Received: 15 November 2024 | Accepted: 7 March 2025

DOI: 10.1002/rmb2.12644

Reproductive Medicine and Biology

WILEY

ORIGINAL ARTICLE

Serum copper to zinc ratio and risk of endometriosis: Insights from a case–control study in infertile patients

Yanping Liu | Guihong Cheng | Hong Li | Qingxia Meng

Center of Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School of Nanjing Medical University, Suzhou, China

Correspondence
Hong Li and Qingxia Meng, Center of Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School of Nanjing Medical University, Suzhou, China.
Email: hongli@njmu.edu.cn (H. L.) and mqx593204@163.com (Q. M.)

Funding information
Gusu Health Talents Project, Grant/Award Number: GSWS2023012 and GSWS2023111; The National Key Research and Development Program of China, Grant/Award Number: 2022YFC2702901

Abstract

Purpose: Endometriosis is a prevalent gynecological disorder, yet data on the role of trace metal elements in its risk remain limited. We aimed to investigate the relationship between serum copper (Cu), zinc (Zn), iron (Fe), magnesium (Mg) levels, and the Cu/Zn ratio with the risk of endometriosis.

Methods: This study involved 568 infertile patients diagnosed with endometriosis, compared to 819 infertile patients without endometriosis (Control group). Basic characteristics, hormonal parameters, and essential trace elements of the patients were measured and analyzed.

Results: The findings indicated a notable decrease in serum Zn levels in the endometriosis group compared to controls, alongside a significant increase in the Cu/Zn ratio ($p < 0.001$). Restricted cubic spline (RCS) analysis revealed a linear relationship between Zn levels and the Cu/Zn ratio and endometriosis risk. Moreover, Zn levels exhibited a negative correlation with endometriosis risk (p trend = 0.005), while the Cu/Zn ratio displayed a positive correlation with endometriosis risk, even after adjusting for age, body mass index (BMI), and baseline hormones (p trend < 0.001). Compared to the first quartile of Cu/Zn ratio after adjustment, the odds ratios (ORs) with 95% confidence intervals (CIs) for the second and fourth quartiles were 1.97 (1.37, 2.83) and 2.63 (1.80, 3.84), respectively.

Conclusions: This study provided evidence of decreased serum Zn levels and an increased Cu/Zn ratio being associated with an elevated risk of endometriosis among infertile patients. These findings offer valuable real-world data, enriching our understanding of endometriosis.

KEYWORDS
copper, copper/zinc ratio, endometriosis, infertility, zinc

1 | INTRODUCTION

Endometriosis is a prevalent gynecological disorder that significantly impairs fertility among women of reproductive age. Common clinical manifestations of endometriosis include dysmenorrhea, irregular menstruation, dyspareunia, and infertility.[1] Nonetheless, a considerable number of patients are primarily diagnosed with endometriosis upon seeking medical assistance for infertility issues.

Epidemiological studies indicate that the incidence of endometriosis among women experiencing infertility ranges from 30% to 50%.[2,3] Endometriosis exerts multifaceted effects on fertility, attributed to alterations in pelvic anatomy, compromised ovarian reserve, oocyte and embryo quality deterioration, and disrupted endometrial receptivity.[4,5] The pathological mechanisms of endometriosis involve various factors such as aberrant inflammatory response, hormonal imbalance, genetic predisposition, and immune dysregulation, yet they remain elusive.[6]

Trace metal elements play crucial roles in various biochemical processes in the human body, garnering increasing attention for their involvement in the pathogenesis of endometriosis. Hall et al.[7] performed a cross-sectional study utilizing NHANES 1999–2006 data, revealing a link between urinary cadmium concentrations and endometriosis prevalence in the U.S. population. Likewise, Shen et al. found that concentrations of arsenic, cadmium, lead, and mercury in both serum and follicular fluid were positively correlated with an increased risk of endometriosis.[8] Additionally, Lai et al.[9] noted a correlation between decreased Zinc (Zn) levels and a higher risk of endometriosis in infertile women. Conversely, Su et al.[10] observed that elevated Zn levels in both blood and follicular fluid were linked to an increased risk of endometriosis. This inconsistency in research findings regarding the relationship between Zn levels and the risk of endometriosis may be attributed to differences in study design, sample size, characteristics, and analytical methods.

Zn, an essential trace nutrient, acts as a crucial modulator of immune function, exhibiting potent antioxidant and anti-inflammatory properties vital for maintaining cellular redox homeostasis.[11] Zn deficiency compromises the activity of antioxidant enzymes, exacerbating oxidation stress (OS) conditions.[12] Conversely, copper (Cu) exhibits multifaceted involvement in OS. By catalyzing the Fenton reaction, Cu promotes the production of reactive oxygen species, which can subsequently induce lipid peroxidation, thereby amplifying OS responses.[13] Moreover, Cu participates in redox reactions, leading to the depletion of the antioxidant glutathione and disruption of cellular antioxidant defense mechanisms.[14] Both Cu and Zn serve as necessary enzyme cofactors in antioxidant defenses and neurotransmitter synthesis.[15] The intricate balance and levels of Cu and Zn within cells are pivotal for maintaining redox balance and regulating OS. The human body intricately manages and regulates the levels and proportions of trace elements crucial for blood circulation and cellular storage. When the system malfunctions, abnormalities in the levels or ratios of metal ions arise. In clinical settings, the Cu/Zn ratio holds more significance than the concentrations of these metals individually.[16,17] The potential significance of OS in the development of endometriosis is attracting attention.[18,19] Imbalances in the ratio of Cu to Zn can lead to pathological conditions associated with OS.[20,21] Nevertheless, limited and contentious research concerning the association between serum Zn levels and the Cu/Zn ratio with endometriosis exists.

In this context, we conducted a retrospective analysis of the distribution characteristics of Cu, Zn, iron (Fe), and magnesium (Mg) levels, as well as the ratio of Cu to Zn among 1387 infertility patients. Additionally, we investigated the correlation between these elements and the risk of endometriosis.

2 | MATERIALS AND METHODS

2.1 | Subjects

This case–control study was conducted at the Reproductive and Genetic Center of Suzhou Municipal Hospital (Suzhou, Jiangsu Province, China). Detailed clinical data were retrieved from the Clinical Reproductive Medical Management System. The participant recruitment process is illustrated in Figure 1. From January 2018 to December 2022, women undergoing their first in vitro fertilization (IVF) cycle due to tubal factor, male factor, or endometriosis-related infertility were enrolled. Written informed consent was obtained from all participants, and the study protocol was approved by the institutional ethics committee.

Based on clinical, imaging, and surgical findings, participants were categorized into the endometriosis group or the control group. The inclusion criteria for the endometriosis group required fulfillment of at least one of the following conditions: (1) laparoscopic and histopathological confirmation of endometriosis; or (2) a high clinical suspicion of endometriosis, with characteristic imaging findings on transvaginal ultrasound (TVUS) or magnetic resonance imaging (MRI), such as ovarian endometriomas or deep infiltrating endometriosis. The control group consisted of women with no history of endometriosis, as confirmed by medical records and the absence of endometriotic lesions on TVUS or MRI. For individuals who had undergone laparoscopic surgery for other indications, the absence of intraoperative evidence of endometriosis was required for inclusion.

Exclusion criteria for both groups included the presence of concomitant gynecological disorders that could confound the study outcomes, such as hydrosalpinx, salpingitis, acute pelvic inflammatory disease, and ovarian malignancies. Additionally, women with systemic diseases known to affect immune or metabolic function, including polycystic ovary syndrome, hyperprolactinemia, hypogonadism, thyroid dysfunction, autoimmune diseases, or chromosomal abnormalities, were excluded. Other exclusion criteria encompassed a history of smoking, long-term medication use, or hormonal therapy (such as gonadotropin-releasing hormone agonists, oral contraceptives, or progestins) within the past 6 months. These factors were excluded due to their potential impact on estrogen metabolism, ovarian function, and systemic inflammatory responses, which could introduce bias and confound the interpretation of study findings.

2.2 | Hormone and trace metal elements assays

Serum samples collected during the early follicular phase (7.00–9.00 a.m.) were utilized to measure the baseline levels of sex

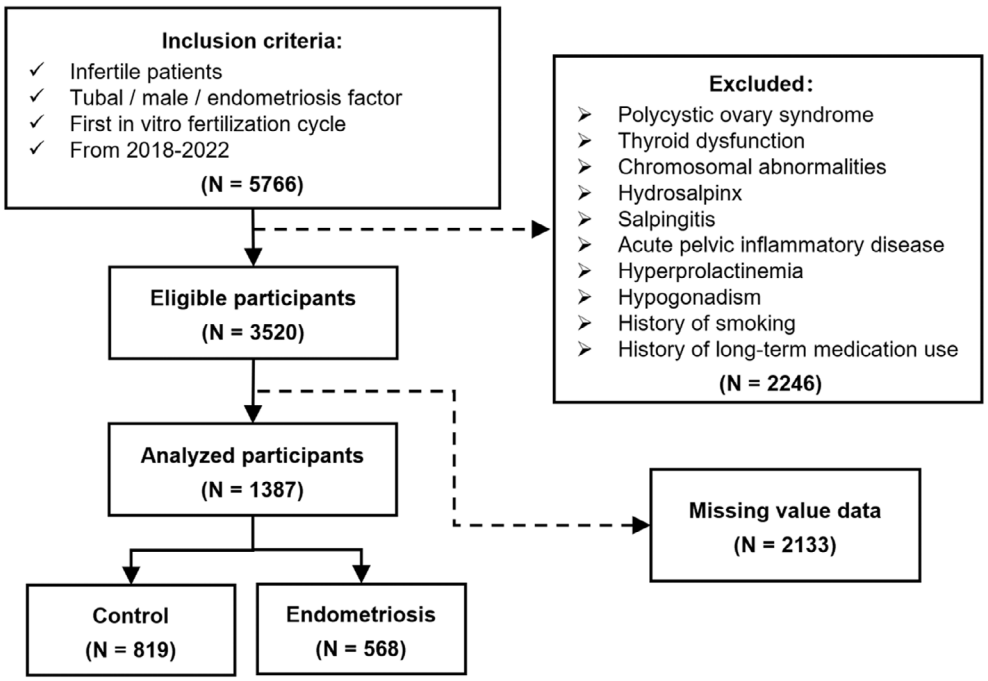

FIGURE 1 Flow chart of recruitment of participants in the study.

hormones. Hormone levels, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), estradiol (E2), progesterone (P), prolactin (PRL), and anti-Müllerian hormone (AMH), were assessed using an automated electrochemiluminescence immunoassay system (Hitachi Model 7170, Japan). Participants fasted overnight before blood collection, and serum samples were separated for subsequent analysis of trace metal concentrations. The concentrations of copper (Cu), zinc (Zn), iron (Fe), and magnesium (Mg) were measured using specific assay kits according to the provided experimental protocols. The products/kits being utilized are described as follows: Quick Auto Neo Cu kit (SHINO-TEST CORPORATION, Japan), Quick Auto Neo Fe kit (SHINO-TEST CORPORATION, Japan), Zn Assay Kit (Metallogenic Co., Ltd., Japan), and Mg-HR II Kit (FUJIFILM, Japan).

2.3 | Statistical analyses

The software package for social science statistics (SPSS, version 27) was used for the statistical analysis. The normality of data distribution for continuous variables was assessed using the Shapiro–Wilk test. For data with a normal distribution, we presented mean values along with their standard deviations (SD) and compared them using the *T*-test. For data not following a normal distribution, we reported median values along with the 25th and 75th percentiles and conducted comparisons using non-parametric tests (Mann–Whitney *U* test) for continuous variables between groups. Categorical data were expressed as proportions or percentages (%), with intergroup comparisons conducted using the chi-square test. RCS was employed to examine the linear correlation between trace metal levels and the risk of endometriosis. RCS was plotted using the rms package in R (version 4.2.1), with the 25th, 50th, 75th, and 95th percentiles chosen as fitting nodes for each parameter. Logistic regression was utilized to compute odds ratios (ORs) and 95% confidence intervals (CIs) to evaluate the correlation between levels of trace metals and the risk of endometriosis. Continuous variables were transformed into categorical variables based on quartiles, and p for trend tests were conducted.[22] A significance threshold of $p < 0.05$ was applied for determining statistical significance.

3 | RESULTS

3.1 | Baseline profile of study participants

Out of 1387 patients experiencing infertility, 568 were diagnosed with endometriosis (Endometriosis group), while 819 were identified as the Control group, which included cases of tubal factor infertility or male factor infertility. Table 1 displays the clinical and biochemical profiles of the participants. No significant differences were observed between the two groups regarding mean age and duration of infertility (all p-values > 0.05). The mean body mass index (BMI) was significantly lower in the Endometriosis group (21.39 ± 2.74) compared to the control group (22.15 ± 3.06), with a p-value less than 0.001. There was a significant difference in the type of infertility, with 58.80% of individuals in the Endometriosis group experiencing primary infertility, compared to 41.50% in the control group ($p < 0.001$). Regarding baseline hormones, the Endometriosis group exhibited significantly higher levels of FSH ($p = 0.003$) and

TABLE 1 Baseline characteristics of Control and Endometriosis groups.

Variables	Control, $N=819$	Endometriosis, $N=568$	$t/z/\chi^2$	p
Age (years)	30.77 ± 3.52	30.79 ± 3.37	−0.068	0.946
Age strata (%)			1.388	0.239
≤35	88.90 (728/819)	90.80 (516/819)		
>35	11.10 (91/819)	9.20 (52/819)		
BMI (kg/m^2)	22.15 ± 3.06	21.39 ± 2.74	4.861	<0.001
BMI strata (%)			12.676	0.002
<18.5	9.77 (80/819)	12.50 (71/568)		
18.5–24.9	72.77 (596/819)	76.58 (435/568)		
>24.9	17.46 (143/819)	10.92 (62/568)		
Type of infertility (%)			40.132	<0.001
Primary	41.50 (340/819)	58.80 (334/568)		
Secondary	58.50 (479/819)	41.20 (234/568)		
Infertile duration (years)	3 (2, 4)	3 (2, 4)	−1.2	0.23
Baseline hormones				
FSH (mIU/mL)	7.56 ± 2.00	7.91 ± 2.33	−2.962	0.003
LH (mIU/mL)	4.83 ± 2.67	4.96 ± 4.29	−0.692	0.489
E2 (pg/mL)	38.0 (29.0, 51.0)	41.0 (30.0, 55.7)	−2.49	0.013
P (ng/mL)	0.59 (0.42, 0.80)	0.60 (0.45, 0.79)	−0.313	0.754
PRL (ng/mL)	15.27 (11.47, 20.68)	15.67 (11.82, 20.52)	−0.621	0.535
T (ng/mL)	0.48 ± 0.19	0.47 ± 0.21	1.495	0.135
AMH (μg/L)	3.70 (2.43, 5.39)	3.48 (2.27, 4.81)	−2.143	0.032

Abbreviations: AMH, anti-mullerian hormone; BMI, body mass index; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; P, progesterone; PRL, prolactin; T, testosterone.

E2 ($p=0.013$) compared to the control group. Conversely, the Endometriosis group exhibited a significantly lower median level of AMH compared to the control group [3.48 (2.27, 4.81) vs. 3.70 (2.43, 5.39), Endometriosis vs. control, μg/L; $p=0.032$]. Levels of LH, P, PRL, and T did not exhibit significant differences between the two groups (all p-values > 0.05).

3.2 | Distribution of serum metal element levels among study participants

The distribution of serum metal element concentrations was presented in Table 2. Specifically, the levels of serum Cu, Zn, Fe, and Mg are displayed as the minimum, 25th, 50th (median), 75th, and maximum values. The median Zn concentration showed a significant decrease in the endometriosis group compared to the control group (14.6 vs. 15.1 μmol/L, Endometriosis vs. Control; $p<0.001$). However, the levels of three other metallic elements—Cu ($p=0.226$), Fe ($p=0.363$), and Mg ($p=0.083$)—exhibited comparable values across both groups. Of particular note, the endometriosis group exhibited a significantly higher Cu-toto-Zn ratio compared to the control group (median of Cu/Zn %: 107.39 vs. 104.83, Endometriosis vs. Control; $p<0.001$).

3.3 | Correlation of serum metal levels and the risk of endometriosis

To determine whether there was a linear correlation between serum metal levels and the risk of endometriosis, we conducted restricted cubic spline analyses (RCS) after controlling for confounding factors (age, BMI, and baseline hormone levels). As shown in Figure 2, Zn levels and the Cu/Zn % exhibited a linear relationship with the risk of endometriosis (Zn: P for nonlinearity = 0.154; Cu/Zn %: P for nonlinearity = 0.164). Moreover, Zn exhibited a negative association with the risk of endometriosis, whereas the Cu/Zn % demonstrated a positive association. Cu, Fe, and Mg levels showed a non-linear relationship with the risk of endometriosis.

We further evaluated the ORs and 95% CIs for the association between serum metal element levels and the risk of endometriosis (Table 3). Initially, we categorized serum metal element levels into quartiles. Subsequently, we conducted an ordinal trend test using logistic regression models constructed with the median of serum metal element levels and Cu/Zn ratio quartiles. Cu, Fe, and Mg levels exhibited no notable correlation with the risk of endometriosis, whether in univariate or multivariate analysis (all p trend > 0.05). Conversely, serum Zn levels were inversely correlated with the risk of developing endometriosis (p trend = 0.005). Compared to individuals

TABLE 2 Distribution of serum metal element concentrations in Control and Endometriosis groups.

	Control, $N=819$					Endometriosis, $N=568$					
Elements	**Min**	**P_{25}**	**P_{50}**	**P_{75}**	**Max**	**Min**	**P_{25}**	**P_{50}**	**P_{75}**	**Max**	***p***
Cu (μmol/L)	5.4	13.95	15.6	17.45	37	7.57	14.27	15.77	17.49	48.12	0.226
Zn (μmol/L)	8.9	13.6	15.1	16.8	26.5	5.6	13	14.6	16.5	27.1	<0.001
Fe (μmol/L)	1.0	11.2	14.8	19.4	41.8	1.4	10.45	14.93	19.1	71.9	0.363
Mg (mmol/L)	0.69	0.86	0.91	0.95	1.27	0.71	0.87	0.92	0.97	1.21	0.083
Cu/Zn (%)	29.67	88.89	104.83	119.31	235.77	42.53	93.14	107.39	125.89	291.60	<0.001

Abbreviations: Cu, copper; Fe, iron; Max, maximum; Mg, magnesium; Min, minimum; P_{25}, 25th percentiles; P_{50}, 50th percentiles; P_{75}, 75th percentiles; Zn, zinc.

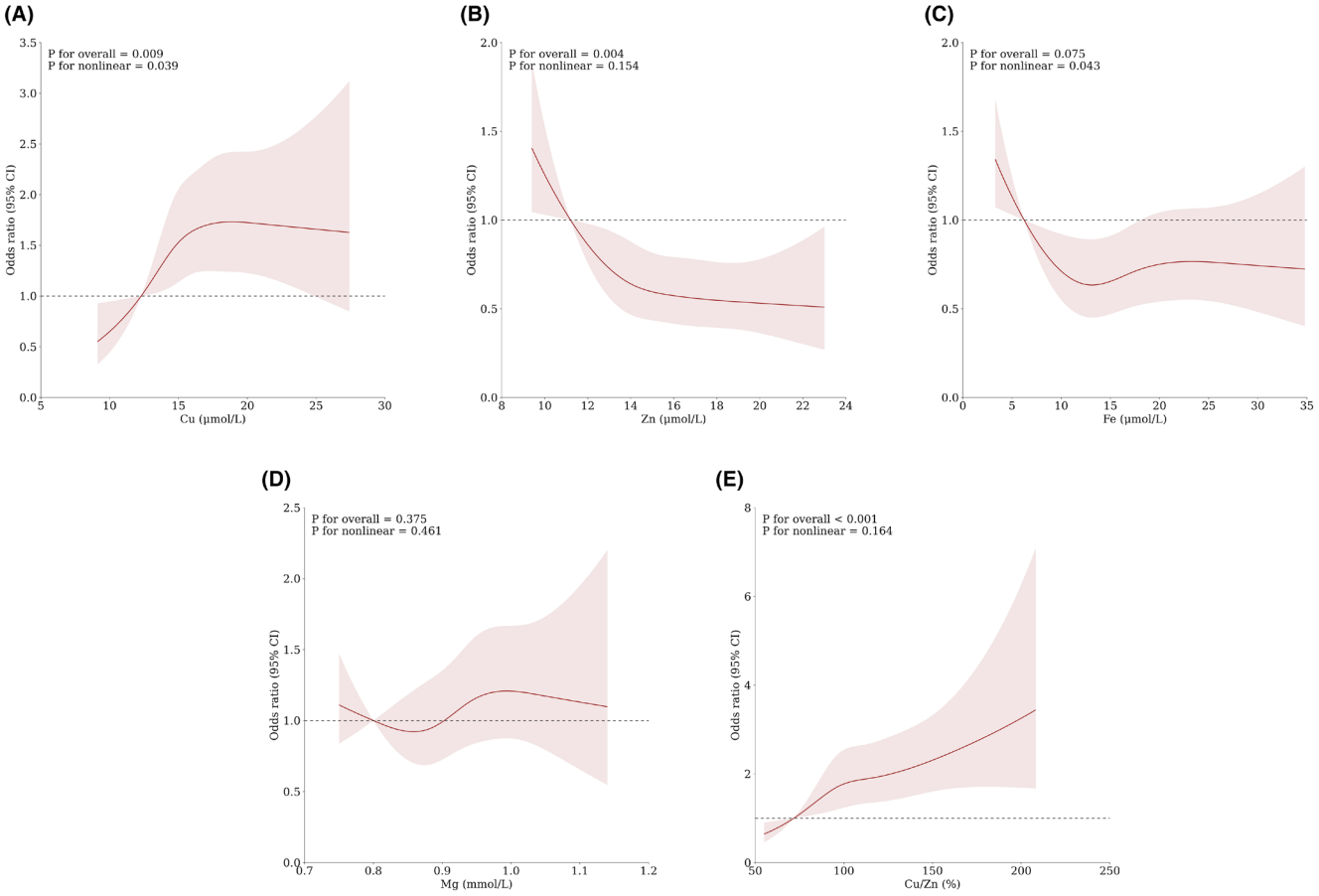

FIGURE 2 Restricted cubic spline curves depicting the odds ratios of endometriosis risk in relation to serum metal levels and Cu/Zn (%). (A) Cu and endometriosis; (B) Zn and endometriosis; (C) Fe and endometriosis; (D) Mg and endometriosis; (E) Cu/Zn ratio and endometriosis. The solid red line represents the odds ratio, while the red shaded area indicates the 95% confidence interval. The distribution percentiles for increasing weights are at the 25th, 50th, and 75th percentiles. The model is adjusted for age, body mass index, and baseline hormones.

in the fourth quartile of Zn, the ORs (95% CIs) for the first and second quartiles were 1.54 (1.13, 2.08) and 1.41 (1.04, 1.91), respectively. However, no significance was observed after confounding for age, BMI, and baseline hormones (p trend = 0.065). Furthermore, the results showed a positive association between the Cu/Zn ratio and the risk of endometriosis (p trend < 0.001), which remained significantly positive after adjusting for age, BMI, and baseline hormones (p trend < 0.001). Compared to the first quartile of Cu/Zn ratio after adjustment, the ORs (95% CIs) for the second and fourth quartiles were 1.97 (1.37, 2.83) and 2.63 (1.80, 3.84), respectively.

4 | DISCUSSION

The current study analyzed a large cohort, including 819 patients with tubal or male factor infertility and 568 patients diagnosed

TABLE 3 Odds ratios (95% confidence intervals) for the association between serum trace metal concentrations and the risk of endometriosis.

			Univariate		Multivariate	
Elements	**Cases/Total (*N*)**	**Median (range)**	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***
Cu (μmol/L)						
Q1	139/348	13.10 (5.40, 14.08)	1.00 (1.00, 1.00)	Ref	1.00 (1.00, 1.00)	Ref
Q2	148/356	14.98 (14.09, 15.70)	1.19 (0.88, 1.62)	0.253	1.28 (0.89,1.83)	0.185
Q3	148/337	16.53 (15.73, 17.45)	1.31 (0.97, 1.78)	0.081	1.55 (1.07,2.26)	0.022
Q4	142/346	18.82 (17.47, 48.12)	1.17 (0.86, 1.58)	0.32	1.62 (1.10,2.39)	0.015
p for trend				0.371		0.058
Zn (μmol/L)						
Q1	164/352	12.30 (5.60, 13.30)	1.54 (1.13, 2.08)	0.006	1.59 (1.10, 2.29)	0.013
Q2	158/355	14.20 (13.40, 14.90)	1.41 (1.04, 1.91)	0.026	1.44 (0.999, 2.09)	0.051
Q3	121/335	15.70 (15.00, 16.60)	0.99 (0.73, 1.36)	0.976	1.19 (0.82, 1.73)	0.352
Q4	125/345	18.10 (16.60, 27.10)	1.00 (1.00, 1.00)	Ref	1.00 (1.00, 1.00)	Ref
p for trend				0.005		0.065
Fe (μmol/L)						
Q1	156/348	8.30 (1.00, 10.90)	1.27 (0.94, 1.72)	0.125	1.27 (0.88, 1.83)	0.196
Q2	128/354	13.00 (11.00, 14.90)	0.88 (0.65, 1.20)	0.428	0.90 (0.62, 1.30)	0.568
Q3	150/342	17.00 (14.95, 19.30)	1.22 (0.90, 1.65)	0.203	1.35 (0.94, 1.95)	0.105
Q4	134/343	23.2 (19.30, 71.90)	1.00 (1.00, 1.00)	Ref	1.00 (1.00, 1.00)	Ref
p for trend				0.066		0.091
Mg (mmol/L)						
Q1	138/361	0.83 (0.69, 0.86)	1.00 (1.00, 1.00)	Ref	1.00 (1.00, 1.00)	Ref
Q2	141/360	0.89 (0.87, 0.91)	1.04 (0.77, 1.40)	0.796	1.20 (0.83, 1.73)	0.328
Q3	144/346	0.94 (0.92, 0.96)	1.15 (0.85, 1.56	0.357	1.26 (0.88, 1.81)	0.215
Q4	145/320	1.00 (0.97, 1.27)	1.34 (0.99, 1.82)	0.061	1.55 (1.06,2.26)	0.024
p for trend				0.245		0.159
Cu/Zn (%)						
Q1	117/347	80.95 (29.67, 90.55)	1.00 (1.00, 1.00)	Ref	1.00 (1.00, 1.00)	Ref
Q2	158/347	98.28 (90.59, 105.66)	1.64 (1.21, 2.23)	0.002	1.97 (1.37, 2.83)	<0.001
Q3	123/347	112.38 (105.67, 122.10)	1.08 (0.79, 1.48)	0.632	1.22 (0.83, 1.79)	0.321
Q4	170/346	138.85 (122.14, 291.60)	1.90 (1.40, 2.58)	<0.001	2.63 (1.80, 3.84)	<0.001
p for trend				<0.001		<0.001

Note: Based on the distribution characteristics, the levels of serum metal elements and the copper-to-zinc ratio were divided into four quartiles, represented as Q1, Q2, Q3, and Q4. Univariate, unadjusted for confounders; multivariate, adjusted for age, BMI, and baseline hormones (FSH, LH, E2, P, PRL, T, AMH).

with endometriosis. The results revealed a significant decrease in serum Zn levels and a notable elevation in the Cu/Zn ratio in individuals with endometriosis compared to individuals without endometriosis. Logistic regression analysis showed a significant positive association between the Cu/Zn ratio and the risk of endometriosis. These findings provide valuable real-world data, enhancing our understanding of endometriosis from the perspective of trace elements.

Endometriosis is a prevalent and intricate syndrome, with its pathogenesis remaining largely elusive. Among the numerous candidate factors in pathophysiology, oxidative stress is purported to exert a significant influence.[18,23] Reports suggest that individuals with endometriosis may exhibit elevated oxidative stress parameters, accompanied by a notable decrease in plasma superoxide dismutase 1 (SOD1) levels and an increase in lipid peroxidation enzymes.[24,25] Extensive literature substantiates the involvement of trace elements in oxidative stress pathways.[26,27] In recent years, there has been a growing focus on the association between trace element homeostasis imbalances and endometriosis risk.

Cu ranks as the third most prevalent trace metal in the human body, serving as a crucial cofactor for numerous key enzymes essential in various cellular processes and metabolic functions.[28] It can act both as an antioxidant, reducing oxidative damage, and as a pro-oxidant, leading to OS and disease progression, such as

Alzheimer's disease.[29] Certain studies have observed higher levels of Cu in the serum of individuals with endometriosis compared to healthy women.[30–32] This implies that elevated serum Cu levels might be linked to an increased risk of endometriosis, although the specific mechanisms require further investigation. In our study, while the serum Cu concentrations in the endometriosis group exhibited a slight increase compared to those in the control group, no significant statistical disparity was detected (median of Cu: 15.77 vs. 15.6, Endometriosis vs. Control). The latest case–control study, involving 451 blood samples, reported findings consistent with ours.[10] It is important to note that in both our study and the aforementioned case–control study,[10] the control group consisted of infertility patients rather than healthy women. This difference in control group selection could have contributed to the absence of statistically significant differences in serum Cu levels, warranting further investigation.

Zn is an indispensable micronutrient essential for various physiological functions in the human body, encompassing enzymatic reactions, DNA synthesis, immune responses, wound healing, as well as growth and development processes.[11] As a cofactor for numerous enzymes, Zn plays a crucial role in preserving the structural integrity of proteins and cell membranes.[33] Furthermore, Zn assumes a critical role in modulating oxidative-reductive balance, exerting antioxidant effects, and shielding cells from oxidative damage, often in conjunction with other antioxidants like vitamin E.[34] Zn levels impact the activity of various antioxidant enzymes, such as Cu/Zn superoxide dismutase, which contributes to preventing DNA damage.[35] Multiple clinical observations have indicated decreased serum Zn concentrations in individuals afflicted with endometriosis.[9,36] Messalli et al. compared serum Zn levels between 42 patients with endometriosis and 44 healthy patients. They noted a significant difference, with serum Zn concentration being lower in the endometriosis group ($1294 \pm 62.22\,\mu g/L$).[36] In a cross-sectional study, Lai et al. likewise found markedly lower median blood Zn concentrations in infertile women with endometriosis compared to those without the condition (11.62 vs. 4.47 mg/L).[9] However, a case–control study by Su et al. yielded opposite results, showing that women with endometriosis had significantly higher median Zn levels (8666.14 vs. 4847.88 μg/L).[10] In this study, we observed that the Zn concentration in the serum was significantly lower in the Endometriosis group compared to the Control group (14.6 vs. 15.1 μmol/L). Furthermore, serum Zn levels exhibited a negative correlation with the risk of endometriosis, although no significance was observed after adjusting for age, BMI, and baseline hormones. We hypothesize that multiple mechanisms may underlie the observed Zn deficiency in women with endometriosis. Chronic inflammation and oxidative stress, hallmarks of the disease, could increase Zn utilization and depletion due to its essential role in antioxidant defense and immune regulation. Additionally, disrupted trace element homeostasis in endometriosis may lead to increased Cu accumulation and reduced Zn levels, potentially due to competition for transporters and metallothionein regulation. Although our study did not assess dietary intake or absorption, nutritional deficiencies or impaired Zn absorption could also contribute to lower serum levels in affected individuals. Overall, there are inconsistencies in Zn concentrations in the blood of patients with endometriosis, but the abnormal activity of Zn correlates with the condition, suggesting Zn's involvement in the multifaceted pathogenesis of the disease.

The human body employs a highly regulated system to maintain trace metal homeostasis, with Cu and Zn exhibiting a well-documented competitive interplay. These metals share common intestinal transporters, including the divalent metal transporter 1 and Cu Transporter 1,[37] leading to competitive absorption, wherein an excess of one element can impair the uptake of the other. Additionally, high Zn intake induces metallothionein expression in enterocytes, which preferentially binds Cu, sequestering it and promoting its excretion.[38] In systemic circulation, Cu and Zn compete for binding to transport proteins such as albumin, further influencing their distribution and metabolic availability.[39,40] This antagonistic relationship is crucial for maintaining homeostasis, and one common imbalance observed is elevated Cu levels alongside reduced Zn levels. Indeed, in clinical settings, the Cu/Zn ratio often holds greater significance than independent concentrations.[16,17] Recent studies have revealed complex interactions involving the serum Cu/Zn ratio across various diseases. In breast cancer patients, a higher Cu/Zn ratio was found to be correlated with lower overall survival rates following diagnosis, indicating its potential as an independent predictive marker.[41] Similarly, in chronic obstructive pulmonary disease (COPD), elevated serum Cu/Zn ratios were linearly associated with an increased risk of COPD in men.[42] Additionally, the serum Cu/Zn ratio has shown promise as a diagnostic marker for ectopic pregnancy, with higher ratios associated with the condition.[43] Moreover, during pregnancy, a higher plasma Cu/Zn ratio has been independently linked to pregnancy-specific psychological distress symptoms, suggesting a potential role for micronutrients as novel biomarkers for perinatal mood disorders.[44] Overall, a common feature across these diseases is an increase in the Cu/Zn ratio attributed to either decreased serum Zn or increased serum Cu. In this context, the plasma Cu/Zn ratio may represent downstream outcomes of a series of complex mechanisms. These findings suggest that the Cu/Zn ratio may serve as a valuable tool in clinical research as a prognostic and predictive factor for various pathological and pre-pathological states. However, the serum Cu/Zn ratio is rarely mentioned in endometriosis. Our results revealed that the endometriosis group exhibited a higher Cu/Zn ratio compared to the control group. RSC indicates that an increased Cu/Zn ratio is linearly associated with a higher risk of endometriosis. Further logistic regression analysis confirmed a positive correlation between the Cu/Zn ratio and the risk of endometriosis.

We also analyzed two other trace elements, Fe and Mg. There were no significant differences in the distribution of Fe and Mg between the endometriosis and control groups. Additionally, no significant correlations were found between Fe and Mg levels and the risk of endometriosis. Among the four trace elements examined in this study, Cu showed a slight increase in endometriosis patients. At the same time, Zn levels were significantly lower, resulting in a

significantly elevated Cu/Zn ratio. Overall, imbalances in Zn and Cu, particularly the marked increase in the Cu/Zn ratio, may reveal certain pathological mechanisms of endometriosis. The Cu/Zn ratio has been extensively studied across various pathological conditions due to its critical role in maintaining physiological balance, particularly in modulating oxidative stress, inflammation, and immune function. Patients with endometriosis exhibit elevated serum Cu levels, potentially due to increased Cu demand during inflammation and tissue repair. With its antioxidant properties, Zn protects cells from oxidative stress; however, endometriosis patients often experience heightened oxidative stress, and Zn deficiency could exacerbate this issue. The elevated Cu/Zn ratio affects antioxidant defense and immune function, possibly promoting the progression of endometriosis. Given that endometriosis often presents with nonspecific symptoms, leading to delayed diagnosis, identifying reliable biomarkers is of paramount importance. The Cu/Zn ratio may serve as a helpful adjunct in the diagnostic process by reflecting systemic inflammation and oxidative stress, potentially aiding in the identification of at-risk patients and prompting further clinical evaluation. Additionally, monitoring Cu/Zn dynamics could offer valuable insights into disease progression and therapeutic response, as interventions targeting inflammation or Zn supplementation may contribute to restoring its balance. While an elevated Cu/Zn ratio is not exclusive to endometriosis, its diagnostic potential lies in its ability to complement established diagnostic modalities, such as ultrasonography, magnetic resonance imaging, laparoscopy, and histopathological examination. Although further validation through prospective studies is warranted, current evidence suggests that the Cu/Zn ratio holds promise as a noninvasive biomarker for the early detection and clinical management of endometriosis.

Growing evidence supports Zn's role in disease prevention and management, with its translational potential in medicine increasingly recognized. Zn supplementation has been shown to significantly improve glycemic control in patients with diabetes by reducing fasting blood glucose, postprandial blood glucose, and glycated hemoglobin levels.[45] Additionally, Zn has demonstrated beneficial effects on lipid metabolism, with meta-analyses indicating reductions in total cholesterol, low-density lipoprotein cholesterol, and triglycerides, suggesting a potential role in mitigating the risk of atherosclerosis.[46] In elderly individuals, Zn supplementation has been reported to decrease infection rates, reduce inflammatory cytokine levels, and lower oxidative stress markers while simultaneously increasing plasma Zn concentrations and immune response biomarkers.[47,48] In the context of endometriosis, studies indicate that affected women have lower dietary antioxidant intake, including Zn.[49] Antioxidant supplementation may reduce dysmenorrhea and oxidative stress, though findings are limited by study heterogeneity and bias.[50] Interestingly, conflicting data suggest that Zn may play both a protective and a potentially detrimental role in endometriosis, possibly due to its involvement in enzymatic activity, immune modulation, and interactions with MMPs.[51] While Zn possesses antioxidant and immunomodulatory properties that may help counteract oxidative stress and immune dysregulation, Zn supplementation alone is unlikely to fully correct the underlying pathological mechanisms of the disease. Moreover, excessive Zn intake may disrupt Cu homeostasis and other trace element balances, further complicating its therapeutic potential. Thus, Zn supplementation should be considered within a broader therapeutic framework integrating antioxidant and anti-inflammatory strategies for endometriosis management. Given the current lack of direct clinical trials specifically addressing Zn supplementation in endometriosis, future prospective studies are warranted to clarify the dose–response relationship, potential threshold effects, and clinical utility of dietary Zn intake as a supportive therapeutic approach for managing endometriosis symptoms.

This study included a substantial number of participants, enhancing the reliability and generalizability of the findings. To the best of our knowledge, this is the first study to evaluate the relationship between the Cu/Zn ratio and the risk of endometriosis, adding a new dimension to understanding endometriosis. These findings offer practical insights that could influence future diagnostic and therapeutic strategies. However, several limitations need to be noted. Firstly, it was a single-center retrospective analysis, thus inherently constituting bias and confounding factors despite our adjustments for potential confounders. Secondly, we selectively studied essential trace elements in serum. At the same time, the follicular fluid, urine, and endometrial tissue levels were also worth investigating, as this would have provided a more comprehensive understanding. Additionally, we lacked data on dietary intake, inflammatory processes, and oxidative stress markers—factors that could have enriched the analysis of serum Cu/Zn correlation and potentially enhanced interpretation and inference. Therefore, future research should involve multicenter, multi-tissue, multi-parameter studies to capture a more holistic perspective. Similarly, exploring the correlation between the Cu/Zn ratio and endometriosis severity, including pain symptoms, rASRM score, endometrioma size, and the presence of deep endometriosis, would offer valuable clinical insights.

Our study revealed an association between decreased serum Zn levels and an increased Cu/Zn ratio with the risk of endometriosis, emphasizing the predictive potential of the Cu/Zn ratio. Considering the limitations of this study, further multicenter prospective research incorporating additional tissues such as follicular fluid, urine, and endometrial tissue, as well as more parameters including oxidative stress-related markers, is necessary. Moreover, investigating the causal relationships and biological mechanisms involved is warranted.

ACKNOWLEDGMENTS

We thank all study participants and appreciate the support from the Center of Reproduction and Genetics and the Department of Laboratory Medicine at Suzhou Municipal Hospital for their help with data collection and methodological guidance.

FUNDING INFORMATION

This research was funded by The National Key Research and Development Program of China (2022YFC2702901) and the Gusu Health Talents Project (GSWS2023012 and GSWS2023111).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data is available upon request.

ETHICAL APPROVAL

The research involving human participants received ethical approval from the Reproductive Medicine Ethics Committee of Suzhou Municipal Hospital.

INFORMED CONSENT

The participants consented in writing to take part in the study.

ORCID

Yanping Liu https://orcid.org/0009-0009-9729-2890

REFERENCES

1. Giudice LC, Kao LC. Endometriosis. Lancet. 2004;364(9447):1789–99.
2. Meuleman C, Vandenabeele B, Fieuws S, Spiessens C, Timmerman D, D'Hooghe T. High prevalence of endometriosis in infertile women with normal ovulation and normospermic partners. Fertil Steril. 2009;92(1):68–74.
3. Leone Roberti Maggiore U, Chiappa V, Ceccaroni M, Roviglione G, Savelli L, Ferrero S, et al. Epidemiology of infertility in women with endometriosis. Best Pract Res Clin Obstet Gynaecol. 2024;92:102454.
4. Macer ML, Taylor HS. Endometriosis and infertility: a review of the pathogenesis and treatment of endometriosis-associated infertility. Obstet Gynecol Clin North Am. 2012;39(4):535–49.
5. Practice Committee of the American Society for Reproductive M. Endometriosis and infertility. Fertil Steril. 2006;86(5 Suppl 1):S156–S160.
6. Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol. 2014;10(5):261–75.
7. Hall MS, Talge NM, Upson K. Urinary cadmium and endometriosis prevalence in a US nationally representative sample: results from NHANES 1999–2006. Hum Reprod. 2023;38(9):1835–42.
8. Shen L, Liang C, Li D, Zhang Z, Wang X, Jiang T, et al. The association between exposure to multiple toxic metals and the risk of endometriosis: evidence from the results of blood and follicular fluid. Sci Total Environ. 2023;855:158882.
9. Lai GL, Yeh CC, Yeh CY, Chen RY, Fu CL, Chen CH, et al. Decreased zinc and increased lead blood levels are associated with endometriosis in Asian women. Reprod Toxicol. 2017;74:77–84.
10. Su X, Yue X, Zhang Y, Shen L, Zhang H, Wang X, et al. Elevated levels of Zn, Cu and Co are associated with an increased risk of endometriosis: results from a casecontrol study. Ecotoxicol Environ Saf. 2024;271:115932.
11. Sharma A, Patni B, Shankhdhar D, Shankhdhar SC. Zinc – an indispensable micronutrient. Physiol Mol Biol Plants. 2013;19(1):11–20.
12. Tiwari MK, Hagglund PM, Moller IM, Davies MJ, Bjerrum MJ. Copper ion/H_2O_2 oxidation of Cu/Zn-superoxide dismutase: implications for enzymatic activity and antioxidant action. Redox Biol. 2019;26:101262.
13. Spasojevic I, Mojovic M, Stevic Z, Spasic SD, Jones DR, Morina A, et al. Bioavailability and catalytic properties of copper and iron for Fenton chemistry in human cerebrospinal fluid. Redox Rep. 2010;15(1):29–35.
14. Xu J, Xu Y, Sun L, Lu B, Yan X, Wang Z, et al. Glucose oxidase loaded Cu^{2+} based metal-organic framework for glutathione depletion/reactive oxygen species elevation enhanced chemotherapy. Biomed Pharmacother. 2021;141:111606.
15. Lee KH, Cha M, Lee BH. Neuroprotective effect of antioxidants in the brain. Int J Mol Sci. 2020;21(19):7152.
16. Ivanova ID, Pal A, Simonelli I, Atanasova B, Ventriglia M, Rongioletti M, et al. Evaluation of zinc, copper, and Cu:Zn ratio in serum, and their implications in the course of COVID-19. J Trace Elem Med Biol. 2022;71:126944.
17. Mravunac M, Szymlek-Gay EA, Daly RM, Roberts BR, Formica M, Gianoudis J, et al. Greater circulating copper concentrations and copper/zinc ratios are associated with lower psychological distress, but not cognitive performance, in a sample of Australian older adults. Nutrients. 2019;11(10):2503. https://doi.org/10.3390/nu11102503
18. Ansariniya H, Yavari A, Javaheri A, Zare F. Oxidative stress-related effects on various aspects of endometriosis. Am J Reprod Immunol. 2022;88(3):e13593.
19. Van Langendonckt A, Casanas-Roux F, Donnez J. Oxidative stress and peritoneal endometriosis. Fertil Steril. 2002;77(5):861–70.
20. Bahi GA, Boyvin L, Meite S, M'Boh GM, Yeo K, N'Guessan KR, et al. Assessments of serum copper and zinc concentration, and the Cu/Zn ratio determination in patients with multidrug resistant pulmonary tuberculosis (MDR-TB) in Cote d'Ivoire. BMC Infect Dis. 2017;17(1):257.
21. Ozturk P, Belge Kurutas E, Ataseven A. Copper/zinc and copper/selenium ratios, and oxidative stress as biochemical markers in recurrent aphthous stomatitis. J Trace Elem Med Biol. 2013;27(4):312–6.
22. Li D, Jiang T, Wang X, Yin T, Shen L, Zhang Z, et al. Serum essential trace element status in women and the risk of endometrial diseases: a case-control study: serum essential trace element status in women and the risk of endometrial diseases: a case-control study. Biol Trace Elem Res. 2023;201(5):2151–61.
23. Chen C, Zhou Y, Hu C, Wang Y, Yan Z, Li Z, et al. Mitochondria and oxidative stress in ovarian endometriosis. Free Radic Biol Med. 2019;136:22–34.
24. Donabela FC, Meola J, Padovan CC, de Paz CC, Navarro PA. Higher SOD1 gene expression in cumulus cells from infertile women with moderate and severe endometriosis. Reprod Sci. 2015;22(11):1452–60.
25. Prieto L, Quesada JF, Cambero O, Pacheco A, Pellicer A, Codoceo R, et al. Analysis of follicular fluid and serum markers of oxidative stress in women with infertility related to endometriosis. Fertil Steril. 2012;98(1):126–30.
26. Oteiza PI. Zinc and the modulation of redox homeostasis. Free Radic Biol Med. 2012;53(9):1748–59.
27. Wandt VK, Winkelbeiner N, Bornhorst J, Witt B, Raschke S, Simon L, et al. A matter of concern - trace element dyshomeostasis and genomic stability in neurons. Redox Biol. 2021;41:101877.
28. Bost M, Houdart S, Oberli M, Kalonji E, Huneau JF, Margaritis I. Dietary copper and human health: current evidence and unresolved issues. J Trace Elem Med Biol. 2016;35:107–15.
29. Sensi SL, Granzotto A, Siotto M, Squitti R. Copper and zinc dysregulation in Alzheimer's disease. Trends Pharmacol Sci. 2018;39(12):1049–63.
30. Lin Y, Yuan M, Wang G. Copper homeostasis and cuproptosis in gynecological disorders: Pathogenic insights and therapeutic implications. J Trace Elem Med Biol. 2024;84:127436.
31. Pollack AZ, Louis GM, Chen Z, Peterson CM, Sundaram R, Croughan MS, et al. Trace elements and endometriosis: the ENDO study. Reprod Toxicol. 2013;42:41–8. https://doi.org/10.1016/j.reprotox.2013.05.009
32. Turgut A, Ozler A, Goruk NY, Tunc SY, Evliyaoglu O, Gul T. Copper, ceruloplasmin and oxidative stress in patients with advanced-stage endometriosis. Eur Rev Med Pharmacol Sci. 2013;17(11):1472–8.

33. Weiss A, Murdoch CC, Edmonds KA, Jordan MR, Monteith AJ, Perera YR, et al. Zn-regulated GTPase metalloprotein activator 1 modulates vertebrate zinc homeostasis. Cell. 2022;185(12):2148–63.e27.
34. Faure P. Protective effects of antioxidant micronutrients (vitamin E, zinc and selenium) in type 2 diabetes mellitus. Clin Chem Lab Med. 2003;41(8):995–8.
35. Jomova K, Makova M, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, et al. Essential metals in health and disease. Chem Biol Interact. 2022;367:110173.
36. Messalli EM, Schettino MT, Mainini G, Ercolano S, Fuschillo G, Falcone F, et al. The possible role of zinc in the etiopathogenesis of endometriosis. Clin Exp Obstet Gynecol. 2014;41(5):541–6.
37. Espinoza A, Le Blanc S, Olivares M, Pizarro F, Ruz M, Arredondo M. Iron, copper, and zinc transport: inhibition of divalent metal transporter 1 (DMT1) and human copper transporter 1 (hCTR1) by shRNA. Biol Trace Elem Res. 2012;146(2):281–6.
38. Richards MP. Recent developments in trace element metabolism and function: role of metallothionein in copper and zinc metabolism. J Nutr. 1989;119(7):1062–70.
39. Nishime K, Kondo M, Saito K, Miyawaki H, Nakagawa T. Zinc burden evokes copper deficiency in the Hypoalbuminemic hemodialysis patients. Nutrients. 2020;12(2):577.
40. Masuoka J, Saltman P. Zinc(II) and copper(II) binding to serum albumin. A comparative study of dog, bovine, and human albumin. J Biol Chem. 1994;269(41):25557–61.
41. Bengtsson Y, Demircan K, Vallon-Christersson J, Malmberg M, Saal LH, Ryden L, et al. Serum copper, zinc and copper/zinc ratio in relation to survival after breast cancer diagnosis: a prospective multicenter cohort study. Redox Biol. 2023;63:102728.
42. Kunutsor SK, Voutilainen A, Laukkanen JA. Serum copper-to-zinc ratio and risk of chronic obstructive pulmonary disease: a cohort study. Lung. 2023;201(1):79–84.
43. Tok A, Ozer A, Baylan FA, Kurutas EB. Copper/zinc ratio can be a marker to diagnose ectopic pregnancy and is associated with the oxidative stress status of ectopic pregnancy cases. Biol Trace Elem Res. 2021;199(6):2096–103.
44. Hulsbosch LP, Boekhorst M, Gigase FAJ, Broeren MAC, Krabbe JG, Maret W, et al. The first trimester plasma copper-zinc ratio is independently related to pregnancy-specific psychological distress symptoms throughout pregnancy. Nutrition. 2023;109:111938. https://doi.org/10.1016/j.nut.2022.111938
45. Jayawardena R, Ranasinghe P, Galappatthy P, Malkanthi R, Constantine G, Katulanda P. Effects of zinc supplementation on diabetes mellitus: a systematic review and meta-analysis. Diabetol Metab Syndr. 2012;4(1):13.
46. Ranasinghe P, Wathurapatha WS, Ishara MH, Jayawardana R, Galappatthy P, Katulanda P, et al. Effects of zinc supplementation on serum lipids: a systematic review and meta-analysis. Nutr Metab. 2015;12:26.
47. Prasad AS, Beck FW, Bao B, Fitzgerald JT, Snell DC, Steinberg JD, et al. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. Am J Clin Nutr. 2007;85(3):837–44.
48. Mocchegiani E, Romeo J, Malavolta M, Costarelli L, Giacconi R, Diaz LE, et al. Zinc: dietary intake and impact of supplementation on immune function in elderly. Age (Dordr). 2013;35(3):839–60.
49. Mier-Cabrera J, Aburto-Soto T, Burrola-Méndez S, Jiménez-Zamudio L, Tolentino MC, Casanueva E, et al. Women with endometriosis improved their peripheral antioxidant markers after the application of a high antioxidant diet. Reprod Biol Endocrinol. 2009;7:54.
50. Meneghetti JK, Pedrotti MT, Coimbra IM, da Cunha-Filho JSL. Effect of dietary interventions on endometriosis: a systematic review and meta-analysis of randomized controlled trials. Reprod Sci. 2024;31(12):3613–23.
51. Huang Y, Wei Y, Liang F, Huang Y, Huang J, Luo X, et al. Exploring the link between dietary zinc intake and endometriosis risk: insights from a cross-sectional analysis of American women. BMC Public Health. 2024;24(1):2935.

How to cite this article: Liu Y, Cheng G, Li H, Meng Q. Serum copper to zinc ratio and risk of endometriosis: Insights from a case–control study in infertile patients. Reprod Med Biol. 2025;24:e12644. https://doi.org/10.1002/rmb2.12644