA on metabolic disorders. The data demonstrate that HA was an important risk factor in metabolic disorders in women with PCOS. Serum T concentration (>1.67 nmol/L) and F–G score (>3) significantly increased the risk of metabolic disorders ($p < 0.05$).

TABLE 2 Baseline characteristics of women among the three groups.

Characteristics	OD-HA group (*n* = 138)	OD-PCOM group (*n* = 161)	HA-PCOM group (*n* = 143)	*p*-value [a]
Age (years)	32.9 ± 8.3	33.7 ± 7.9	34.1 ± 9.5	0.769
Ferriman–Gallwey score	4.9 ± 1.7★	2.8 ± 1.0	4.3 ± 1.4★	0.034
Marital status				0.872
Single	21 (15.2)	27 (16.8)	25 (17.5)	
Married	117 (84.8)	134 (83.2)	118 (82.5)	
Smoking	10 (7.2)	13 (8.1)	12 (8.4)	0.935
Family history				
Diabetes mellitus	19 (13.8)	25 (15.5)	20 (14.0)	0.893
Hypertension	16 (11.6)	18 (11.2)	17 (11.9)	0.981
Coronary heart disease	14 (10.1)	15 (9.3)	13 (9.1)	0.951
Thyroid disease	13 (9.4)	14 (8.7)	16 (11.2)	0.757
Basal concentration				
FSH (mIU/mL)	7.3 ± 2.0	6.8 ± 1.9	6.5 ± 1.7	0.665
LH (mIU/mL)	14.8 ± 4.3	11.6 ± 2.7	13.6 ± 3.8	0.413
PRL (ng/mL)	13.5 ± 3.6	12.7 ± 3.3	15.7 ± 4.2	0.338
E_2 (pmol/L)	89.2 ± 17.4	81.3 ± 16.8	95.3 ± 18.4	0.527
P (nmol/L)	0.9 ± 0.5	1.0 ± 0.6	0.8 ± 0.4	0.901
T (nmol/L)	2.4 ± 0.8★	1.2 ± 0.5	2.3 ± 0.9★	0.042
LH/FSH	2.0 ± 0.5	1.7 ± 0.4	2.2 ± 0.6	0.376
AMH (ng/mL)	3.9 ± 0.8	3.6 ± 0.7	4.1 ± 1.2	0.259
History of drug therapy▲				
Antibiotic	20 (14.5)	22 (13.7)	19 (13.3)	0.956
Vitamins	16 (11.6)	19 (11.8)	14 (9.8)	0.834
Sedative-hypnotics	5 (3.6)	7 (4.3)	4 (2.8)	0.770
History of GDM	2 (1.4)	1 (0.6)	2 (1.4)	0.744

[a]Variance analysis or chi-square test. Data given as mean ± SD or number (%).
★Vs. OD-PCOM group. t-test, $p < 0.05$.
▲Drugs used in the last 6 months.
FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin; P, progesterone; E_2, estradiol; T, testosterone; GDM, gestational diabetes mellitus.

TABLE 3 Comparison of general metabolic parameters among the three groups.

Parameters	OD-HA group (*n* = 138)	OD-PCOM group (*n* = 161)	HA-PCOM group (*n* = 143)	*p*-value [a]
Blood pressure				
SBP (mmHg)	105.3 ± 13.2	103.6 ± 12.9	101.2 ± 11.7	0.710
DBP (mmHg)	72.4 ± 8.6	71.3 ± 9.7	69.5 ± 8.4	0.698
BMI (kg/m^2)	25.3 ± 6.5★	22.7 ± 5.1	24.9 ± 5.3★	0.046
Waist circumference (cm)	83.4 ± 14.9★	77.2 ± 15.3	82.5 ± 13.8★	0.039
Hip circumference (cm)	95.2 ± 18.3	96.1 ± 19.2	95.7 ± 17.5	0.457
WHR	0.9 ± 0.4★	0.8 ± 0.3	0.9 ± 0.4★	0.048

[a]Variance analysis. Data given as mean ± SD.
★Vs. OD-PCOM group. t-test, $p < 0.05$.
SBP, systolic blood pressure; DBP, diastolic blood pressure.

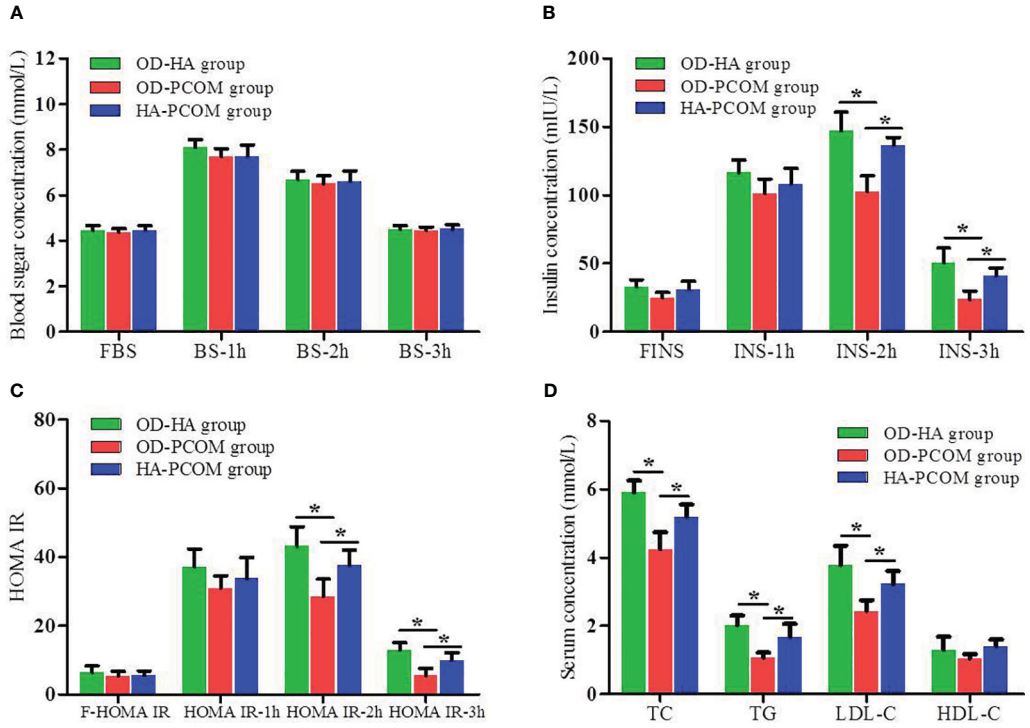

FIGURE 2
Comparison of the results of OGTT, IRT, and blood lipid among the three groups. **(A)** Blood sugar concentration at different time points after oral glucose powder. **(B)** Insulin concentration at different time points after oral glucose powder. **(C)** HOMA IR at different time points after oral glucose powder. **(D)** Blood lipid. (*$p < 0.05$).

Discussion

PCOS is a kind of heterogeneous disease with metabolic disorders. Studies have confirmed that the interaction among genetic factors, metabolic factors, and environmental factors plays an important role in the pathogenesis of PCOS (18). The clinical manifestations of women with PCOS are disparate in different countries, races, and regions, which show the polymorphism of the disease. HA and IR are important links in the pathogenesis of PCOS, which influence each other and form a vicious cycle (19). Furthermore, IR is also the core pathological mechanism of MS (20). Several studies have shown that most women with PCOS presented as IGT, IR and compensatory HI, abdominal obesity, metabolic disorders, and MS (8, 21). Therefore, on the basis of

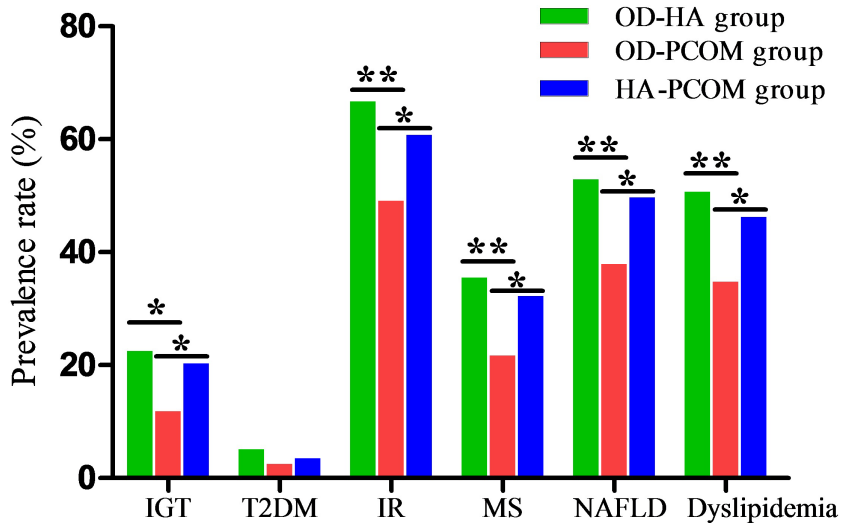

FIGURE 3
Comparison of prevalence rates of metabolic disorders among the three groups (*$p < 0.05$, **$p < 0.01$).

TABLE 4 Multivariate logistic regression analysis of the effect of HA on metabolic disorders in women with PCOS.

	Standardized β	OR	95% CI	*p*-value
IGT				
T (>1.67 nmol/L)	0.438	1.860	1.233–2.867	0.016
F–G score (>3)	0.204	1.572	1.104–2.139	0.021
IR				
T (>1.67 nmol/L)	0.526	2.009	1.236–3.451	0.012
F–G score (>3)	0.709	2.408	1.309–4.287	0.010
MS				
T (>1.67 nmol/L)	0.319	1.670	1.139–2.402	0.020
F–G score (>3)	0.367	1.803	1.208–2.609	0.018
NAFLD				
T (>1.67 nmol/L)	0.178	1.490	1.100–2.009	0.025
F–G score (>3)	0.210	1.621	1.119–2.233	0.021
Dyslipidemia				
T (>1.67 nmol/L)	0.618	2.013	1.239–3.812	0.011
F –G score (>3)	0.478	1.924	1.244–2.987	0.015

F–G, Ferriman–Gallwey.

symptomatic treatment, the long-term complications of women with PCOS should be paid more attention, especially endocrine and metabolic problems.

Our findings indicated that the metabolic characteristics of different phenotypes in reproductive-aged women with PCOS were different. The BMI, waist circumference, and WHR in women from the OD-HA group and HA-PCOM group were significantly higher than those of women from the OD-PCOM group. The serum insulin concentration and HOMA IR of 2 h and 3 h after oral glucose powder in women from the OD-HA group and HA-PCOM group were significantly higher than those from the OD-PCOM group. Furthermore, the serum TC, TG, and LDL-C in women from the OD-HA group and HA-PCOM group were significantly higher than those from the OD-PCOM group. However, no significant difference was found when comparing the above parameters between women in the OD-HA group and HA-PCOM group. In addition, our research also compared the prevalence rates of metabolic disorders among different phenotypes in reproductive-aged women with PCOS. The prevalence rates of metabolic abnormalities were different due to the differences in race, region, lifestyle, age, diagnostic criteria, etc.

Earlier studies of different phenotypes in PCOS displayed that the prevalence of IR and MS in oligomenorrhoeic but normoandrogenemic (PO) women were lower than in PHO women (PCO morphology, hyperandrogenemic, and oligomenorrhoeic), and these results suggested that normoandrogenemic and oligomenorrhoeic women with PCOS are metabolically similar to control women with significantly fewer metabolic features than women with PCOS who are also hyperandrogenemic (22). Data in this study indicated that the prevalence rates of IGT, T2DM, IR, MS, NAFLD, and dyslipidemia of women with PCOS were 17.9%, 3.6%, 58.4%, 29.4%, 46.6%, and 43.4%, respectively. Studies have shown that the prevalence rates of IR and dyslipidemia in women with PCOS were 50–70% and 70%, respectively, and women with PCOS are vulnerable to higher concentrations of TC, LDL-C, and TG (8). The results of investigation from Beijing showed that the prevalence rate of MS in women with PCOS was 31.9% (23). Furthermore, the increased concentrations of TC and TG not only promoted the adverse effect of LDL on metabolism, but also weakened the protective effect of HDL-C on metabolism. Taken together, the above changes in blood lipid could promote the occurrence and development of atherosclerosis and MS. In addition, we also compared the prevalence rates of metabolic disorders among different phenotypes of women with PCOS. Data displayed that the prevalence rates of IGT, IR, MS, NAFLD, and dyslipidemia of women in the OD-HA group and HA-PCOM group were significantly higher than those of women in the OD-PCOM group. However, no significant difference was found when comparing the above parameters between women in the OD-HA group and those in the HA-PCOM group. Moreover, no significant difference was found when comparing the prevalence rate of T2DM among the three groups.

Multivariate regression analysis revealed that HA was an important risk factor in metabolic disorders in women with PCOS. Serum T concentration (>1.67 nmol/L) and F–G score (>3) significantly increased the risk of metabolic disorders. HA plays an important role in metabolic disorders. A number of previous studies have reported that women with PCOS with HA are more prone to metabolic disorders (24). A systematic review in 2020 showed that metabolic disorders in women with PCOS were closely related to HA and IR (25). A study of women with PCOS from the Netherlands found that the occurrence risk of IR and MS increased significantly in women with HA (26). Similarly, Li et al. pointed out that waist circumference combined with free testosterone index (FAI) could be used to predict IR and MS in women with PCOS (27). HA was associated with abnormal fat metabolism. Adipose tissue is the key target of androgen action. Roland et al. reported higher fasting glucose and IGT in prenatally androgenized female mice (28). A recent study reported that the visceral adipose tissue mass of patients with PCOS with HA phenotype was significantly increased, and serum androgen concentration was correlated with IR (29). In brief, the relationship between HA and metabolic disorders is very complex. HA can cause metabolic disorders in several ways, including fat metabolism, glucose-regulating pathways, and islet beta-cell dysfunction (30).

This study still has some limitations. First, the endocrine and metabolic disorders of PCOS are complex. Other androgen metabolic indicators, such as FAI and sex hormone binding globulin, were not detected. Second, some risk factors for metabolic abnormalities were not examined in this study, such as molecular markers of chronic inflammation. Therefore, the accuracy and repeatability of this study need to be proven in the future.

Conclusion

The metabolic characteristics of different phenotypes in reproductive-aged women with PCOS were different. The PCOS phenotypes of OD-HA and HA-PCOM are vulnerable to metabolic disorders compared to OD-PCOM. HA plays a crucial role in increasing the risk of metabolic disorders in women with PCOS. Targeting HA is likely to become an effective approach in the treatment of PCOS metabolic disorders. However, the metabolic characteristics of PCOS are very complex, and it is not just HA that affects metabolic disorders. In fact, HA and metabolic disorders may influence each other and form a vicious cycle. Therefore, the mechanism and specific relationship between HA and metabolic disorders need to be further studied in the future.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by The First Affiliated Hospital of Xi'an Jiaotong University Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

XW: Writing – review & editing, Data curation, Investigation. LW: Writing – review & editing, Conceptualization, Writing – original draft. EB: Investigation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Dairy consumption and its association with anthropometric measurements, blood glucose status, insulin levels, and testosterone levels in women with polycystic ovary syndrome: a comprehensive systematic review and meta-analysis

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Background: We conducted a systematic review and meta-analysis on dairy consumption and its association with anthropometric measurements, blood glucose status, insulin levels, and testosterone levels in women with Polycystic Ovary Syndrome.

Methods: This study conducted a comprehensive literature search using electronic databases like MEDLINE, Scopus, PubMed, Web of Science, and Google Scholar to identify observational and interventional studies investigating the relationship between dairy product consumption and Polycystic Ovary Syndrome. A meta-analysis was performed on clinical trial studies that examined the effect of a low starch/low dairy diet in Polycystic Ovary Syndrome subjects. Statistical analyses were performed using Stata version 16.0 (Stata Corporation, College Station, Texas, USA), and statistical significance was defined as p-value < 0.05.

Results: Of the 1,313 citations reviewed, our systematic review identified 11 studies that met the inclusion criteria, comprising six case-control studies, four clinical trials, and one cross-sectional study. The case-control studies found limited evidence of an association between dairy consumption and Polycystic Ovary Syndrome. The result of the clinical trial studies in meta-analysis showed that reducing dairy intake along with reducing starch intake led to statistically significant improvements in anthropometric and metabolic measures including mean weight (Standardized mean difference: -8.43 (95% CI: -9.01, -7.86)), Body mass index (-3.14 (95% CI: -3.35, -2.92), waist circumference (-6.63 (95% CI: -10.70, -2.57)) and Waist-to-Height Ratio (-0.04 (95% CI: -0.07, -0.01), insulin

fasting (-18.23 (95% CI: -22.11, -14.36)), insulin 120 minutes (-94.05 (95% CI: -157.67, -30.42)), HbA1c (-0.27 (95% CI: -0.37, -0.17)), Ferryman-Gallwey score (-2.07 (95% CI: -2.98, -1.16)) and total testosterone (-9.97 (95% CI: -14.75, -5.19)). No significant reduction was found in fasting glucose, 2 hours glucose, percent of fat mass, and mean free testosterone after intervention.

Conclusions: The findings of this systematic review show limited evidence about the association between dairy consumption and Polycystic Ovary Syndrome. The interventional studies suggest that a low-dairy/low-starch diet may improve some anthropometric and metabolic measures in women with Polycystic Ovary Syndrome.

KEYWORDS

dairy consumption, polycystic ovary syndrome (PCOS), anthropometric measurements, blood glucose, insulin levels, women, systematic review, meta-analysis

Introduction

Polycystic ovary syndrome (PCOS) is a common hormonal disorder in women of reproductive age, characterized by irregular menstrual cycles, elevated androgen levels, and polycystic ovaries (1). The prevalence of PCOS varies between ranging from 4% to 18% (2). The wide range of prevalence can be attributed to the complex pathophysiology of PCOS, the variable clinical presentation, and the lack of adequate evidence-based data due to the existence of several diagnostic guidelines (3).

The exact causes of PCOS remain largely unknown, but it is believed that hormonal imbalances, including an excess of androgens and/or insulin, play a role (4). Additionally, environmental factors such as geography, diet, socioeconomic status, and environmental pollutants may also contribute to the development and management of PCOS (5).

Many women with PCOS are also overweight or obese, and weight loss can be challenging due to insulin resistance and compensatory hyperinsulinemia (6). The management of PCOS focuses on improving both the reproductive and metabolic symptoms (7), and lifestyle modifications, such as physical activity and diet, are critical for improving adverse outcomes associated with the condition (8, 9). Specifically, dietary modifications that reduce hyperinsulinemia may help improve fatty acid oxidation, facilitate weight loss, and prevent further weight gain (10).

Recent research has highlighted the potential significance of dairy products in the diet of women with PCOS, particularly in relation to carbohydrate metabolism disorders such as insulin resistance or type 2 diabetes. Dairy products contain valuable nutrients such as calcium, vitamin D, and high-quality proteins, which can have beneficial effects on overall health (11). Studies indicate that the consumption of carbohydrates from dairy and starch-based foods elicits a higher postprandial insulin response compared to carbohydrates from non-starchy vegetables and fruits (12, 13). To evaluate the differences in dairy consumption in women with and without PCOS and the effect of dairy diets on PCOS complications, a comprehensive systematic review and meta-analysis of all available studies was conducted.

Materials and methods

This study was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. This study was approved by the ethics committee of Alborz University of Medical Sciences with the code IR.ABZUMS.REC.1401.061.

All eligible studies that assessed the relationship between dairy consumption and PCOS, were included in this systematic review.

Search strategy

The review question was formulated using the PECO framework in observational study and PICO in interventional study. Population (P) was women, Exposure (E) in observational study and intervention study (I) was dairy product, Comparison (C) was control group (if applicable) and Outcome (O) was Polycystic Ovary Syndrome. the Search strategy was performed based on PECO and PICO.

A comprehensive literature search was performed in the electronic databases (including MEDLINE/PubMed, Scopus, Web of Science, and Google Scholar) until 17 June 2023 to identify eligible studies investigating the association of PCOS with dairy product consumption. The search was conducted in each database using a combination of the key terms in two domains (1): PCOS and (2) Dairy product (Table 1).

TABLE 1 Search strategy.

PubMed
((Polycystic Ovary Syndrome) OR ("Polycystic Ovary Syndrome" [Mesh])) AND (("Dairy Products" [Mesh]) OR (dairy)) OR ("Milk" [Mesh])) OR (milk)) OR ("Cheese" [Mesh])) OR (Cheese)) OR ("Butter" [Mesh])) OR (butter)) OR ("Buttermilk" [Mesh])) OR (buttermilk)) OR ("Yogurt" [Mesh])) OR (yogurt)) OR (curd)) OR ("Whey" [Mesh])) OR (whey))
Scopus
((TITLE-ABS-KEY (polycystic AND ovary AND syndrome) OR TITLE-ABS-KEY (pcos) OR (TITLE-ABS-KEY (polycystic AND ovary AND syndrome) OR TITLE-ABS-KEY (pcos) AND ((TITLE-ABS-KEY (Dairy Products) OR TITLE-ABS-KEY (dairy) OR TITLE-ABS-KEY (milk) OR TITLE-ABS-KEY (cheese) OR TITLE-ABS-KEY (butter) OR TITLE-ABS-KEY (buttermilk) OR TITLE-ABS-KEY (yogurt) OR TITLE-ABS-KEY (curd) OR TITLE-ABS-KEY (whey) (TITLE-ABS-KEY (dairy)(
ISI/WOS
(Polycystic Ovary Syndrome (All Fields) or (PCOS) AND (dairy (All Fields) or milk (All Fields) or Cheese (All Fields) or butter (All Fields) or buttermilk (All Fields) or yogurt (All Fields) or curd (All Fields) or whey (All Fields))

Selection process

All retrieved articles were imported into EndNote X8.2, and duplicates were automatically removed from the list. Two researchers independently screened titles, abstracts, and full texts, respectively. Furthermore, they examined the reference list of eligible studies to identify any potentially eligible citations. Disagreements between researchers were resolved by discussing the full texts.

Eligibility criteria

Our systematic review included interventional studies that examined the effects of dairy product consumption on PCOS-related complications and met the following criteria (1): the intervention involved a dietary intervention with dairy products, and (2) the study participants were women with PCOS. In addition, observational studies comparing the dairy product intake in women with and without PCOS were also included. Studies that were not written in English, as well as letters to the editor and editorials, were excluded from our analysis."

Data extraction

Data extraction from the included studies was conducted by two researchers using Microsoft Excel 2013. The following information was extracted: author details, country of origin, study design, sample size (for both intervention and control groups), age of participants (mean and standard deviation (SD)), anthropometric measurements (including weight, Body mass index (BMI), waist circumference (WC), and Waist-to-Height Ratio (WHtR)), blood glucose tests (including fasting glucose, 2-hour glucose, percentage of fat mass, fasting insulin, insulin at 120 minutes, and HbA1c), variables used for confounding adjustment, PCOS diagnosis, eligibility criteria, assessment tool, time to event (in weeks), and effect size (mean and SD). Any discrepancies between the two researchers were resolved through discussion and review of the full-text articles.

Quality assessment

Two researchers assessed the quality of the studies using the National Institutes of Health (NIH) quality assessment scales for before-and-after studies without control groups, cross-sectional, and case-control studies (14). Any discrepancies between the researchers were resolved through discussion and review of the full-text articles.

Statistical analysis

To assess heterogeneity among the studies, we used both the Chi-squared and I^2 tests. A random-effects model was employed if the heterogeneity was statistically significant (Chi-squared; P-value<0.10); otherwise, a fixed-effects model was used. We conducted a meta-analysis of clinical trial studies that investigated the impact of a low starch/low dairy diet on PCOS patients before and after the intervention. For these studies, we summarized the mean and standard deviation using the standard mean difference (SMD) and 95% confidence interval (CI). Meta-analysis was performed for the following endpoints: anthropometric measurements, blood glucose levels, Ferryman-Gallwey score, and testosterone levels. All statistical analyses were conducted using Stata version 16.0 (Stata Corporation, College Station, Texas, USA

Results

In the initial literature search, a total of 1,313 citations were retrieved from electronic databases, including 50 from PubMed, 320 from Scopus, and 256 from ISI/Web of Science. An additional 687 citations were identified through a manual search on Google Scholar. After removing the duplicates automatically, 776 studies were assessed using titles and abstracts. Then, 107 studies were evaluated for full texts. Finally, 11 articles were found to be relevant to our topic. Out of the 11 studies, 6 were case-control, 4 were clinical trials, and 1 was cross-sectional. The detailed selection process is illustrated in Figure 1.

The reasons for excluding the studies were as follows: not investigating the intended outcomes of this review study (n= 68), study design (n=15)), not examining dairy products as a separate item (n= 9), being repetitive (n=3), and using non-English language in the text.

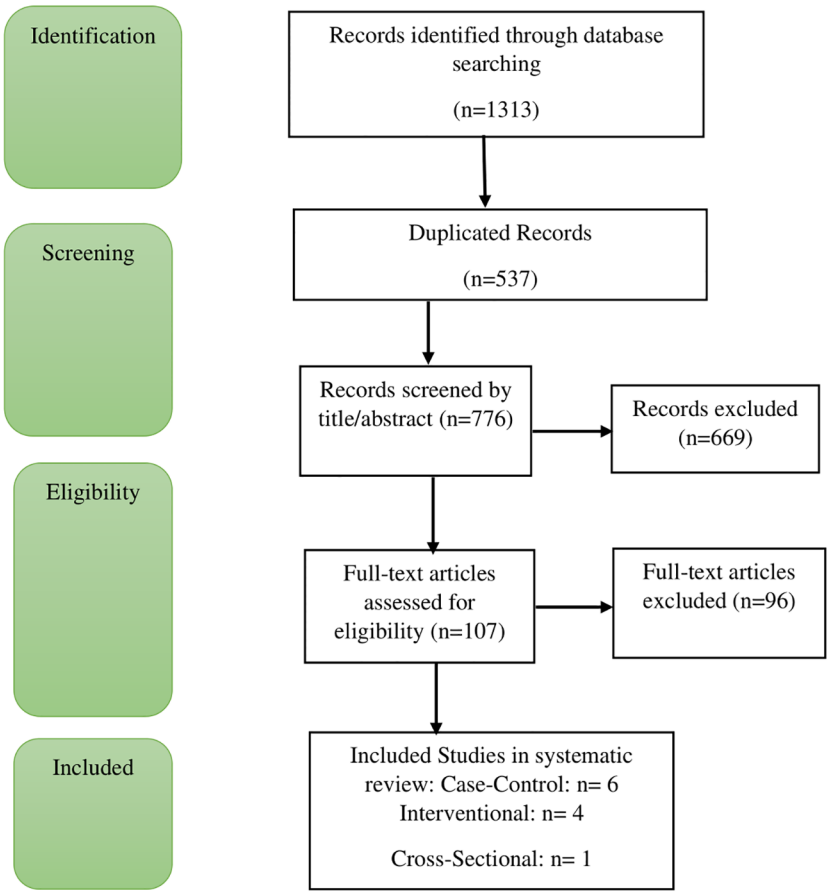

FIGURE 1
PRISMA flowchart of literature search and selection process. Adapted from PRISMA 2020 flow diagram templates, licensed under CC BY 4.0.

Case-control studies

Description

In this systematic review, six case-control studies were reviewed, of which five studies were conducted in Iran and one study in Italy. A total of 1,011 individuals with PCOS and 887 controls with different diagnostic criteria participated in these studies (Table 2).

Among the included studies, two studies used the Androgen Excess Society (AES) criteria for diagnosing PCOS. One study used a clinical diagnosis, while the remaining four studies used the Rotterdam criteria. The dietary intake assessment tool varied among the studies, with four using the Food Frequency Questionnaire (FFQ), one using the United States Department of Agriculture (USDAQ), and another using the 7 Days Food Diary Questionnaire (7DFQ).

The number of participants with PCOS ranged from 99 to 347, while the number of controls ranged from 38 to 291 across the included studies. The eligibility criteria considered in each study are listed in Table 2. The quality assessment of the case-control studies is presented in Table 3. Two studies fulfilled 10 out of the 12 assessment criteria, while four studies fulfilled 9 out of the 12 criteria. All studies fulfilled at least 9 out of the 12 assessment criteria, indicating a high level of quality across the studies.

Main findings

Out of the six case-control studies that were included in study, four of them measured overall dairy consumption. Of these, two studies reported lower dairy consumption in individuals with PCOS compared to non-infected individuals, while the other two studies reported no significant difference. One study examined the consumption of high-fat (p=0.065) and low-fat (p=0.530) dairy products separately, but there was no significant difference between the two groups. The findings from the case-control studies, including those related to dairy and cheese consumption, are presented in Table 4.

In one study, milk consumption was found to be significantly higher in individuals with PCOS after adjusting for confounding factors (P=0.028), but there was no significant difference between the two groups in terms of milk consumption by milk type. In five of the studies reviewed, dairy consumption in the last 12 months was measured using a scale of g/day, while in one study, it was measured over the last 7 days using the same scale. Additionally, the amount of dairy consumption was measured using a scale of g/day in all studies reviewed, except for one study in which it was measured by scoring on a scale of 0-10.

Two studies examined cheese consumption separately. In one study, there was a significant difference in cheese consumption

TABLE 2 Characteristics of the included case-control Studies.

ID	Author	Country	Design	Sample size		Age(year) Mean ± SD		confounders	PCOS diagnosis	Eligibility criteria	Assessment tool
				PCOS	Control	PCOS	Control				
1	Shishehgar et al. (2016) (22)	Iran	CC	142	140	28.5 ± 4.9	28.9 ± 5.8	BMI, age	AES criteria	Non pregnant or not on lactation, without hyper PRL, TD, NCAH, CD, chronic disease (liver, kidney disease, HTN, DM, cancer), Not using of insulin or sensitizing agents, OCP, medication affecting appetite or weight, weight loss diet or exercise	FFQ
2	Hosseini et al. (2017) (19)	Iran	CC	99	198	29.0 ± 5.5	29.2 ± 6.0	BMI, age	AES criteria	-Controls: Healthy women -Case: PCOS without Androgen secreting tumors, congenital adrenal hyperplasia, CD, TD, severe insulin resistance syndrome, DM, Hyper PRL, HTN, CVD, use of androgenic or anabolic drugs	FFQ
3	Badri-Fariman et al. (2021) (20)	Iran	CC	120	120	20-48	20-48	Age, BMI	Rotterdam criteria	-Controls: without PCOS diagnosis, with normal menstrual - Case: PCOS without CVD, liver, and kidney diseases, smoking, taking drugs that can affect the metabolism of hormones and body composition, having strenuous physical activities, not consent to participate	USDAQ
4	Rajaeieh et al. (2014) (23)	Iran	CC	347	38	29.5 ± 4.8	29.5 ± 4.8	–	Clinical D.	Lack of precocious puberty, not having uterus cancer and typical (chronic) diseases, not being pregnant, without specific diet.	FFQ
5	Altieri et al. (2012) (24)	Italy	CC	100	100	27·7 ± 5·2	28·4 ± 5·8	age	Rotterdam criteria	-No other causes of HA such as Hyper PRL, CS, CAH -Exclusion criteria: presence of serious concomitant illness, based on clinical examination and routine laboratory findings, including DM, other endocrine and metabolic disturbances, eating disorders, use of OCP, Insulin-sensitizing, glucose lowering, lipid-lowering or psychoactive medications within 6 months from assessment	7DFQ
6	Zirak Sharkesh et al. (2021) (21)	Iran	CC	203	291	28.98 ± 5.43	30.15 ± 6.21	age	Rotterdam criteria	No history of hypothyroidism, hyper PRL, CS, adrenal hyperplasia, drug use including OCP, hormonal drugs, and glucocorticoids, taking mineral supplements and vitamins, having a special diet for the past 6 months, smoking, alcohol intake, subjects more than a year has passed since their diagnosis of PCOS, pregnant or lactating women underreporting or over reporting of energy Exclusion criteria: intake less than 800 kcal and more than 4200 kcal and not answering more than 40 items in the FFQ	FFQ

CC, Case Control; AES, Androgen Excess Society; PRL, Prolactin; TD, Thyroid Dysfunction; NCAH, Nonclassic 21-hydroxylase deficiency; CD, cushing's disease; HTN, Hypertention; DM, Diabetes Mellitus; OCP, Oral Contraceptive Pills; CVD, Cardiovascular Disease; HA, Hyperandrogenism; CS, cushing's syndrome; CAH, Congenital Adrenal Hyperplasia; FFQ, Food Frequency Questionnaire; USDAQ, United States Department of Agriculture (USDA) Food Security Questionnaire; 7DFQ, 7 Days Food Diary Questionnaire.

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TABLE 3 Quality of the included case-control studies.

Criteria	Shishehgar et al. (22)	Hosseini et al. (19)	Badri-Fariman et al. (20)	Rajaeieh et al. (23)	Altieri et al. (24)	Z Sharkesh et al. (21)
1. Was the research question or objective in this paper clearly stated and appropriate?	Y	Y	Y	Y	Y	Y
2. Was the study population clearly specified and defined?	Y	Y	Y	Y	Y	Y
3. Did the authors include a sample size justification?	Y	Y	Y	Y	Y	Y
4. Were controls selected or recruited from the same or similar population that gave rise to the cases (including the same timeframe)?	Y	Y	Y	Y	Y	Y
5. Were the definitions, inclusion and exclusion criteria, algorithms or processes used to identify or select cases and controls valid, reliable, and implemented consistently across all study participants?	Y	Y	Y	Y	Y	Y
6. Were the cases clearly defined and differentiated from controls?	Y	Y	Y	Y	Y	Y
7. If less than 100 percent of eligible cases and/or controls were selected for the study, were the cases and/or controls randomly selected from those eligible?	N	N	N	N	N	N
8. Was there use of concurrent controls?	Y	Y	Y	Y	Y	Y
9. Were the investigators able to confirm that the exposure/risk occurred prior to the development of the condition or event that defined a participant as a case?	N	N	N	Y	Y	N
10. Were the measures of exposure/risk clearly defined, valid, reliable, and implemented consistently (including the same time period) across all study participants?	Y	Y	Y	Y	Y	Y
11. Were the assessors of exposure/risk blinded to the case or control status of participants?	N	N	N	N	N	N
12. Were key potential confounding variables measured and adjusted statistically in the analyses? If matching was used, did the investigators account for matching during study analysis?	Y	Y	Y	Y	Y	Y

TABLE 4 Comparison of dairy consumption in PCOS and healthy women in case-control studies.

					Findings		
ID	Author	Dairy Type	Unit	Duration		Association direction	Description
1	Shishehgar et al. (22)	• Low fat dairy • High fat dairy	g/day	Past 12 months	NS NS	- -	-No significant difference between PCOS G. and control G. regarding consumption of Low fat dairy [Median (IQR): 206.42 (54.76-351.83) Vs. 187.03(101.10-327.19) g/day, respectively; P value = 0.530] and High fat dairy(g/day) [73.06(13.77-233.41) vs. 108.23(33.11-240) g/day, respectively; P value = 0.065)]
2	Hosseini et al. (19)	Dairy products	Score: 0-10	Past 12 months	+	Inverse	-PCOS G. had a lower mean score compared to control G. (Mean (SD): (4.8 (2.6) vs. 6.0 (2.7), respectively; P value< 0.001)
3	Badri-Fariman et al. (20)	Dairy products	g/day	Past 12 months	+	Inverse	-PCOS G reported a lower dairy consumption compared to control G ((Mean (SD): 2.52 (1.58) vs. 5.69 (1.17), respectively; P value< 0.001)

(Continued)

TABLE 4 Continued

					Findings		
ID	Author	Dairy Type	Unit	Duration		Association direction	Description
4	Rajaeieh et al. (23)	-milk(overall) -Total dairy products -Skim milk -Low-fat milk -Whole milk -Cocoa milk -Other milks -Total milk -Cream -Ice cream -Low–fat yogurt -High–fat yogurt -Total yogurt -Dough -Curd -Cheese	g/day	Past 12 months	Milk(overall): + Others: NS	Direct Others: -	-No significant association between total score of dairy products and odds of PCOS G in univariate and multivariate models. -They only found a significant direct association between milk intake (g/day) and odds of PCOS after adjusting for confounding factors (OR (95% CI): …. P = 0.028).
5	Altieri et al. (24)	-Cheese -Ice cream -Low-fat milk and yogurt -Whole milk and yogurt	g/day	Past 7 days	Cheese: + Others: NS	Direct Others: -	PCOS G. compare to control G. had a higher mean of cheese consumption (Mean (SD): 59·2(37·1) Vs. 49·6(28·8); P value= 0·049), respectively; but no significant differences was observed between two groups regarding other assessed items.
6	Zirak Sharkesh et al. (21)	Dairy products	g/day	Past 12 months	NS	–	No significant difference between PCOS G and control G groups regarding mean dairy products consumption [Mean (SD): 122.23 (97.45) Vs. 139.29 (83.55) g/day, respectively; P value = 0.038]

NS, Not Significant; IQR, Interquartile Range; SD, Standard Deviation.

between individuals with PCOS and controls. In the other study, there was no significant difference observed.

Interventional studies

Description

No studies were identified that specifically examined the effectiveness of reducing or increasing the amount or type of dairy consumption separately. However, four interventional studies were found that evaluated the effect of simultaneously reducing dairy and starch consumption (12, 15–17). The eligible women in these studies were between 18 and 45 years of age. In these studies, participants consumed a high-fat, low-carbohydrate liquid meal (HSFLM) consisting of 8 floz (237 ml) of chocolate Ensure (Abbott Laboratories, Chicago, Illinois), which contained a total of 6 g of fat (1 g of saturated fat (SFA), 3 g of polyunsaturated fat, and 2 g of monounsaturated fat), 40 g of carbohydrate, and 9 g of protein. To increase the proportion of SFA and make the liquid meal an SFA-rich high-fat meal, 32 g of butter, 5 g of coconut oil, and 19 g of palm oil were added to the chocolate Ensure. After the addition of dietary fat and lecithin, the total grams of fat in the high-fat meal equaled 56 g, which constituted 68% of total calories.

All four studies were conducted in the United States and included a total of 51 participants, with a range of 10 to 24 participants per study. In all four studies, PCOS was diagnosed using the Rotterdam criteria. The eligibility criteria for each study are summarized in Table 5. The quality assessment of the before-and-after intervention studies with no control group is outlined in Table 6. All studies fulfilled 10 out of the 12 assessment criteria, indicating a high level of quality across the studies.

Main findings

In the clinical studies included in the meta-analysis, high levels of heterogeneity were observed in the association between reducing dairy product consumption and the mean of anthropometric, blood glucose, Ferryman-Gallwey score, and testosterone level components (p-value<0.001). Therefore, a random-effect model was used to analyze the data. After reducing dairy product consumption, the results demonstrated statistically significant reductions in mean weight (SMD: -8.43 (95% CI: -9.01, -7.86)), BMI (-3.14 (95% CI: -3.35, -2.92)), WC (-6.63 (95% CI: -10.70, -2.57)), and WHtR (-0.04 (95% CI: -0.07, -0.01)) compared to pre-intervention levels. (Figure 2). Furthermore, a notable association was found between reducing dairy product consumption and the mean levels of fasting insulin (-18.23 (95% CI: -22.11, -14.36)), insulin at 120 minutes (-94.05 (95% CI: -157.67, -30.42)), and HbA1c (-0.27 (-0.37, -0.17)). However, no significant reduction was observed in the mean levels of fasting glucose, 2-hour glucose, and percentage of fat mass before and after the intervention (Figure 3).

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TABLE 5 Characteristics of the Included Interventional Studies. (Randomization: no).

ID	Author	Country	Sample size (Loss to FU)	Final sample size	Age(year) Mean ± SD/ Range	PCOS diagnosis	Eligibility criteria	Intervention	Duration (week)
1	Pohlmeier et al. (2014) (12)	USA	13 (3)	10	29.6 ± 4.6	Rotterdam criteria	Overweight and obese women with at least one polycystic ovary by ultrasound, without adrenal enzyme defects, tumors, DM2, late onset 21-hydroxylase deficiency, medical condition requiring supervision, and oligomenorrhea, and not using insulin sensitizers, OCP, cyclic progesterone for one month prior to the study.	A low starch/low dairy diet	8
2	Phy et al. (2015) (15)	USA	28 (4)	24	29.8 ± 4.0	Rotterdam criteria	Overweight and obese women with at least one polycystic ovary by ultrasound, without adrenal enzyme defects, tumors, DM2, late onset 21-hydroxylase deficiency, medical condition requiring supervision, and oligomenorrhea, and not using insulin sensitizers, OCP, cyclic progesterone for one month prior to the study.	A low starch/low dairy diet	8
3	Phy et al. (2013 a) (16)	USA	21 (3)	18	18 to 45	Rotterdam criteria	Women with PCOS aged 18 to 45 years	A low starch/low dairy diet	8
4	Phy et al. (2013 b) (17)	USA	21 (3)	18	18 to 45	Rotterdam criteria	Women with PCOS aged 18 to 45 years	A low starch/low dairy diet	8

FU, Follow Upp; DM, Diabetes Mellitus.

TABLE 6 Quality of the Included Interventional Studies (14).

Criteria	Pohlmeier et al. (12)	Phy et al. (2015) (15)	Phy et al. (2013 a) (16)	Phy et al. (2013 b) (17)
1. Was the study question or objective clearly stated?	Y	Y	Y	Y
2. Were eligibility/selection criteria for the study population prespecified and clearly described?	Y	Y	Y	Y
3. Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest?	Y	Y	Y	Y
4. Were all eligible participants that met the prespecified entry criteria enrolled?	Y	Y	Y	Y
5. Was the sample size sufficiently large to provide confidence in the findings?	Y	Y	Y	Y
6. Was the test/service/intervention clearly described and delivered consistently across the study population?	Y	Y	Y	Y
7. Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	Y	Y	Y	Y
8. Were the people assessing the outcomes blinded to the participants' exposures/interventions?	N	N	N	N
9. Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	Y	Y	Y	Y
10. Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	Y	Y	Y	Y
11. Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	N	N	N	N
12. If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level?	Y	Y	Y	Y

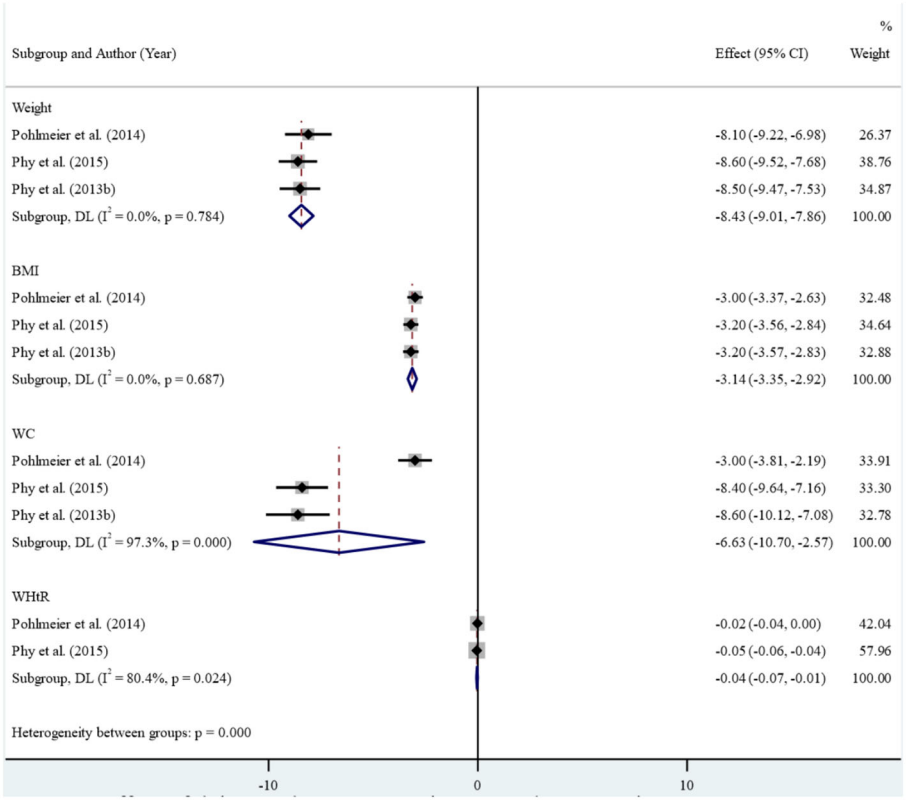

FIGURE 2
Effect of low dairy-low starch diet on anthropometric measurements.

Regarding the Ferryman-Gallwey score and testosterone level components, there was a statistically significant decrease in the mean of total testosterone (-9.97 (95% CI: -14.75, -5.19)) and Ferryman-Gallwey score (-2.07 (95% CI: -2.98, -1.16)) after the intervention compared to before. However, there was no significant association observed between the two groups in terms of the mean of free testosterone after the intervention (Figure 4).

Cross-sectional studies

Description

This cross-sectional study assessed the dietary quality of 100 women diagnosed with PCOS. All participants met the Rotterdam criteria for PCOS diagnosis and were within the reproductive age range of 18–45 years. Anthropometric measurements, including weight, height, WC, BMI, and hip circumference (HC) were obtained. Dietary intake was evaluated using the Brazilian Healthy Eating Index – Revised (BHEI-R), where higher scores reflect better dietary quality (18). The quality of this study was assessed and outlined in Table 7. It fulfilled 10 out of the 14 assessment criteria, indicating a high level of quality.

The prevalence of overweight and obesity was 30.0% and 66.0%, respectively, with 90.0% exhibiting increased visceral fat mass, indicating a heightened risk of metabolic complications. Additionally, 64.0% were categorized as sedentary, while 36.0% were classified as less active (18).

Main finding

The mean BHEI-R score was 56.1 ± 12.0 points, with 56.0% of participants reporting an inadequate diet and 44.0% reporting a diet that needs improvement'. The BHEI-R score showed negative correlations with measures of obesity, including BMI (r = 0.248; P = 0.013), body weight (r = 0.220; P = 0.028), and WC (r = 0.278; P = 0.005).

Discussion

In this study, we reviewed 11 studies that met our inclusion criteria out of the 1,313 citations reviewed. These studies consisted of six case-control studies, four clinical trials, and one cross-sectional study. The meta-analysis of the clinical trials studies revealed statistically significant improvements in various clinically relevant measures when compared to the pre-intervention period. These included reductions in weight, BMI, waist circumference, waist-to-height ratio, fasting insulin, insulin at 120 minutes, HbA1c, and Ferryman-Gallwey score. However, there were no significant changes observed in fasting glucose, 2-hour glucose, percent of fat mass, and mean free testosterone levels between the two groups following the intervention.

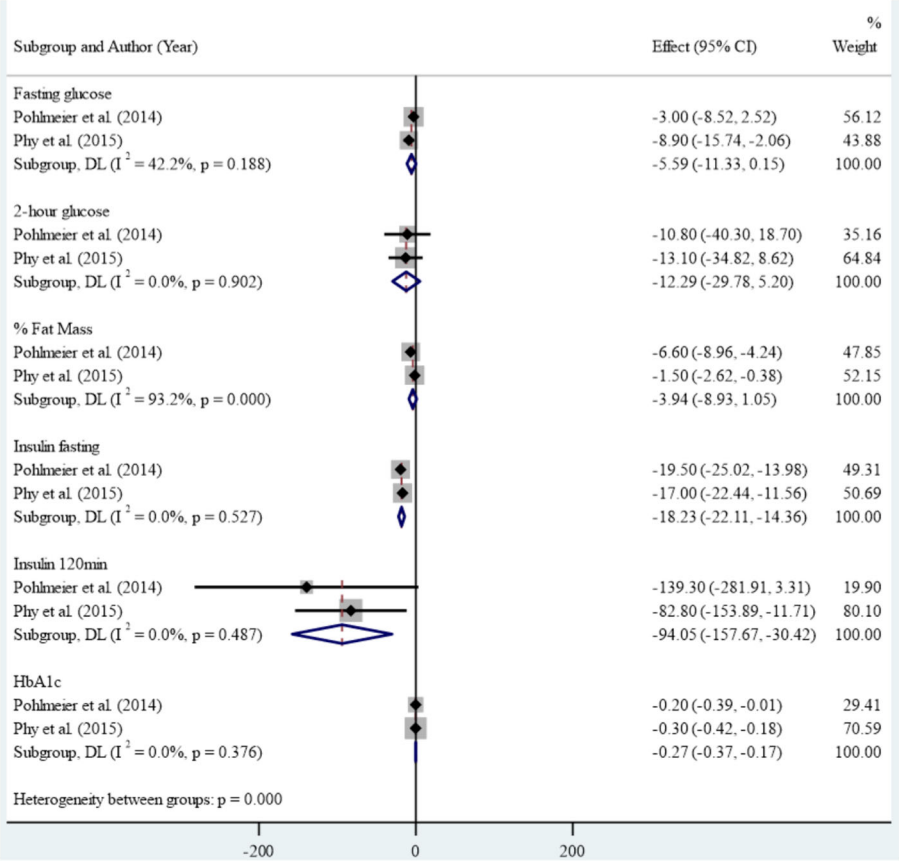

FIGURE 3
Effect of low-diary-low starch diet on blood glucose tests.

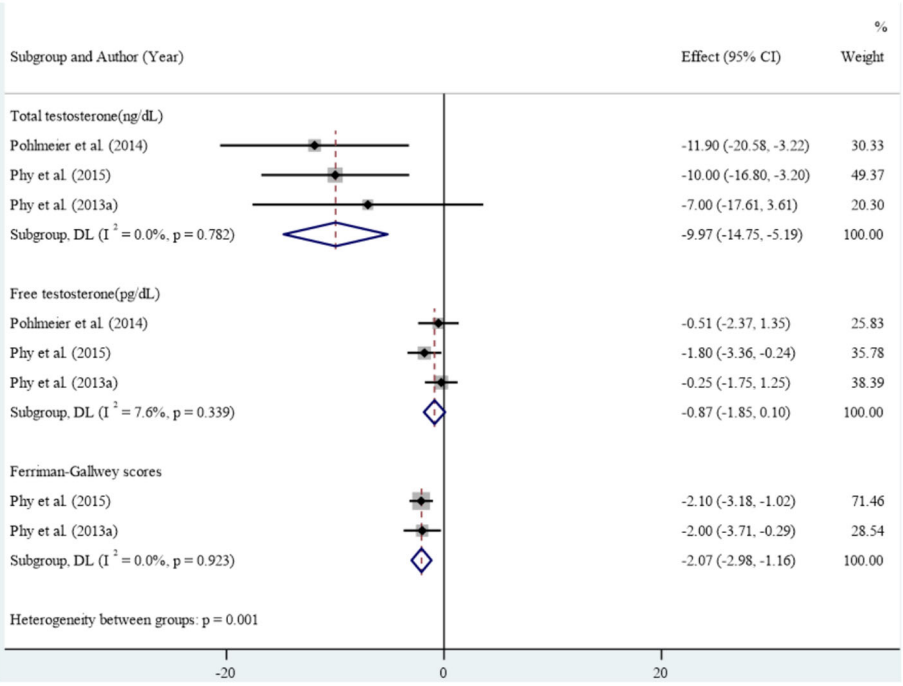

FIGURE 4
Effect of low dairy-low starch diet on Ferrhman-Gallwey score and testosterone level.

TABLE 7 Quality of the Included Cross-Sectional Studies (14).

Criteria	Rodrigues AM
1. Was the research question or objective in this paper clearly stated?	Y
2. Was the study population clearly specified and defined?	Y
3. Was the participation rate of eligible persons at least 50%?	Y
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Y
5. Was a sample size justification, power description, or variance and effect estimates provided?	Y
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Y
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	N
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Y
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Y
10. Was the exposure(s) assessed more than once over time?	N
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Y
12. Were the outcome assessors blinded to the exposure status of participants?	N
13. Was loss to follow-up after baseline 20% or less?	Y
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Y

Main findings

In general, our research suggests that there is controversy regarding association between dairy consumption and PCOS occurrence. Some studies, such as Hosseini et al. and Badri-Fariman et al., detected an inverse correlation between dairy consumption and PCOS (19, 20), while others, such as Zirak Sharkesh et al. and Shishehgar et al., found no significant relationship (21, 22). Rajaeieh et al.'s study showed a direct relationship between milk consumption and PCOS, while Altieri et al. found a direct relationship with cheese consumption (23, 24).

Overall, it is impossible to conclude that dairy consumption is a risk factor for PCOS, and further research is needed to determine whether reducing dairy consumption can improve PCOS symptoms.

All four interventional studies reviewed found that a low-dairy/low-starch diet had a positive impact on anthropometric factors and glycemic control, as demonstrated in the studies conducted by Pohlmeier et al., Phy et al., and Phy et al. b (1, 16, 17). Additionally, the diet was shown to have positive effects on testosterone levels in the studies conducted by Phy et al., Phy et al. a, and Pohlmeier et al. (1, 15, 17) Overall, these studies suggest that a low-dairy/low-starch diet may be effective in improving laboratory and anthropometric factors in patients with PCOS. The results suggest that reducing the intake of dairy and starch in the diet may lead to beneficial impacts on laboratory measures in people with polycystic ovary syndrome.

Janiszewska et al.'s study showed that including whole milk and dairy products in the diet of women with polycystic ovary syndrome could be beneficial due to their positive effect on the risk of developing type 2 diabetes mellitus in women (25). However, this finding was not supported by experimental and clinical studies, which instead showed that reducing dairy and starch consumption had a positive effect on glycemic control, as demonstrated in our study.

Temperament as the final homogeneous quality results from the interactions of opposite qualities- heat, coldness, moisture, and dryness - the four philosophical elements when they combine and form the composite materials of the world. Based on this, everything, including food and medicine and even conditions such as weather and climate, has its temperament, which is determined by the change it imposes on human temperament. Due to this effect, their temperament can cause human disease or be used to maintain health or treat diseases in different people. Large groups of diseases are caused by bad temperaments provoked in a part or the whole human body. Therefore, disease prevention or treatment may be facilitated by recognizing and treating the underlying temperament disorder. The temperament of the whole body or specific organs has been observed by sages with scenarios of signs and symptoms accumulated over the centuries (26, 27).

Iranian medical texts describe milk as consisting of three parts: fat, water, and cheese (carbohydrates and protein). The fat part of milk has a hot and dry temperament, while the cheese part has a cold and dry temperament, and the water has a cold and wet temperament. This means that as milk fat content increases, its temperament becomes warmer, and if the fat content is low, its coldness increases. For example, high-fat milk and dairy products are warmer than low-fat options, and buttermilk and yogurt are colder than milk (28, 29).

In traditional Iranian medicine, PCOS is considered a disease with a cold and wet temperament (30). Therefore, it is important to carefully observe the avoidance of dairy products with a cold and wet temperament in people with PCOS (31).

Chavarro's study suggested that high intake of low-fat dairy foods may increase the risk of anovulatory infertility, while intake of high-fat dairy foods may decrease this risk. Lactose, the main carbohydrate in milk and dairy products is not believed to affect fertility within the usual range of intake levels in humans (32, 33).

Shishehgar's study found that PCOS cases consumed less high-fat dairy than controls, but the difference was not statistically significant (22). However, in other studies, the temperament of the consumed dairy product has not been considered, which may be a reason for the different and contradictory results.

In observational studies, in most cases, the consumption of dairy products has been lower in the polycystic ovary syndrome group, but in interventional studies, reducing dairy consumption has had a positive effect on improving anthropometric and laboratory factors.

To make more accurate and informed decisions regarding the consumption of dairy products in PCOS patients, it is important to conduct both interventional and descriptive studies that take into account the temperament of dairy products. This may help to clarify the relationship between dairy consumption and PCOS symptoms and provide more specific recommendations regarding the types and amounts of dairy products that are safe and beneficial for PCOS patients.

Limitations

One limitation of our study was the absence of intervention studies that solely investigated the impact of reducing or increasing dairy consumption. All four interventional studies that we analyzed focused on the effect of simultaneously reducing dairy and starch consumption. Another limitation was the lack of studies that considered the type of dairy products consumed and the temperament of patients with PCOS. These factors are important in assessing the impact of dairy products on the disease and could influence the results of the studies.

Conclusion

In summary, the findings of this systematic review show limited evidence about the association between dairy consumption and PCOS. The interventional studies suggest that reducing dairy consumption in combination with reducing starch intake may improve some anthropometric and metabolic measures in PCOS women, including weight, BMI, waist circumference, Waist-to-Height Ratio, insulin fasting, insulin 120 minutes, HbA1c. However, more research with larger sample sizes and more diverse populations is needed to fully understand the effects of dairy consumption on PCOS.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

HR: Writing – original draft, Writing – review & editing, Formal Analysis, Methodology, Validation, Visualization. ES: Formal Analysis, Methodology, Validation, Writing – original draft, Writing – review & editing, Software. HH: Methodology, Validation, Writing – original draft, Writing – review & editing, Visualization. MM: Writing – original draft, Writing – review & editing, Data curation, Investigation, Project administration, Resources, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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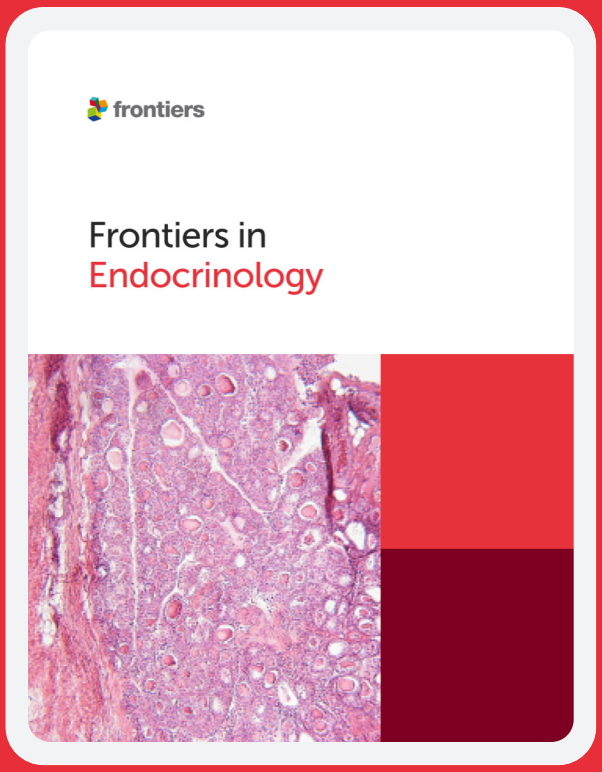

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