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Cysteine mediates the association of essential trace elements with polycystic ovary syndrome: Does ambient particulate matters exposure modify the mediation effect of cysteine

Rui Dong [a,d,1], Shiwei Ren [a,d,1], Xin Wang [b,1], Jing Yuan [c,1], Tao Yin [c], Dongyang Zhang [d], Qian Li [c], Jing Yang [a], Zihan Sun [d], Yajing Liu [c,e,f,g,h,i], Linsheng Yang [d], Fangbiao Tao [d,e,h,i], Zhiguo Zhang [c,e,f,g,h,i,*], Dongmei Ji [c,e,f,g,h,i,j,*], Jing Wei [k,**], Chunmei Liang [a,c,e,f,g,h,i,***]

[a] Department of Public Health Inspection and Quarantine, school of Public Health, Anhui Medical University, No 81 Meishan Road, Hefei, Anhui 230032, China
[b] Department of Ultrasound, the Fourth Affiliated Hospital of School of Medicine, and International School of Medicine, International Institutes of Medicine, Zhejiang University, Yiwu 322000, China
[c] Department of Obstetrics and Gynaecology, the First Affiliated Hospital of Anhui Medical University, No. 218 Jixi Road, Hefei, Anhui 230022, China
[d] Anhui Medical University, No 81 Meishan Road, Hefei, Anhui 230032, China
[e] Key Laboratory of Population Health Across Life Cycle (Anhui Medical University), Ministry of Education of the People's Republic of China, No 81 Meishan Road, Hefei, Anhui 230032, China
[f] Anhui Province Key Laboratory of Reproductive Health and Genetics, No 81 Meishan Road, Hefei, Anhui 230032, China
[g] Anhui Provincial Engineering Research Center of Biopreservation and Artificial Organs, No 81 Meishan Road, Hefei, Anhui 230032, China
[h] Anhui Provincial Institute of Translational Medicine, No 81 Meishan Road, Hefei, Anhui 230032, China
[i] NHC Key Laboratory of Study on Abnormal Gametes and Reproductive Tract (Anhui Medical University), No 81 Meishan Road, Hefei, Anhui 230032, China
[j] Center for Big Data and Population Health of IHM, The First Affiliated Hospital of Anhui Medical University, Hefei, China
[k] MEEKL-AERM, College of Environmental Sciences and Engineering, Institute of Tibetan Plateau, and Center for Environment and Health, Peking University, Beijing 100871, China

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ABSTRACT

Objective: In this study, we aimed to investigate the relationship between essential trace element levels in FF and PCOS and to determine whether this association was mediated by the antioxidant stress indicator cysteine (Cys). In addition, the effect of ambient PM exposure on the mediation effect of Cys was examined.
Methods: A case–control study was conducted, which involved 420 patients with PCOS and 408 controls. The levels of eight trace elements (Cr, Mn, Se, Sn, Cu, Co, Fe, and Zn) and Cys in the FF were detected. The exposure levels of PMs in the participants within 30 days before FF collection were estimated. The combined effect of trace elements on PCOS development was evaluated using Bayesian kernel machine regression (BKMR). Moreover, the major trace elements were selected. Subsequently, the mediating effect of Cys on the linear relationship between elements and PCOS was analyzed using the conventional mediation model, and the mediating effect of Cys on the non-linear association was tested using the piecewise mediation model. Next, the relationships between PMs and Cys were examined using a distributed lag linear model to determine the presence of an inverse association. Finally, stratification analyses of PM exposure were performed to demonstrate that PMs can modify the mediation effect of Cys.
Results: The BKMR indicated that increased levels of trace elements in FF were related to a reduced odds of PCOS. The mediation analysis identified Cys as a significant mediator of the inverse relationships observed between Sn

* Corresponding author at: Department of Obstetrics and Gynaecology, the First Affiliated Hospital of Anhui Medical University, No. 218 Jixi Road, Hefei, Anhui 230022, China.
** Corresponding author.
*** Corresponding author at: Department of Public Health Inspection and Quarantine, school of Public Health, Anhui Medical University, No 81 Meishan Road, Hefei, Anhui 230032, China.
E-mail addresses: zzg_100@163.com (Z. Zhang), jidongmei@ahmu.edu.cn (D. Ji), weijing_rs@163.com (J. Wei), liang_chun_mei@126.com (C. Liang).
[1] These authors have equal contributions to this work.

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(ACME (average causal mediation effect): −0.01 [-0.02, 0.00]), Co (ACME: −0.01 [-0.02, 0.00]), Cu (ACME: −0.01 [-0.03, 0.00]) and PCOS. Furthermore, an inverse relationship was observed between PM exposure and Cys level in FF. Interestingly, stratification analyses revealed that in the lower PM exposure group, Co and Cu were negatively associated with PCOS by positively mediating the Cys level change, with mediation proportions of 14.0 % and 50.6 %, respectively; in contrast, in the higher PM exposure group, the mediation effect of Cys was absent.

Conclusions: Sn, Co, and Cu were found to be negatively associated with PCOS as they positively mediated the changes in the Cys levels. In addition, exposure to PMs may modify the mediation effect of Cys on the above-mentioned negative association. Further research is needed to verify the modifying effect of PM and explore the underlying mechanisms.

1. Introduction

Infertility is defined as the inability to achieve a clinical pregnancy despite 12 months of regular, unprotected sexual intercourse (Gnoth et al., 2005). Ovulatory dysfunction, male factor infertility, and tubal disease are the prevalent causes of infertility (Carson and Kallen, 2021). Polycystic ovary syndrome (PCOS), the most common reproductive endocrinological disorder, affects approximately 20 % of women of reproductive age. The characteristics of PCOS include menstrual irregularities, clinical or biochemical hyperandrogenism, and polycystic ovary morphology (Alesi et al., 2022). PCOS can lead to infertility owing to irregularities in the menstrual cycle; thus, identifying the cause of the condition is crucial. Genetic susceptibility, epigenetics, oxidative stress, nutritional composition, and environmental factors may contribute to the development of PCOS (Stener-Victorin and Deng, 2021).

Essential trace elements are naturally occurring chemical elements that are present in biological tissues. Although they account for only 0.01 % of the body weight, they are indispensable to life. These elements play a crucial role in female reproductive function, being involved in folliculogenesis, ovulation processes, and the regulation of follicle numbers (Pokorska-Niewiada et al., 2021; Tatarchuk et al., 2016; Yin et al., 2024). Currently, the association between essential trace elements and PCOS remains controversial (Kirmizi et al., 2020; Kurdoglu et al., 2012; Sharma et al., 2022). According to a previous study, chronic copper (Cu) overload may cause oxidative stress, alter the hormone levels, and contribute to PCOS (Li et al., 2017). A prospective study observed that women with PCOS exhibited significantly lower manganese (Mn) levels, especially in the presence of insulin resistance (Chakraborty et al., 2013). Furthermore, women with PCOS and insulin resistance have been reported to have significantly lower levels of magnesium (Mg) and chromium (Cr) but higher levels of zinc (Zn) and copper (Cu) in their serum. A case–control study noted that decreased plasma concentrations of selenium (Se) are considerably linked to an increased odds of PCOS (Coskun et al., 2013).

Ambient particulate matter (PM), a mixture of solid and liquid particles suspended in the air, has gradually attracted significant attention in recent years owing to its widespread exposure and serious disease burden on humans (Lelieveld et al., 2015; Li et al., 2021). Lin et al. and Kim et al. documented that higher exposure levels of $PM_{2.5}$ and longer exposure durations exacerbated PCOS (Kim et al., 2022; Lin et al., 2019). An investigation conducted in the Nurses' Health Study II observed that PM exposure was positively correlated with ovulation disorders (Mahalingaiah et al., 2016). An in vivo study stated that $PM_{2.5}$ can induce ovarian dysfunction and cause damage to the ovaries, leading to a decrease in natural fertility (Zhou et al., 2020). Epidemiological and experimental studies have also shown that maternal exposure to $PM_{2.5}$ before or during pregnancy can substantially reduce the antioxidant capacity of reproductive organs and ultimately impair reproductive function (Wang et al., 2021).

Oxidative stress (OS) is a pathological state attributed to the imbalance between the excessive production of reactive oxygen species (ROS) and the body's limited capacity to detoxify them. This imbalance can aggravate the pathogenesis of PCOS, potentially worsening its clinical manifestations and complications (Zeber-Lubecka et al., 2023). Excessive ROS generation is a likely contributing factor to the pathogenesis of PCOS (Agarwal et al., 2012; Gonzalez et al., 2006). Cysteine (Cys), a sulfur-containing amino acid in humans, functions as a monothiol-reducing agent and a biomarker of OS (Nairn et al., 2015). Additionally, Cys is a component of glutathione (GSH), a crucial antioxidant involved in cellular reduction processes and phospholipid metabolism in the liver (Gasmi et al., 2023).

Some essential trace elements, such as Se, Cu, Mn, tin (Sn), Cr, and cobalt (Co), are crucial to many metalloenzymes and proteins involved in cellular metabolism (Hansch and Mendel, 2009; Yin et al., 2024). Mn and Cu superoxide dismutases are responsible for defending against ROS, both requiring their respective catalytic elements (Mn and Cu) for activation (Alfano and Cavazza, 2020; Fukai and Ushio-Fukai, 2011). Se, a cofactor of glutathione peroxidase (GSH-Px), can remove excess peroxides from the human body (Zoidis et al., 2018). Several studies have opined that exposure to PMs can escalate ROS concentrations in the human body, contributing to lipid peroxidation, DNA mutation, and oxidative damage to proteins (Gurgueira et al., 2002; Liu et al., 2022). In addition, evidence on the association between the levels of essential trace elements in FF and PCOS is limited, and the role of Cys in this association remains to be confirmed. Although PMs exposure has been linked to systemic increases in ROS, its influence on Cys levels in FF is not well characterized. Moreover, how PMs exposure modifying the mediating effect of Cys has yet to be elucidated.

To address this research gap, we performed a case-control study of 828 women with infertility. First, we systematically analyzed both individual-element associations and the joint effects of these essential trace-element mixtures on PCOS. Next, we assessed whether these associations were mediated by cysteine (Cys). Finally, we explored whether the mediation effect was modified by ambient PMs exposure. By answering these questions, the study aims to provide new insights for targeted prevention strategies for women with PCOS.

2. Materials and methods

2.1. Study design and population

Between May 2020 and December 2021, a case—control study including 828 female participants (420 PCOS and 408 controls) was conducted at the Reproductive Medicine Center of the First Affiliated Hospital of Anhui Medical University. The inclusion criteria for the case group were as follows: (1) women aged 20–40 years; (2) a diagnosis of PCOS confirmed by the Rotterdam Criteria (Goodarzi et al., 2011), which combine medical history, reproductive hormone profiles, and transvaginal ultrasound results. Participants had to meet at least two of the three Rotterdam-defined PCOS features (Liang et al., 2022): 1) oligoovulation or anovulation; 2) polycystic ovarian morphology via transvaginal ultrasound examination; 3) clinical or biochemical signs of hyperandrogenism. (3) The exclusion criteria was any history of endometriosis, diminished ovarian reserve, recurrent miscarriage, genetic disorders, thyroid dysfunction, endocrine tumors, or autoimmune diseases. The inclusion criteria for the control group were: (1) women attending our reproductive center during the same period whose infertility was attributable to tubal or pelvic factors (e.g., chronic salpingitis, tubal occlusion) or male-factor infertility; (2) age matched to the case group; and (3) clinical evaluation confirming that they did not meet the Rotterdam criteria for PCOS. The control group was subject to the same exclusion criteria as the case group. All participants provided their signed informed consent form, agreeing to utilize their discarded FF samples for scientific research in the present study. The related demographic and clinical information of the participants was obtained from their respective medical records systems and questionnaires. The research protocol was approved by the ethics committee of Anhui Medical University (20160270).

2.2. Exposure assessment

Daily data on PMs (PM_{10}, $PM_{2.5}$, and PM_1) at a spatial resolution of 1 km were collected from the China High Air Pollutants database (CHAP), which provides full coverage and high–quality datasets of ground air pollutants (Wei et al., 2019, 2021a,b). The CHAP databases integrate various sources, including ground measurement data, remote sensing products, atmospheric reanalysis, and artificial intelligence. Based on the latitude and longitude of each female subject's residence, daily average exposure data were assigned for PMs within the 30 days preceding FF collection for each subject.

Monthly data on air temperature and relative humidity at a 1-km resolution were obtained from the National Earth System Science Data Center, National Science & Technology Infrastructure of China.

2.3. Sample collection and element detection

The FF samples were collected during oocyte retrieval, isolated by centrifugation, and stored at –80°C until further determination. The concentrations of Fe, Cr, Co, Zn, Mn, Se, Sn, and Cu in FF were simultaneously detected by inductively coupled plasma mass spectrometry (ICP−MS; PerkinElmer NexION 350X, Shelton, CT, USA). Briefly, 200 μL of FF was diluted to 5 mL with solution 1 (1 % HNO_3+0.05 % TritonX-100). Instrument ionization drift was then corrected with an internal standard solution. The standard curves were configured during detection with a multiple–element standard stock solution (10 μg/mL; Perkin Elmer, USA; N9300233). Multiple metal standard solutions (10 μg/mL; Perkin Elmer; N9303832) were used as internal standards. The determination method was as our previously published study (Wang et al., 2024). The limits of detection (LODs) for Cr, Mn, Co, Cu, Se, Fe, Zn, and Sn were 0.0087, 0.0051, 0.0010, 0.0045, 0.8501, 0.2176, 0.0457, and 0.0010 μg/L, respectively. To evaluate the accuracy and precision of the method, we detected the spiked recovery rate and the intraday and interday precision of the quality control samples (Supplemental Table 2).

2.4. Measurement of Cys

The levels of Cys in the FF samples were measured using the CheKine™ Micro Cysteine Colorimetric Assay Kit. Briefly, 900 μL of the substrate diluent and 100 μL of the substrate were premixed, to which 100 μL of the reaction buffer, 100 μL of the chromogen solution, and 20 μL of the sample were added, and the mixture was incubated at room temperature for 15 min. The absorbance was then measured at 600 nm by using a microplate reader.

2.5. Statistical Analysis

The demographic characteristics of the participants were described as the mean ±standard deviation (SD) or as the number (percentages). The concentrations of trace elements and Cys were reported using the geometric means (GMs) and five selected percentiles (P_5, P_{25}, P_{50}, P_{75}, and P_{95}). The concentrations of eight trace elements below the LODs were assigned a value equal to their LOD divided by $\sqrt{2}$. Trace elements were natural logarithms transformed to normalize the distribution before inclusion in the subsequent statistical models.

Initially, the least absolute shrinkage and selection operator (LASSO) model was applied to obtain a subset of predictors. The 10–fold cross-validation method was applied to select the optimal regularization parameter λ (penalty function) that caused the regression coefficients of some variables to shrink toward zero, obtaining a model with excellent equation-fitting performance and the minimum number of variables (Liu et al., 2023). By selecting the variables corresponding to non–zero coefficients, we can maximize the prediction ability for the target variable, thereby simplifying the model and avoiding collinearity and overfitting (Chen and Yang, 2021). Based on the LASSO model results, Cr, Sn, Cu, Se, Mn, and Co were selected for further analysis. Second, we assessed the odds ratios (ORs) and 95 % confidence intervals (CIs) for the relationships between the ln–transformed concentration of the abovementioned six essential trace elements and PCOS using binary logistic regression, respectively. After deriving the P values for each of the mentioned elements, we derived the corresponding P values after False Discovery Rate (FDR) adjustment. $P_{FDR} < 0.05$ was considered to indicate statistical significance. The restricted cubic spline (RCS) models were adopted to determine whether the abovementioned relationship was linear or non-linear. Then, the joint effect of six elements on PCOS was examined with the Bayesian kernel machine regression (BKMR) model. BKMR is a mixed modeling method that accommodates flexibility and non-linearities. (Bobb et al., 2015; Liang et al., 2022). Briefly, the individual effect of each essential element was estimated by shifting it from its 25th to its 75th percentile while holding the other five elements fixed at their 25th, 50th or 75th percentile. The combined effects of Cr, Sn, Cu, Se, Mn, and Co concentrations were assessed by comparing the effect estimates at the 25th, 30th, …, and 75th percentiles with that at the 50th percentile. In addition, the posterior inclusion probability (PIP), the probability that a given element is included in the mixture, was used to estimate the contributions of each essential trace element to the overall mixture effect on disease, by running a Markov chain Monte Carlo sampler with 20,000 iterations. The mediation effect of Cys in the relationship between Co, Sn, Cu, Se, Mn, and Cr and PCOS was assessed using conventional mediation or the piecewise mediation model as appripriate. The average causal mediation effect (ACME) denotes the effect of an individual element on PCOS via the Cys. $P_{ACME} < 0.05$ indicated a mediating effect.

A generalized linear model (GLM) combined with distributed lag linear (DLM) was used to explore the associations between ambient PMs and Cys. In this model, the exposure–response and lag–response relationships were simultaneously modeled (Gasparrini et al., 2010). The following GLM with DLMs was used: Log (Yt) = α + cb (PMs, lag) + ns (temp, monthly) + ns (relative humidity (RH), monthly) + other covariates, where t was the lag day of investigation; Yt represents the Cys concentration on the lag day t; α was the intercept; cb (PMs, lag) was a cross–basis matrix, wherein a linear exposure–response function was applied to estimate the lag–response function of PMs exposure. The meteorological factors, including temp (temperature) and RH, were used to control for their potential confounding effects. This model included other covariates such as age, infertile type, BMI, occupational type, dysmenorrhea status, education level, and habitation and smoking status (Liang et al., 2022; Wang et al., 2024). For the DLM, we used a natural cubic spline with 3 degrees of freedom and exposure lags 0–30 (Gasparrini, 2011). The optimal model was chosen by minimising the Akaike Information Criterion (AIC). We set the PM exposure level associated with the lowest estimated effect as the reference and examined the percentage change in each FF Cys for every 1 μg/m^3 increase in PM_1, $PM_{2.5}$, or PM_{10}, which is calculated as follows: $100 \times [\exp^{(1\times\beta)}- 1]$. As the participants were from different cities in Anhui Province, PM exposure possibly varied greatly. Therefore, a region–stratified analysis was performed to observe the association of PM exposure with Cys. Based on previous research (Nassan et al., 2021), we investigated the single-day exposure lag effects (lag0–lag30), which revealed that the usage of 31 days in the DLMs was relatively reasonable.

Finally, we performed stratified analyses to examine the modifying effects of PMs exposure status (lower PMs versus upper PMs) on the mediation effect of Cys in the abovementioned associations.

All statistical analyses were performed with SPSS for Windows (version 22.0; IBM® SPSS® Statistics, Armonk, NY, USA) and R software (version 4.3.1; Vienna, Austria). In all tests, a P value < 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the study population

Table 1 presents the general demographic characteristics of the 828 participants. The mean (SD) ages of those involved in this study were 30.53 (3.01) years and 30.37 (3.38) years in the control and PCOS groups, respectively ($P = 0.471$). Half of the participants were of normal weight. The BMI [mean (SD)] in the controls was 22.39 (3.14) kg/m^2, and that in the cases was 23.60 (3.43) kg/m^2 ($P < 0.001$). The proportion of primary infertility (69.52 %) was higher in patients with PCOS than in controls (60.29 %) ($P = 0.005$). However, the other variables did not differ significantly between the two groups. Thyroid function indicators (including thyroid-stimulating hormone, free triiodothyronine, and free thyroxine) of women in the control group with male factors and tubal factors, as well as the case group, are presented in Supplementary Table 1.

Table 1
The general demographic characteristics of participants [Mean ± SD or n (%)].

Variables	Total (n = 828)	Controls (n = 408)	PCOS (n = 420)	*P*
Age (years)	30.45 ± 3.20	30.53 ± 3.01	30.37 ± 3.38	0.471
BMI (kg/m^2)		22.39 ± 3.14	23.60 ± 3.43	**< 0.001**
Underweight (< 18.5)	60 (7.25)	39 (9.56)	21 (5.00)	
Normal (18.5–24.0)	456 (55.07)	253 (62.01)	203 (48.33)	
Overweight/obesity (> 24.0)	312 (37.68)	116 (28.43)	196 (46.67)	
Education				0.376
High school or below (≤ 12 years)	394 (47.58)	204 (50.00)	190 (45.24)	
Junior college (13–15 years)	248 (29.95)	118 (28.92)	130 (30.95)	
University or above (≥ 16years)	186 (22.46)	86 (21.08)	100 (23.81)	
Living area in Anhui province				0.266
Hefei city	243 (29.35)	107 (26.23)	136 (32.38)	
City parallel to Hefei city	129 (15.58)	65 (15.93)	64 (15.24)	
City in the south of Hefei city	148 (17.87)	75 (18.38)	73 (17.38)	
City in the north of Hefei city	308 (37.20)	161 (39.46)	147 (35.00)	
Infertile type				**0.005**
Primary infertility	538 (64.98)	246 (60.29)	292 (69.52)	
Secondary infertility	290 (35.02)	162 (39.71)	128 (30.48)	
Dysmenorrhea				0.295
No	628 (75.85)	303 (74.26)	325 (77.38)	
Yes	200 (24.15)	105 (25.74)	95 (22.62)	
Smoke				0.357
No	476 (57.49)	228 (55.88)	248 (59.05)	
Yes	352 (42.51)	180 (44.12)	172 (40.95)	
Occupation				0.657
Government official and clerk or enterprise clerk	158 (19.08)	80 (19.61)	78 (18.57)	
Technician	116 (14.01)	60 (14.71)	56 (13.33)	
Businessmen	85 (10.27)	38 (9.31)	47 (11.19)	
Manual worker and others	168 (20.29)	90 (22.06)	78 (18.57)	
Unemployment	301 (36.35)	140 (34.31)	161 (38.33)	

3.2. Concentration distributions of essential trace elements in FF of the participants in the PCOS and control groups, and environmental data on PM exposure

The concentration distributions of eight trace elements in FF are furnished in Supplementary Table 2. The GM concentrations of Cu, Se, Sn, Mn, Co, Fe, and Zn in FF of the PCOS group were lower than those in the control group, with Sn, Mn, and Co exhibiting statistically significant differences ($P < 0.001$). Cr in FF of the case group was significantly higher than that of the control group ($P = 0.010$). The Spearman correlation coefficients between the concentrations of Cr, Cu, Se, Mn, Co, Zn, Fe, and Sn are depicted in Fig. 1 and Supplementary Figure 1.

Supplementary Table 3 presents the distributions of PMs, temperature, and humidity. The average concentrations of PM_{10}, $PM_{2.5}$, and PM_1 were 58.29, 30.59, and 18.68 μg/m^3 , respectively, within the 30 days prior to FF collection. Furthermore, a statistically significant difference in PM exposure was not observed between the PCOS and control groups (Supplementary Table 4).

3.3. The screening model based on LASSO regression

In the variable screening analysis, Mn, Co, Sn, Cu, Se, and Cr were selected using the LASSO model. A model with excellent equation-fitting performance and containing the minimum number of variables was obtained when λ was 0.01985562 (log λ = −3.92). Subsequently, further analyses were conducted on the individual and combined effects of the six elements on PCOS (Supplementary Figure 2).

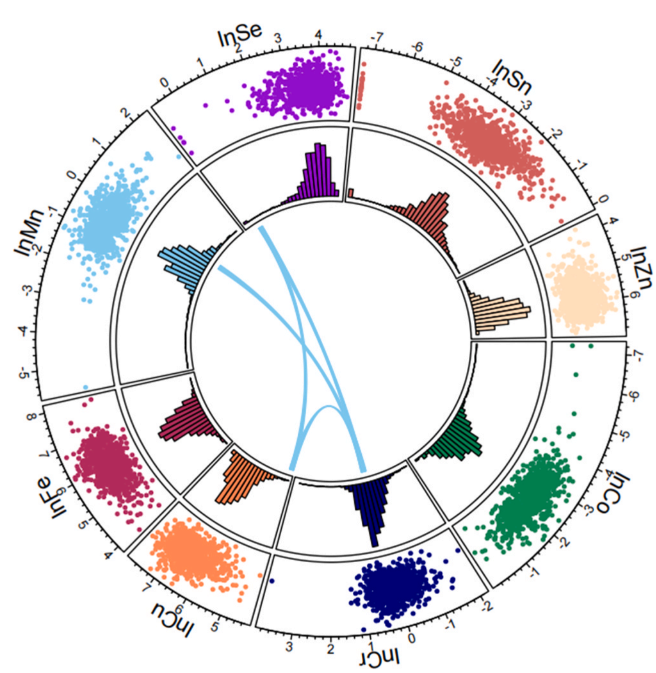

Fig. 1. The levels and correlation of the eight trace elements in study population.

3.4. The levels of six elements and PCOS

The linear or nonlinear associations of ln-transformed levels of Mn, Co, Sn, Cu, Se, and Cr with PCOS are illustrated in Supplementary Figure 3 and Supplementary Table 6. An apparent nonlinear relationship was found between the levels of Mn, Cr, and Sn in FF and PCOS ($P_{nonlinear} < 0.05$). In contrast, a significant linear association was noted between the levels of Co, Cu, and Se in FF and PCOS ($P_{nonlinear} > 0.05$). Supplementary Table 5 presents the associations between the six elements and PCOS when considering them as continuous and categorical variables. Following FDR adjustment for multiple comparisons, six trace elements remained significantly associated with PCOS (Supplemental Table 6). Collectively, the obtained data confirmed that Co, Cu, and Se were negatively linked to PCOS, whereas Mn and Sn exhibited a non-monotonic, U-shaped relationship with PCOS.

3.5. Mixed effects of the six essential trace elements on PCOS based on BKMR

The BKMR model was used to investigate the joint effects of the six-element mixture on PCOS, and the results are presented in Fig. 2A. The results from BKMR indicated a trend of reduced possibility of PCOS with increased element levels, which attained statistical significance when the mixture concentrations were higher than the 60th percentile. In addition, the univariate association between the levels of each of the six elements and PCOS was examined by holding all the other elements constant at the 50th percentile (Fig. 2B). Fig. 2C shows that the 95 % CI of the OR for Cu, Co, Mn, Se, and Sn did not cross 0 and was less than 0, respectivley, indicating negative associations with PCOS. Nonetheless, Cr in FF exhibited a significantly positive association with PCOS. The PIP results are listed in Supplementary Table 6.

3.6. Mediating effect of Cys on the association between the level of each element and PCOS

The adjusted associations between the six elements and Cys in FF, as well as the relationships of Cys with PCOS, are presented in Supplementary Table 7. The elements that fulfilled the conditions for mediation analysis were screened, and mediation analyses were performed for the selected elements. The findings indicated that Cys played a significant mediating role in the association between the levels of Sn, Co, and Cu and PCOS ($P_{ACME} < 0.05$), with mediation proportions of 28.4 %, 13.3 %, and 28.5 %, respectively (Table 2 and Fig. 3). In addition, based on the results of the RCS, stratified analyses were performed on Sn, Mn, and Cr. In both the low and high concentration groups, a significant relationship was found between Sn (all $P < 0.05$) and Cys levels. However, no such association was evident for Mn or Cr (all $P > 0.05$) (Supplementary Table 7). The results of piecewise mediation analysis indicated that Cys significantly mediated the Sn–PCOS association at both low and high Sn levels (low group ACME: 0.01 [0.00, 0.02]; high group ACME: −0.02 [−0.04, 0.00]; total population group ACME: −0.01 [−0.02, 0.00]), precisely revealing the differential mediating effects of Cys across different Sn levels (Supplementary Table 8).

3.7. The association between PM exposure and the levels of Cys in FF based on DLM

To explore whether PM exposure contributed to the alteration in the Cys level, the single-day lag effects of short-term exposure to PMs on Cys were estimated using DLM (Supplementary Figure 5 and Supplementary Table 9). The findings were presented as the percent change (95 % CI) in Cys concentrations with a 1 μg/m^3 increase in PMs at different lag days. In the total population, no association was perceived between exposure to any PM and Cys level (Supplemental Figure 5). However, after stratification analyses, a decreasing trend was observed in the percentage change in Cys in the Hefei region, with a lag of 21–30 days following PM exposure. Specifically, Cys was significantly and negatively associated with PM_1 (lag 27: −0.084 %; lag 28: −0.127 %; lag 29: −0.172 %; and lag 30: −0.218 %), $PM_{2.5}$ (lag 27: −0.042 %; lag 28: −0.065 %; lag 29: −0.089 %; and lag 30: −0.113 %), and PM_{10} (lag 29: −0.031 % and lag 30: −0.038 %) exposure. In the region north of Hefei, a 1 μg/m^3 increment in $PM_{2.5}$ in lag 23 was linked to a 0.026 % decrease in FF Cys. However, no significant association was found between PM exposure and the level of Cys in the region south of Hefei or parallel to it. These findings suggest that PM exposure may be associated with a decrease in the level of Cys in FF.

3.8. PM exposure can modify the mediation effect of Cys

The mean concentrations of higher and lower PM_1, $PM_{2.5}$, and PM_{10} exposure groups were 24.70, 39.51, and 77.08 μg/m^3 and 12.07, 20.60, and 35.77 μg/m^3, respectively (Supplementary Table 11). The demographic characteristics, such as age and BMI, were broadly similar in the higher and lower PM exposure groups (Supplementary Table 10).

Interestingly, in the lower PM exposure group, Co and Cu remained negatively associated with PCOS, positively mediating the change in Cys level, with mediation proportions of 14.0 % and 50.6 %, respectively. In contrast, in the higher PM exposure group, the mediation effect of Cys was absent (Table 2 and Fig. 3). Further exploration of the association between essential trace elements and Cys, using linear regression models and BKMR, revealed that the positive association between certain trace elements and Cys was absent in the higher PM exposure group. Specifically, in the lower PM_1 and PM_{10} exposure group, Co and Cu were positively associated with Cys. However, no such associations were

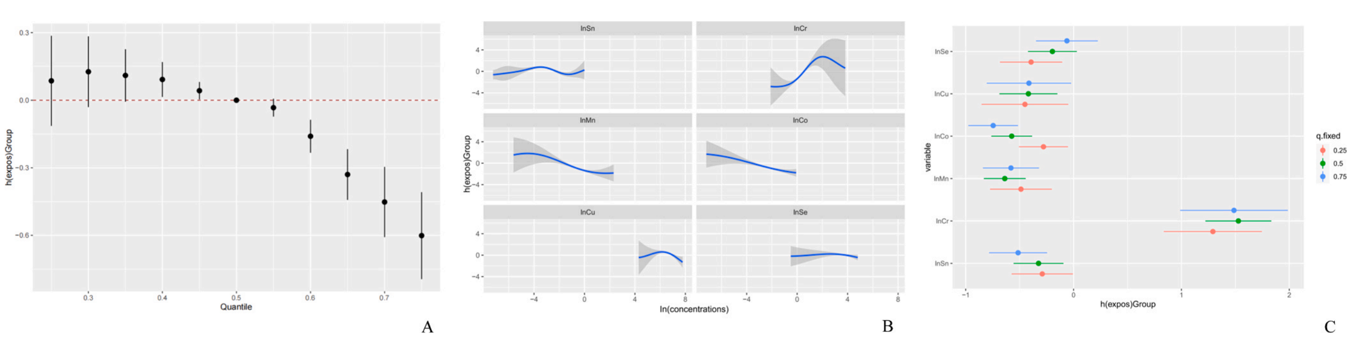

Fig. 2. Joint effect of the mixture (Cr, Cu, Se, Sn, Mn and Co) on PCOS estimated by BKMR model. Note: Age, BMI, education level, occupation of participants, habitation, smoking status, dysmenorrhea, infertile type was adjusted in this model. (A) overall effect of the Cr, Cu, Se Sn Mn and Co [β and 95 % credible intervals (CI)]. The figure plots the estimated change in PCOS when levels are at a particular percentile (ranging from the 25th percentile to 75th percentile) compared to when levels are all at the median. (B) Univariate exposure response function and 95 % credible intervals for Cr, Cu, Se, Sn, Mn or Co with other five elements being fixed at the 50th percentile. (C) Single element association (β and 95 % CI). This plot compares the risk of PCOS when Cr, Cu, Se, Sn, Mn or Co is at the 75th vs. 25th quantile, when all the other elements are fixed at either 25th, 50th, or 75th percentile.

Table 2
Mediation effects of Cys on the associations of essential trace elements with PCOS were modified by PMs exposure (lower versus higher).

Mediation	Element	PMs exposure	ACME	ADE	Total Effect	Proportion Mediated
lnCys	**lnSn**	Total population	−0.01 (−0.02, 0.00) ***P*= 0.018**	−0.03 (−0.05, 0.00) *P* = 0.066	−0.04 (−0.06, −0.01) *P* = 0.024	0.28 (0.03, 0.84) *P* = 0.042
		Lower	−0.01 (−0.03, 0.00) *P* = 0.100	−0.00 (−0.04, 0.04) *P* = 0.830	−0.02 (−0.06, 0.03) *P* = 0.470	0.42 (−3.71, 7.16) *P* = 0.470
		Higher	−0.01 (−0.02, 0.00) *P* = 0.116	−0.04 (−0.06, −0.01) *P* = 0.008	−0.05 (−0.07, −0.02) *P* = 0.002	0.14 (−0.05, 0.54) *P* = 0.118
	lnCo	Total population	−0.01 (−0.02, 0.00) ***P*= 0.006**	−0.07 (−0.08, −0.06) *P* < 0.001	−0.08 (−0.09, −0.07) *P* < 0.001	0.13 (0.04, 0.24) *P* = 0.006
		Lower	−0.01 (−0.03, 0.00) ***P*= 0.016**	−0.07 (−0.09, −0.05) *P* < 0.001	−0.09 (−0.10, −0.06) *P* < 0.001	0.14 (0.02, 0.32) *P* = 0.016
		Higher	−0.01 (−0.02, 0.00) *P* = 0.150	−0.07 (−0.08, −0.04) *P* < 0.001	−0.08 (−0.09, −0.05) *P* < 0.001	0.11 (−0.05, 0.31) *P* = 0.15
	lnCu	Total population	−0.01 (−0.03, 0.00) ***P*= 0.032**	−0.02 (−0.03, 0.01) *P* = 0.088	−0.03 (−0.04, −0.01) *P* = 0.020	0.28 (0.02,1.12) *P* = 0.040
		Lower	−0.02 (−0.05, 0.00) ***P*= 0.030**	−0.00 (−0.03, 0.06) *P* = 0.710	−0.02 (−0.04, 0.04) *P* = 0.310	0.51 (−6.33, 4.27) *P* = 0.320
		Higher	−0.00 (−0.02, 0.01) *P* = 0.492	−0.03 (−0.05, 0.00) *P* = 0.064	−0.03 (−0.04, 0.00) *P* = 0.044	0.11 (−0.34, 0.80) *P* = 0.488

Note: Adjusted for age, BMI, education level, occupation of participants, habitation, smoking status, dysmenorrhea, infertile type. The higher and lower groups of PMs were divided based on the concentration of PM_1.

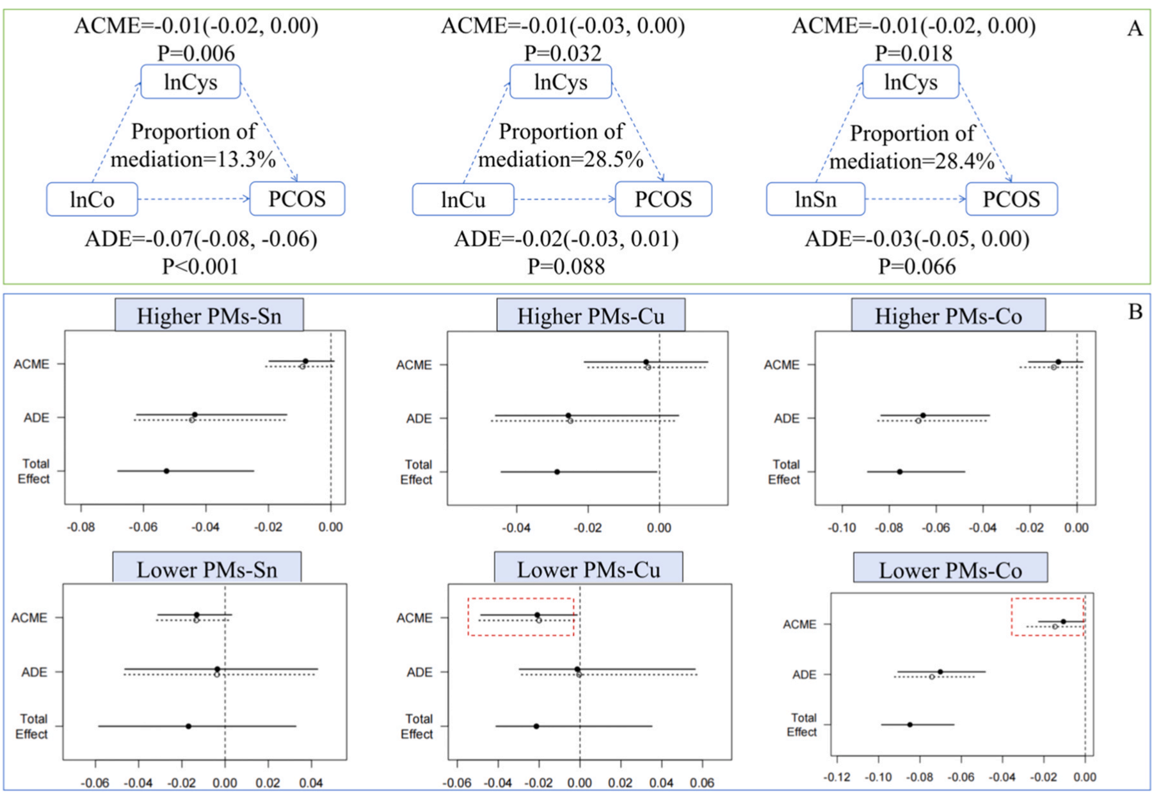

Fig. 3. Adjusted mediation effect of Cys in the relationship between essential trace elements and PCOS. Note: ACME: average causal mediation effect, ADE: average direct effect, TE: total effect. The solid and dotted lines represent the case group and control group, respectively. (A) The Cys mediation effect among all subjects. (B) The Cys mediation effect among PMs exposure in the higher and lower groups. PMs: The higher and lower groups of PMs were divided based on the concentration of PM_1.

evident in higher PM_1 and PM_{10} exposure groups (Supplementary Table 12). Although the BKMR model in the higher PM exposure group showed a positive trend between trace elements and Cys, the significance of this trend was absent (Fig. 4 and Supplemental Figure 4). Thus, higher exposure to PM may mask the protective effect of trace elements on Cys by increasing the OS, exhausting the Cys levels, and interfering with the Cys-mediated pathways.

3.9. *Sensitivity analysis*

As tubal diseases may influence Cys levels, a stratified comparison was performed within the control group (Supplemental Tables 1). This group was categorized into male factor infertility and tubal factor infertility subgroups to evaluate the robustness of the link between trace elements and Cys levels. Furthermore, the association between Cys levels and PCOS was assessed after excluding participants with male factor infertility or tubal factor infertility. Significant differences were not perceived in sensitivity analyses (Supplemental Tables 13 and 14).

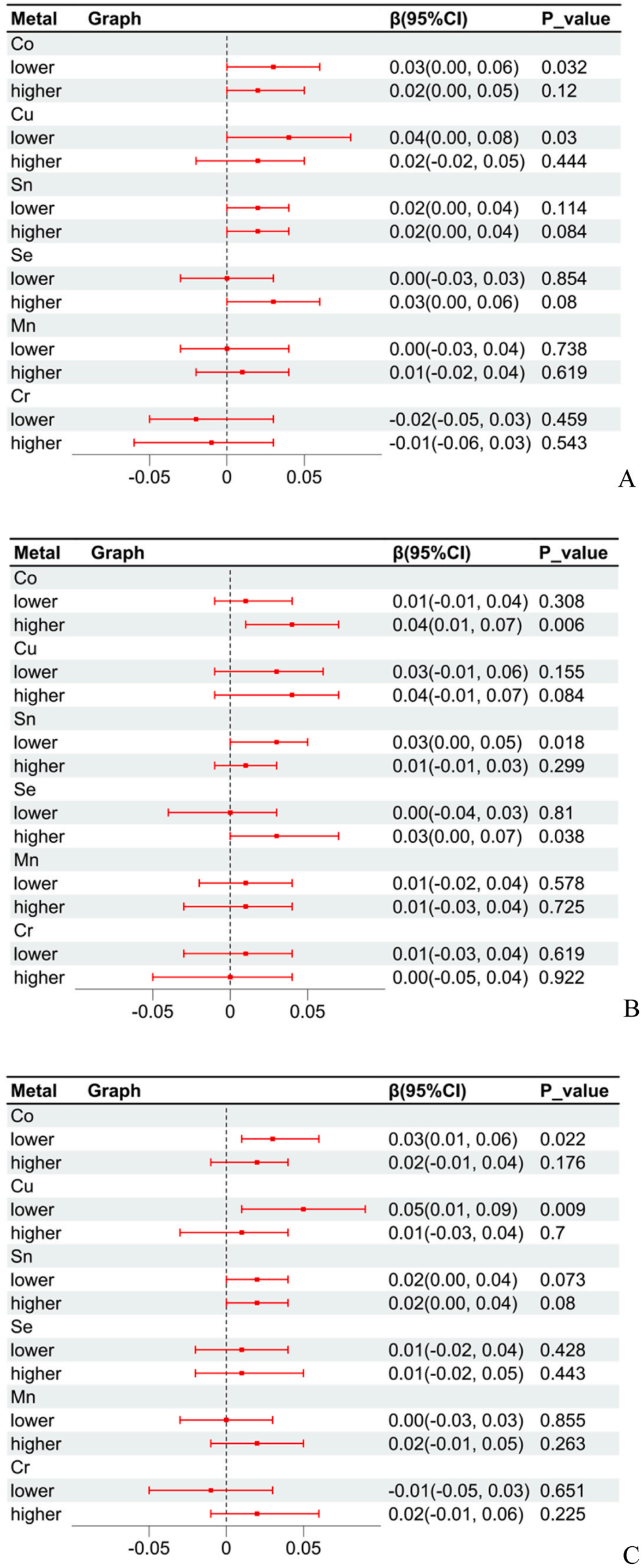

Fig. 4. The association between Cr, Cu, Se, Sn, Mn, Co and Cys level was modified by PMs exposure levels (higher versus lower) via multiple linear regression model. Note: (A) PM_1. (B) $PM_{2.5}$. (C) PM_{10}.

4. Discussion

4.1. Key findings

The findings from this study showed that the concentrations of Mn, Co, Sn, Cu, and Se in FF contributed negatively to PCOS. In contrast, Cr exhibited a positive association with PCOS. Furthermore, a decreased odds of PCOS was evident when the levels of the six-element mixture were all above the 60th percentile. Sn, Co, and Cu were negatively correlated with the possibility of PCOS by positively mediating the change in Cys concentration in FF, with mediation proportions of 28.4 %, 13.3 %, and 28.5 %, respectively. The results from DLMs showed that exposure to PMs during the lag 27–lag 30 days was linked to decreased Cys levels. In addition, PMs exposure modified the mediating effect of Cys in the negative relationship between Co and Cu levels and PCOS. Specifically, in the lower PM exposure status, Co and Cu remained negatively associated with PCOS by positively mediating the change in Cys concentration, whereas in the higher PM exposure group, the mediation effect of Cys was absent.

4.2. Comparison with previous studies

The concentrations of Cr, Co, Sn, Cu, Se, and Mn were evaluated in FF in both patients with PCOS and the control group. The levels of Cu, Mn, and Se were lower than those reported in FF samples from women with infertility in San Francisco, United States (US: 843, 0.82, and 59.4 μg/L, respectively; this study: 656, 0.57, and 35.7 μg/L, respectively) (Butts et al., 2021). Furthermore, the median level of Cu in our participants was lower than that in women with infertility undergoing *in vitro* fertilization at the University of California, San Francisco Center for Reproductive Health (Cu: 1124 μg/L). In contrast, the Mn level was higher (Mn: 0.38 μg/L), and the Co level (Co: 0.09 μg/L) was comparable (Ingle et al., 2017). The differences in Co, Cu, Mn, Se, Cr, and Sn levels between these women with infertility and our study participants could be attributed to differences in race, geographical location, lifestyle, infertility causes, and other factors.

Results from the single-element models suggested that the levels of Co, Sn, Cu, Se, and Mn were related to a decreased odds of PCOS. A case–control study observed that plasma Se and serum Mn concentrations in patients with PCOS were significantly lower than those in the controls (Coskun et al., 2013; Kurdoglu et al., 2012). Kurdoglu et al. reported that serum Co levels did not differ significantly between the two groups (Kurdoglu et al., 2012). However, Sharma et al. revealed that elevated Co, Cu, and Cr levels and decreased Se levels in the serum of patients with PCOS suggest that an imbalance of trace elements may be associated with the pathogenesis of the disease (Sharma et al., 2022). The discrepancies between the results of our investigation and those of the above studies may be attributed to the use of different biological samples and other unknown factors. Furthermore, our research revealed a monotonic and linear negative association between the combined effect of the elements in FF and PCOS, rather than exhibiting a U-shaped relationship. This observation may be explained by the fact that essential element levels in FF are significantly lower than those in serum.

This study observed a negative correlation between PM_1 and $PM_{2.5}$ and Cys concentrations, consistent with previous findings in male cohorts that short-term exposure to $PM_{2.5}$ is associated with decreased Cys levels (Nassan et al., 2021). As the participants in this study were primarily from Hefei and the northern part of Anhui Province, where PM pollution is generally more severe than that in the southern regions, the PM exposure level may explain the regional differences in Cys levels (He et al., 2023; Teng et al., 2022). The following factors may further explain the regional differences: (1) Geographical and climatic factors: Anhui Province spans the Huaibei Plain in the north, the Jianghuai Hills in the center, and the Southern Anhui Mountains in the south (Chu et al., 2024). Compared with the subtropical monsoon climate of the hilly and mountainous topography in Southern Anhui, the temperate monsoon climate of the Huaibei Plain in Northern Anhui significantly drives the dispersion and transport of PM. The dry climate, winter dust storms, and the characteristics of the plain area contribute to the occurrence and spread of particulate pollution in Northern Anhui (Jia et al., 2023). (2) Energy structure and lifestyle factors: in Northern Anhui, coal is the primary energy source for industrial activities, leading to considerable emissions. Moreover, during the winter, the practice of coal-based heating in rural areas of northern Anhui results in a substantial increase in PM exposure (Chen et al., 2021).

4.3. Possible explanations for the findings

Several studies have investigated the association among trace elements, Cys levels, and PCOS; however, the specific mechanisms underlying this relationship remain unclear. The following provides a detailed explanation of the correlation mechanism between essential trace elements and Cys. A recent study further indicates that essential trace elements can activate enzymes critical to female reproductive function (Rayman et al., 2011). GSH-Px, a Se-dependent enzyme involved in antioxidant defense, regulates intracellular hydrogen peroxide levels (Naziroglu, 2009). In *vitro* experiments have reported that Se can reduce ROS formation and increase GSH-Px and GSH levels in the neutrophils of patients with PCOS (Kose and Naziroglu, 2014). Anchordoquy et al. reported that adding Mn to the maturation medium was the primary cause of the reduced DNA damage, reduced frequency of apoptosis, and increased GSH–GSSG contents in oocytes and cumulus cells (Anchordoquy et al., 2014). The antioxidant properties of Mn may play a crucial role in maintaining the integrity of DNA by preventing oxidative damage in cumulus cells. In an animal study, Cu–Zn–SOD deficiency led to increased ROS production in the ovaries, resulting in decreased luteinization and lower serum P4 levels in female mice (Noda et al., 2012). During oocyte maturation, a large GSH reserve with antioxidant properties is crucial for cumulus expansion *in vitro* and for protecting the embryo up to the blastocyst stage (de Matos et al., 1995). Because Cys is the rate-limiting precursor for GSH biosynthesis, its availability directly governs cellular antioxidant capacity. Our study revealed a significant positive relationship between the levels of essential trace elements in FF and Cys concentrations. The finding accorded with the mechanisms described above.

Furthermore, the pathogenesis of Cys and PCOS was further discussed. N-acetylcysteine (NAC) is a highly bioavailable Cys precursor extensively utilized in nutritional supplementation and disease intervention (Samuni et al., 2013; Wu, 2009). Kumar et al. suggested that NAC was associated with mitochondrial function based on its antioxidant capacity (Kumar et al., 2022). Cys may affect mitochondrial function via OS, which has been linked to PCOS (Shukla and Mukherjee, 2020). Moreover, Long et al. reported that conditionally knocking out the *SOD2* gene in mouse oocytes increased the level of ROS in the mitochondrial matrix, resulting in decreased mtDNA copy number (Long et al., 2024), thereby affecting PCOS (Lee et al., 2011; Shukla and Mukherjee, 2020). These findings suggest that trace elements mitigate PCOS by elevating the levels of Cys to maintain mtDNA copy number and enhance mitochondrial function.

According to the findings of an epidemiological study, $PM_{2.5}$ can affect male and female reproductive health by crossing multiple barriers that protect reproductive tissues, accumulating in the reproductive organs and culminating in reproductive dysfunction (Wang et al., 2021). An in vivo study showed that $PM_{2.5}$ exposure triggers OS in ovaries via the NF-κB/IL-6 pathway, resulting in ovarian apoptosis (Zhou et al., 2020). Experimental studies have documented that maternal exposure to $PM_{2.5}$ before conception or during pregnancy could significantly affect the antioxidant capacity of the reproductive system, ultimately disrupting the reproductive function (Wang et al., 2021). In line with our findings, DLM showed that exposure to PM_1 and $PM_{2.5}$ during the 21–30 days before FF collection was negatively associated with the Cys level.

We further clarify the mechanism of the modification effect of PM exposure. Previous studies have established that PM exposure promotes the accumulation of harmful substances including toxic metals, and induces OS (Amador-Munoz et al., 2020; Borgmann et al., 2004). Thus, the body's protective response is activated by significantly increasing metallothionein synthesis, but only at the highest PM levels (Migliaccio et al., 2020; Wong et al., 2017; Pedersen et al., 2014), which requires a substantial amount of Cys (Lakha et al., 2023). In addition, Zhang et al. showed that exposure to $PM_{2.5}$ upregulates the expression of the proinflammatory proteins IL-6 and COX-2 via OS and the TLR4/NF-κB signaling pathway (Zhang et al., 2024). Cys can substantially inhibit the production of proinflammatory cytokines (IL-6) and the expression of adhesion molecules (E-selectin) by suppressing the activation of NF-κB, thus alleviating inflammatory responses (Hasegawa et al., 2012). These processes may divert Cys away from GSH synthesis and into detoxification and anti-inflammatory pathways. Consequently, the positive correlation between trace elements and Cys vanishes, and the Cys-mediated pathway becomes ineffective. This mechanism is consistent with our central finding: the loss of Cys's mediating effect in the high-PM group.

4.4. Strengths and limitations

To the best of our knowledge, this study is the first to integrate ambient PMs (external exposure indicator), essential trace element levels, and Cys (antioxidant) levels in FF to identify critical determinants of PCOS. The results demonstrated that Sn, Co, and Cu in FF were negatively associated with PCOS by positively mediating the change in Cys levels. However, these phenomena were absent in the higher PM exposure group, offering novel insights into how PM exposure interferes with the beneficial roles of essential trace elements and Cys in the FF on PCOS. This study provides a comprehensive understanding of PCOS, emphasizing the moderating role of environmental factors (PM) and the potential impact of OS mechanisms (Cys), offering new directions for future research on PCOS and clinical practice.

However, it is worth noting that the study also has some limitations. First, being a case—control study, it cannot infer the causal relationship among essential trace elements, Cys, PMs, and PCOS. Second, information on PMs (PM_1, $PM_{2.5}$, and PM_{10}) exposure and meteorological factors at the participants' residential addresses was obtained from publicly available environmental datasets. The use of personal monitoring devices may have provided more accurate data on PM concentrations. However, since this is a case-control study that relies on recalling past exposures, it was not feasible to use wearable devices to monitor each participant at the individual level of PM in advance. Third, FF was sampled only from an individual follicle, and the relevant conditions in other follicles could not be analyzed (Butts et al., 2021). Fourth, this study may be subject to information bias. Complete information could not be obtained on the previous medication or antioxidant treatment of the participants. Moreover, detailed individual-level data, such as mask-wearing habits, time–activity patterns, and usage of air purifiers or air conditioning, which could have considerably influenced individual exposure to ambient PM, were not available. Finally, although numerous indicators are available for OS, only Cys was selected as a study factor.

5. Conclusion

Our results showed a negative association between the mixtures of Cr, Mn, Co, Sn, Cu, and Se and PCOS. The level of Cys was significantly and negatively correlated with PCOS, and certain elements (Sn, Co, and Cu) were linked to a decreased PCOS odds by positively mediating the change in Cys level. Furthermore, exposure to PMs modified the mediating effect of Cys in the negative association between Sn, Co, and Cu and PCOS. Further research is needed to verify the modifying effect of PM and explore the underlying mechanism.

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CRediT authorship contribution statement

Fangbiao Tao: Methodology, Conceptualization. **Rui Dong:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Zhiguo Zhang:** Methodology, Conceptualization. **Shiwei Ren:** Visualization, Formal analysis. **Yajing Liu:** Methodology, Conceptualization. **Linsheng Yang:** Methodology, Conceptualization. **Zihan Sun:** Methodology. **Qian Li:** Methodology, Formal analysis. **Jing Yang:** Methodology. **Chunmei Liang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Tao Yin:** Methodology. **Dongyang Zhang:** Methodology. **Dongmei Ji:** Methodology, Conceptualization. **Xin Wang:** Methodology, Data curation. **Jing Wei:** Data curation, Conceptualization. **Jing Yuan:** Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118868.

Data availability

The authors do not have permission to share data.

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