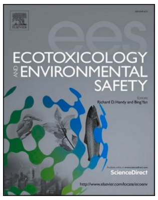
Ecotoxicology and Environmental Safety 272 (2024) 116030

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Associations between multiple metal exposure and fertility in women: A nested case-control study

Xiang Hong [a,1], Wei Wang [a,1], Lingling Huang [a], Jinhua Yuan [b], Xiaoling Ding [c], Hao Wang [b], Qian Ji [b], Fanqi Zhao [a], Bei Wang [a,*]

[a] *Key Laboratory of Environmental Medicine and Engineering of Ministry of Education, Department of Epidemiology and Health Statistics, School of Public Health, Southeast University, Nanjing, Jiangsu, China*
[b] *Nanjing Municipal Centre for Disease Control and Prevention, Nanjing, China*
[c] *Maternal and Child Health Center of Gulou District, Nanjing, China*

ARTICLE INFO

Edited by Dr. RENJIE CHEN

Keywords:
Plasma
Metal
ICP-MS
Fertility
BKMR

ABSTRACT

Metal pollution can cause a decline in female fertility, however, previous studies have focused more on the effect of a single metal on fertility. In this study, we evaluated the effect of metal mixtures on female fertility based on nested case-control samples. The plasma levels of 22 metal elements from 180 women were determined by an inductively coupled plasma mass spectrometer (ICP-MS). Minimum absolute contraction and selection operator (LASSO) penalty regression selected metals with the greatest influence on clinical outcome. Logistic regression was used to analyze the correlation between single metals and fertility while a Bayesian kernel function regression (BKMR) model was used to analyze the effect of mixed metals. Eight metals (Calcium (Ca), Chromium (Cr), Cobalt (Co), Copper (Cu), Zinc (Zn), Rubidium (Rb), Strontium (Sr) and Zirconium (Zr)) were selected by LASSO regression for subsequent analysis. After adjusting for covariates, the logistic model showed that Cu (Odds Ratio(OR):0.33, 95% CI: 0.13 − 0.84) and Co (OR:0.38, 95% CI: 0.15 −0.94) caused a significant reduction in fertility, and identified the protective effect of Zn (OR: 2.96, 95% CI:1.21 −7.50) on fertility. Trend tests showed that increased Cr, Cu, and Rb levels were associated with reduced fertility. The BKMR model showed that Cr, Co, Cu, and Rb had a nonlinear relationship with fertility decline when controlling for the concentrations of other metals and suggested that Cu and Cr might exert an influence on fertility. Analysis showed a negative correlation between Cu, Cr, Co, Rb, and fertility, and a positive correlation between Zn and fertility. Furthermore, we found evidence for the interaction between Cu and Cr. Our findings require further validation and may identify new mechanisms in the future.

1. Introduction

Worldwide, the decline in the total fertility rate has become an indisputable fact, according to the World Fertility and Family Planning, 2020 document released by the United Nations, the global fertility rate has dropped from 3.2 live births per woman in 1990 to 2.50 live births in 2019, furthermore, it is estimated that this indicator will continue to decline over time ("World Fertility and Family Planning, 2020: Highlights," n.d.). The decline in total fertility rate, along with the increase in global life expectancy, will lead to rapid aging of the global population, thus leading to a series of problems such as labor shortage and increased financial pressure (GBD, Demographics Collaborators, 2019, 2020; Ogura and Jakovljevic, 2018; Oura, 2021). The reasons for this decline in total fertility rate include educational factors, social factors, and marriage factors. Furthermore, environmental factors also play an important role in female fertility (Aitken, 2022). It is well-known that the number of germ cells stored in a woman's ovary is fixed from birth, and that these cells are nonrenewable, thus, any pollutants that affect the levels of female hormones or stimulate the reproductive system will inevitably cause damage to female fertility (Canipari et al., 2020). Heavy metals are one of the most important pollutants affecting our environment and have been included in the list of priority chemicals by the U.S. Toxic Substances and Disease Registry (ATSDR) (Dutta et al., 2022; Handan Dökmeci, 2021). Metals can be used as

---

* Correspondence to: 87#, Dingjiaqiao Road, Gulou District, Nanjing City, Jiangsu Province 210009, China.
E-mail address: wangbeilxb@163.com (B. Wang).

[1] These authors contributed equally to this work and should be considered co-first authors.

https://doi.org/10.1016/j.ecoenv.2024.116030
Received 31 October 2023; Received in revised form 23 January 2024; Accepted 24 January 2024

endocrine-disrupting chemicals (EDCs) to interfere with female reproductive function. Exposure to environmental metals during critical periods such as prenatal and neonatal stages may induce damage to reproductive function during later stages of life. One potential reason for this is the negative effects on the hypothalamic-pituitary-gonadal axis (HPG), thus leading to harmful changes in sexual maturity and function (Dutta et al., 2022; Plunk and Richards, 2020; Sengupta et al., 2015).

Due to limitations associated with detection methods, it is usually not possible to measure the concentration of multiple metal elements at the same time, as evident in the existing literature. However, as analysis of the relationship between trace elements and health based on histology and bioinformatics becomes increasingly important, metal groups (the collection of all metal ions in an organism) are also receiving increasing attention (Salt et al., 2008; Zhang, 2017), To detect metal ions in a high-throughput manner, inductively coupled plasma (ICP) spectroscopy has been continuously improved. Researchers have proposed the use of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to quantitatively analyze the composition of multiple elements in life systems (such as cells, tissues, or organs) or biological samples (such as urine, serum, or plasma), as well as the changes of these components under different physiological and pathological conditions (Baxter, 2010; Liu et al., 2014; Zhang et al., 2022). Compared with other methods, ICP-MS has the advantages of high sensitivity, high efficiency, and measurable isotope (Salt et al., 2008).

Heavy metals accumulate in the soil, water, and food chain, and do not easily decompose under natural conditions, therefore, it is not uncommon to study the effects of metals on the body (Canipari et al., 2020). An Australian prospective cohort study found that lower levels of plasma copper in the first trimester had a protective effect on pregnancy complications (Wilson et al., 2018). A previous meta-analysis also showed that increased levels of serum copper are associated with an increased risk of gestational diabetes (Lian et al., 2021). In addition, researchers have found that increased plasma copper levels in pregnant women can lead to increased blood pressure (Liu et al., 2021). Most previous studies have focused on the effects of single metals on the female reproductive system, and far fewer studies have focused on the effects of polymetallic mixtures on the female reproductive system, especially about fertility.

Although there are many studies relating to the effects of metals and female fertility, most studies only focus on the effects of single metals on fertility, however, the effects of environmental metals on human health often exist in the form of co-exposure. There is also some interaction between metals. Therefore, in this study, we determined the concentrations of 22 metals in plasma samples using ICP-MS, identified the metals most closely related to fertility, and then analyzed the interactions between these metals.

2. Methods

2.1. Study population

This study was based on the Free Pre-pregnancy Health Examination Project in the Maternal and Child Center of Gulou district in Nanjing, Jiangsu Province, China, further details relating to this study are available in existing studies (Hong et al., 2022). The specific inclusion and exclusion criteria for the study population were as follows, inclusion criteria: (1) According to the legal age of marriage in China, all included females should be older than 20 years old; and (2) both spouses should report that they are ready to become pregnant. Exclusion criteria: (1) Confirmed pregnancy during pre-pregnancy check-up; (2) self-reported diagnosis of infertility/infertility by one spouse; (3) The woman found through the examination of uterine malformation (including no uterus, no vagina, etc.), or the man found by examination of single/double testis Lateral deletion; (4) The female partner is diagnosed with syphilis infection, or IgM-positive antibodies against giant cell or toxoplasmosis, and needs to be treated before preparing for pregnancy. (5) The man is diagnosed with syphilis and needs to be treated before preparing for pregnancy. This study was conducted with stringent quality control, including the selection and training of investigators, control of the data collection process, and the collection, processing, and preservation of biological samples.

In total, 454 participants preparing for pregnancy were included, these patients were followed up every 3 months to recode the outcome, and the longest follow-up period was 1 year. The specific time of pregnancy was recorded for those who successfully became pregnant and follow-up was completed for those who were not pregnant for 1 year. Eventually, 214 women became pregnant. Based on the 1-year outcome, a nested case-control study was randomly generated from the two groups: a non-pregnancy group ($n = 90$) and a pregnancy group ($n = 90$), the process used to estimate sample size is described in the Supplementary material. This study was reviewed and approved by the Ethics Committee of Zhongda Hospital (Reference: 2018ZDSYLL116-P01). All couples participating in the study were fully informed of the project and signed an informed consent form.

2.2. Data collection

A baseline epidemiological survey was conducted, including patient age, the age difference between husband and wife (male age, female age), occupation (worker/office clerk/other), and education level (high school or below/bachelor's degree or above). We also recorded the menarche's age and pregnancy history. These data were investigated by professionals when they were enrolled into a specific group to ensure that the data were accurate and reliable. Age was calculated by the filing time minus the date of birth, if the date of birth was missing, it was completed by referring to ID card information.

2.3. Outcome measurement

During the follow-up period, the medical staff contacted all female subjects every three months, focusing on the outcome of clinical pregnancy. This information was self-reported by the subjects and confirmed by pelvic ultrasound scans. "Fertility" was used to define the outcome of this study. "Fertility" refers to a woman's proof of being able to become pregnant and is used to indicate a woman's ability to conceive and deliver a baby (Nguyen, 2005).

2.4. Metal concentration measurement

In this study, we collected samples of plasma. First, the plasma samples were pretreated, samples were taken out from a − 80 ℃ freezer and thawed on ice, then 500 μL of blood sample was removed and added to 0.5 mL of pure water and 0.5 mL of nitric acid (Guaranteed Reagent, Fluka, USA). Following ultrafine digestion with a Microwave Digestion System (UltraWave ECR, Milestone, Italy), the volume was fixed to 10 mL. Following pretreatment, we used a quadrupole inductively coupled plasma mass spectrometer (ICP-MS, BRUKER AURORA M90, Analytik Jena, Germany) to determine the levels of 22 metal elements: Lithium (Li), Natrium (Na), Magnesium (Mg), Aluminium (Al), Calcium (Ca), Titanium (Ti), Chromium (Cr), Manganese (Mn), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Gallium (Ga), Selenium (Se), Rubidium (Rb), Strontium (Sr), Zirconium (Zr), Molybdenum (Mo), Cesium (Cs), Barium (Ba), Thallium (Tl), and Lead (Pb), detailed parameter settings are as follows: Plasma flow: 16.50 L/min; Auxiliary flow: 2.00 L/min; Sheath Gas Flow: 0.08 L/min; Nebulizer flow: 1.00 L/min; Sampling depth: 6.50 mm; Power: 1.40 kW. According to the National Health and Nutrition Inspection Survey (NHANES) protocol, we replaced samples in which the plasma metal concentration was lower than the limit of detection (LOD) with $\mathrm{LOD}/\sqrt{2}$ (Williams et al., 2012), none of the metals below the LOD exceeded 50%.

2.5. *Statistical analyses*

For demographic data, we used the MICE package in R to replace missing variables (Division of Analysis, Research, and Practice Integration, National Center for Injury Prevention and Control, U.S. Centers for Disease Control and Prevention, Atlanta, GA 30341, USA et al., 2015), we applied five multiple imputations and 50 iterations. Continuous variables are described by mean and standard deviation and differences between groups were evaluated by the *t*-test or the Kruskal-Wallis test. Classification variables are described by frequency and percentage, and the distribution between groups was described by the chi-squared test or Fisher's exact probability test. All 22 metal elements measured by ICP-MS showed skew. For the original metal concentration data, we report the median, upper, and lower quartiles for all metals. Then, we performed logarithmic conversion (based on 10) to the original metal data. In the follow-up analysis, we only used converted data.

The correlation between metals was determined by calculating Spearman's correlation coefficient matrix. Then, least absolute shrinkage and selection operator (LASSO) penalty regression analysis was used to select the most important metals related to fertility. We chose lambda (λ) with a standard error in the minimum standard (the 1-SE criteria), and generated a model with good performance and the least number of independent variables (Wang et al., 2022). To investigate the risk of single metal exposure to fertility, we compared the second, third, and fourth quartiles with the first quartile. The logistic regression model was used to evaluate odds ratios (ORs) and 95% confidence intervals (95% CIs), and the metal quartile was defined as a continuous variable to test for trends. To ensure the stability of the model, we fitted two models. Only a single metal was included in model 1; however, in model 2, in addition to metal, we considered female age, age difference, occupation, education level, menarche age, and pregnancy history. ORs reflect the one-year pregnancy rate of women with certain characteristics when compared with the control group, thus, an $OR < 1.0$ implied lower levels of fertility.

In addition to the single metal model, we considered the possible collinearity or interaction between metals and applied the Bayesian kernel machine regression (BKMR) model, this strategy allowed us to evaluate the health effects of combined exposure (Bobb et al., 2018, 2015). In the BKMR model, we used the default parameters of Markov Chain Monte Carlo (MCMC). We ran each MCMC sampler for 25,000 iterations in the model and observed the stability of the model by the Trace plot. When the model was fitted, the exposure-response function was visually displayed as follows: (1) when other metals are exposed to a specific quantile, the exposure-response function of one, two, or three metals was displayed along with the results; (2) we also compared the risk of a metal at the 75th percentile with that of exposure at the 25th percentile, where all remaining metal exposures were fixed at a specific quantile, and (3) the cumulative effect on the outcome of exposure-reaction function values when all metals were exposed to a specific quantile and compared to when all metals were exposed to the median (Bobb et al., 2018, 2015), In addition, we further grouped the metals according to whether they are essential to the human body or not and again fitted the BKMR model. We also used Weighted Quantile Sum (WQS) regression to estimate the effect of metal mixtures on fertility, The WQS estimates the impact of all exposure variables on outcomes by constructing a weighted index, testing the association of that index with the outcome, and assessing the relative importance of the impact of individual variables on the outcome through the weights assigned to each exposure variable by the model(Carrico et al., 2015). All statistical analyses were conducted by R (version 4.2.3), including the following R packages: "mice", "glmnet", "bkmr", and "ggplot2". A value of $P < 0.05$ (two-tailed) was regarded as statistically significant.

3. Results

3.1. *Patient characteristics*

A total of 180 participants were included in this study, general demographic characteristics are shown in Table 1. The mean age of women in the non-pregnancy group was 29.8 ± 4.2 years while that in the pregnancy group was 28.5 ± 2.8 years. There was a significant difference between the two groups ($P = 0.012$), although the age difference between spouses did not differ significantly between the two groups (non-pregnancy: 1.3 ± 3.7 years; pregnancy: 1.3 ± 2.6 years; $P = 0.908$). The number of women with a bachelor's degree or above was greater and there were statistical differences in the outcomes of women with different education levels ($P = 0.012$). There was no significant difference between the two groups in terms of the age difference between spouses, type of occupation, age of menarche, and history of pregnancy ($P > 0.05$).

3.2. *Exposure to multiple metals*

Table 2 shows the concentrations of eight types of metals. In the pregnancy group, the median concentrations of Ca, Cr, Co, Cu, Zn, Rb, Sr, and Zr were respectively 4655.00 μg/L, 8.14 μg/L, 0.07 μg/L, 51.08 μg/L, 74.72 μg/L, 23.35 μg/L, 2.49 μg/L, and 0.06 μg/L. In the Non-pregnancy group, the median concentrations of Ca, Cr, Co, Cu, Zn, Rb, Sr, and Zr were respectively 4700.00 μg/L, 8.88 μg/L, 0.07 μg/L, 56.47 μg/L, 75.32 μg/L, 27.33 μg/L, 2.47 μg/L, and 0.06 μg/L. Information relating to different quantile concentrations of the other metals is shown in Supplementary Table 1.

Correlation analyses for the 22 metals are shown in Fig. 1. The correlation coefficients ranged from − 0.08 to 0.93, and most metals were positively correlated. The correlation coefficients for Ca and Cu, Cr and Cu, Cu and Zn, Rb and Cr, and Zr and Zn were 0.82, 0.60, 0.67, 0.46, and 0.50, respectively. The complete matrix of correlation coefficients between the 22 metals and their corresponding P-values are presented in Supplementary Table 2 and Supplementary Table 3.

3.3. *The effects of single metals on fertility*

As shown in Fig. 2, metals that exerted significant influence on outcomes were selected by LASSO regression, when $\log(\lambda) = -3.44$,

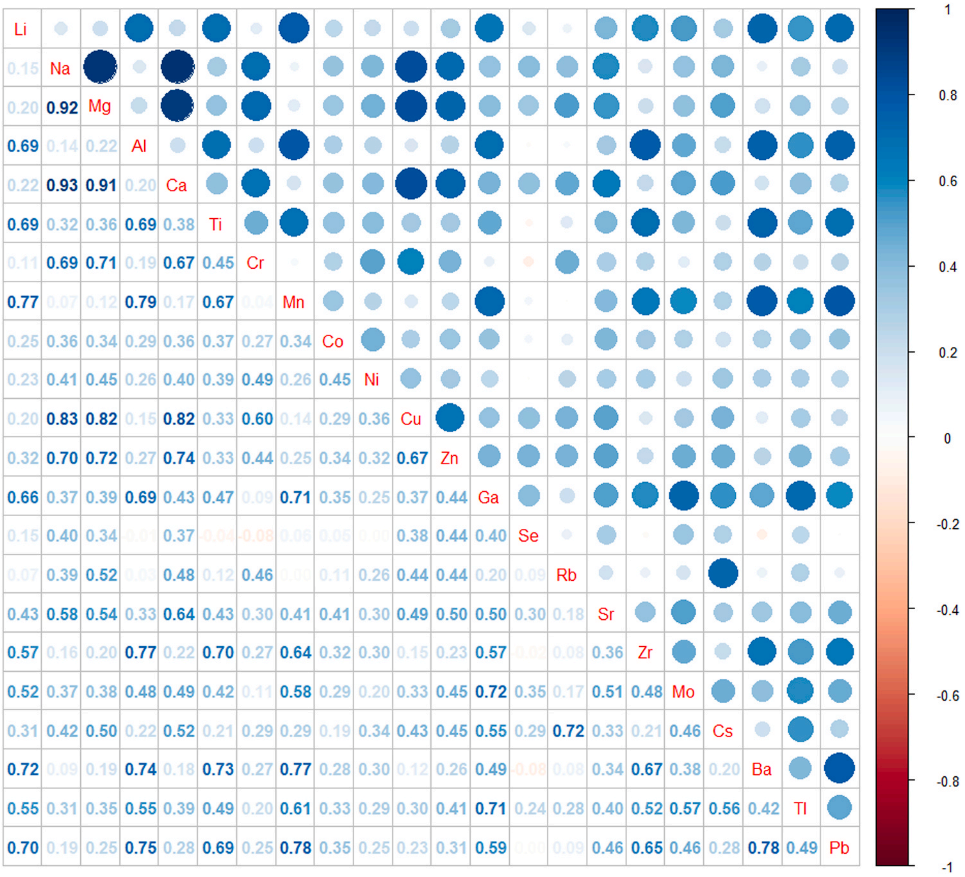
Table 1
General characteristics of the study population.

Variables		Total (N = 180)	Non-pregnancy (N = 90)	Pregnancy (N = 90)	p
Female age		29.2 ± 3.7	29.8 ± 4.2	28.5 ± 2.8	0.012
Age difference[a]		1.3 ± 3.2	1.3 ± 3.7	1.3 ± 2.6	0.908
Occupation	Worker	4(2.2%)	2(2.2%)	2(2.2%)	0.255
	Office clerk	124 (68.9%)	57(63.3%)	67(74.4%)	
	Others	52 (28.9%)	31(34.4%)	21(23.3%)	
Educational level	High school or below	27 (15.0%)	20(22.2%)	7(7.8%)	0.012
	Bachelor's degree or above	153 (85.0%)	70(77.8%)	83(92.2%)	
Age at menarche		13.5 ± 1.4	13.6 ± 1.5	13.4 ± 1.4	0.408
Pregnancy history	No	144 (80.0%)	68(75.6%)	76(84.4%)	0.192
	Yes	36 (20.0%)	22(24.4%)	14(15.6%)	

a. Age difference=Male age-Female age.

Table 2
Concentrations of 8 metals in the nested case-control study.

Metal	Non-pregnancy (N = 90)			Pregnancy (N = 90)			P
	P25	P50	P75	P25	P50	P75	
Ca(μg/L)	3652.50	4700.00	5510.00	3692.50	4655.00	5390.00	0.5769
Cr(μg/L)	7.37	8.88	10.32	5.65	8.14	9.41	0.0070
Co(μg/L)	0.06	0.07	0.10	0.05	0.07	0.10	0.1224
Cu(μg/L)	46.26	56.47	69.89	41.11	51.08	60.65	0.0037
Zn(μg/L)	63.48	75.32	93.47	67.08	74.72	93.91	0.7979
Rb(μg/L)	20.52	27.33	41.55	17.94	23.35	32.08	0.0133
Sr(μg/L)	1.96	2.47	3.02	2.14	2.49	3.17	0.3174
Zr(μg/L)	0.04	0.06	0.09	0.04	0.06	0.14	0.2265



Li																					
0.15	Na																				
0.20	0.92	Mg																			
0.69	0.14	0.22	Al																		
0.22	0.93	0.91	0.20	Ca																	
0.69	0.32	0.36	0.69	0.38	Ti																
0.11	0.69	0.71	0.19	0.67	0.45	Cr															
0.77	0.07	0.12	0.79	0.17	0.67	0.04	Mn														
0.25	0.36	0.34	0.29	0.36	0.37	0.27	0.34	Co													
0.23	0.41	0.45	0.26	0.40	0.39	0.49	0.26	0.45	Ni												
0.20	0.83	0.82	0.15	0.82	0.33	0.60	0.14	0.29	0.36	Cu											
0.32	0.70	0.72	0.27	0.74	0.33	0.44	0.25	0.34	0.32	0.67	Zn										
0.66	0.37	0.39	0.69	0.43	0.47	0.09	0.71	0.35	0.25	0.37	0.44	Ga									
0.15	0.40	0.34	-0.01	0.37	-0.04	-0.08	0.06	0.05	0.00	0.38	0.44	0.40	Se								
0.07	0.39	0.52	0.03	0.48	0.12	0.46	0.00	0.11	0.26	0.44	0.44	0.20	0.09	Rb							
0.43	0.58	0.54	0.33	0.64	0.43	0.30	0.41	0.41	0.30	0.49	0.50	0.50	0.30	0.18	Sr						
0.57	0.16	0.20	0.77	0.22	0.70	0.27	0.64	0.32	0.30	0.15	0.23	0.57	-0.02	0.08	0.36	Zr					
0.52	0.37	0.38	0.48	0.49	0.42	0.11	0.58	0.29	0.20	0.33	0.45	0.72	0.35	0.17	0.51	0.48	Mo				
0.31	0.42	0.50	0.22	0.52	0.21	0.29	0.29	0.19	0.34	0.43	0.45	0.55	0.29	0.72	0.33	0.21	0.46	Cs			
0.72	0.09	0.19	0.74	0.18	0.73	0.27	0.77	0.28	0.30	0.12	0.26	0.49	-0.08	0.08	0.34	0.67	0.38	0.20	Ba		
0.55	0.31	0.35	0.55	0.39	0.49	0.20	0.61	0.33	0.29	0.30	0.41	0.71	0.24	0.28	0.40	0.52	0.57	0.56	0.42	Tl	
0.70	0.19	0.25	0.75	0.28	0.69	0.25	0.78	0.35	0.25	0.23	0.31	0.59	0.00	0.09	0.46	0.65	0.46	0.28	0.78	0.49	Pb

Fig. 1. Spearman's correlations for the mixed exposure of participants to 22 metals.

eight metals were selected, including Ca, Cr, Co, Cu, Zn, Rb, Sr, and Zr. The model for single metals related to fertility established by logical regression is shown in Fig. 3. In model 1, without adjusting for confounding factors, the results showed negative associations of Cr, Co, Cu, and Rb with fertility and positive associations of Zn with fertility. In model 2, we further adjusted female age, age difference, occupation, education level, age of menarche, and pregnancy history and found that Cu (OR: 0.33, 95% CI: 0.13 –0.84) remained statistically significant with regards to fertility reduction and exhibited a linear trend for reduced fertility (*P*-trend=0.045). Co is negatively related to fertility at Q4 (OR: 0.38, 95% CI: 0.15 –0.94) and Q2 (OR: 0.38, 95% CI:0.15 –0.92) concentrations when compared to the reference group. Positive correlation between Zn and fertility remained (OR: 2.96, 95% CI:1.21 –7.50) and there was evidence of a dose-response relationship between Cr (*P*-trend=0.043) and Rb (*P*-trend=0.049) and fertility decline.

3.4. *The effect of mixed metals in the plasma on fertility*

Fig. 4(A) shows a potential nonlinear dose-response relationship between metals and fertility, when the levels of the other seven metals were fixed at the 50th percentile, we found that Ca, Zn, and Zr were positively correlated with fertility, while Cr, Co, Cu, and Rb were inversely related to fertility, all of these relationships were non-linear. Next, we fixed the levels of the seven metals at different quantiles (25th, 50th, or 75th percentile) while increasing the level of the remaining metal from the lower quartile to the upper quartile. The effect of adding one IQR on fertility outcome is shown in Fig. 4(B), Positive correlation between Ca and fertility was meaningful only when the concentration of the other metals was fixed at the 25th and 50th percentiles. The positive correlation between Zr and fertility was meaningful only when the concentration of the other metals was fixed at the 50th percentile. The negative correlation between Co and fertility was significant when the concentration of the other metals was at the 25th and 50th percentiles and the negative correlation between Cu and

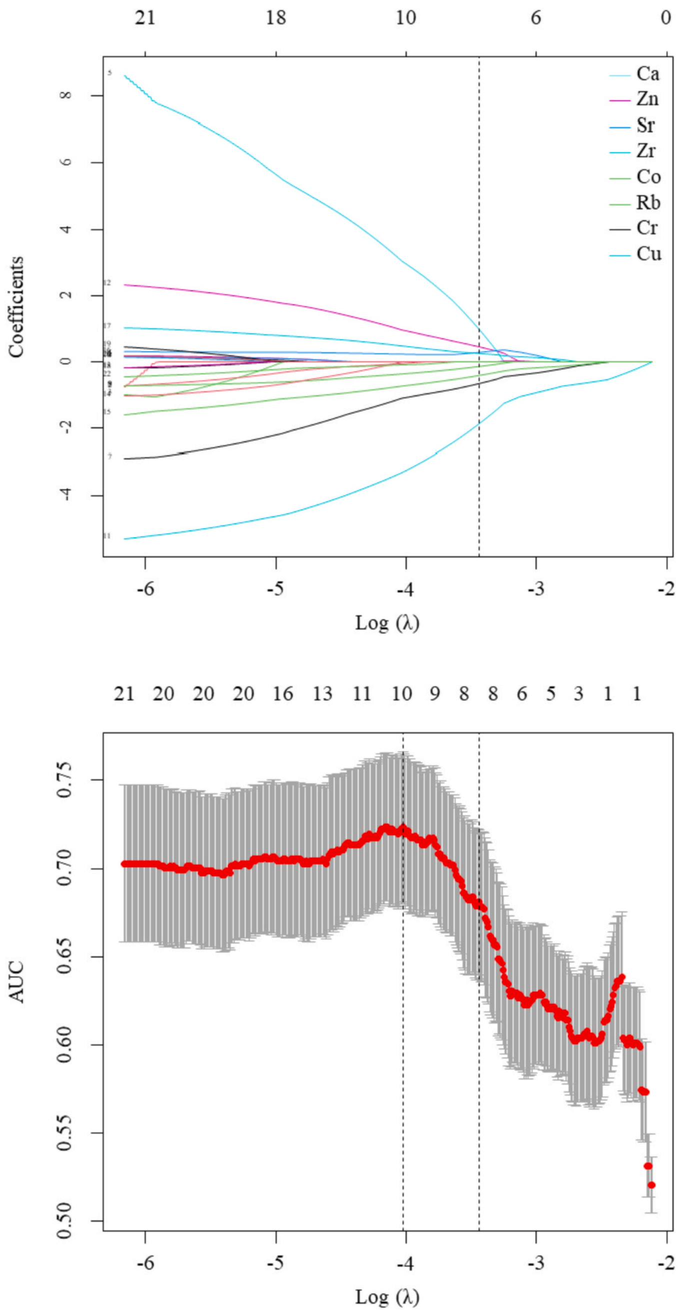


Fig. 2. LASSO penalized regression analysis for the associations between 22 plasma metals and fertility. (A) Changes in LASSO regression coefficients and lambda; (B) Results from a 10-fold cross-validation of the LASSO model.

fertility was significant when the levels of the other metals were at the 50th and 75th percentile. Compared with the logistic regression single-metal model, after accounting for other metal levels, the negative correlation of Cu and Co with fertility remained, meanwhile, we also found that the positive correlation of Ca and Zr with fertility remained. Fig. 5 depicts the overall effect of the mixture compared with a situation when the levels of each metal were fixed at the median. When the levels of all metals were between the 30th and 45th percentile, the overall effect of the mixture was negatively correlated with fertility, this negative correlation is meaningf.

Potential interactions between metals are shown in the Supplementary Material. Supplementary Figure 1 shows that the negative correlation between mixture and fertility increased with increasing concentrations of Cu and Cr, and the bivariate dose-response curve of Cu and Cr became smoother with increasing concentrations of Cr, thus indicating that the interaction between Cu, Cr, and fertility was less than additive, and that there was interaction between Cu and Cr, the remaining metal bivariate dose-response curves were essentially parallel or overlapping, suggesting that there may be no interaction. More intuitive interactions are shown in Supplementary Figure 2, such as higher fertility at lower concentrations of copper and chromium, and low fertility at elevated concentrations of both.

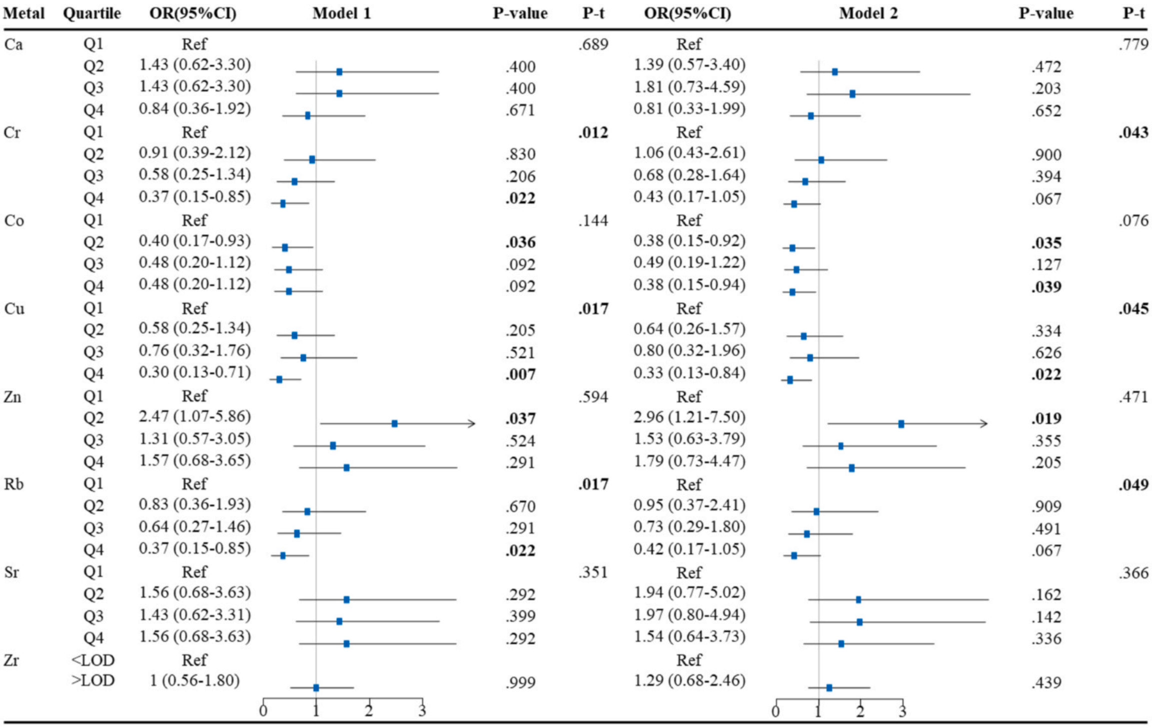
3.5. *Sensitivity analysis*

The eight metals screened out by LASSO regression are divided into two groups according to whether they are essential elements for the human body. Group 1 is essential elements for the human body, including the metals Ca, Co, Cu, and Zn; Group 2 is non-essential elements for the human body, including the metals Cr, Rb, Sr, and Zr. We grouped and analyzed when fitting the BKMR model. As shown in Supplementary Table 5, the Bayesian posterior probabilities of the two groups are 0.92 and 0.87 respectively. The elements with the highest Bayesian posterior probabilities within the group are Cu (condPIP=0.91) in group 1 and Cu in group 2 respectively. Sr (condPIP=0.65). As demonstrated in Supplementary Figure 3, the nonlinear dose-response relationship for 8 metals did not change after grouping, and the negative correlation between Cu and fertility remained relatively clear. The results in Supplementary Figure 4 show that after grouping the eight metals, only Cu has a statistically significant negative correlation with fertility when the other seven metals are fixed at the 50% and 75% quartiles. In the WQS regression model, the metals are ranked according to their contribution to the total effect of exposure. The weights are divided into positive weights and negative weights, details are presented in Supplementary Figure 5, in negative weights, Cr, Co, Cu, and Rb weights are heavier than the reference standard and dominate, and in positive weights, Zr, Zn weights are heavier than the reference standard and dominate.

4. Discussion

This nested case-control study investigated the relationship between pre-pregnancy metal exposure levels in women and fertility. Our analysis showed that high levels of exposure to Cr, Co, Cu, and Rb were negatively associated with fertility and that Zn was positively associated with female fertility, at least within a certain range. Further exploration using the BKMR model showed that these results were similar to those arising from logistic regression when controlling the levels of other metals and that there may be a certain interaction between Cu and Cr in terms of fertility effects.

Cu is a necessary trace element for the human body and a component of several proteins involved in maintaining life. The main sources of Cu in the human body are food and drinking water (de Romaña et al., 2011; Grzeszczak et al., 2020). In the existing literature, there are few studies relating to fertility outcomes following metal exposure, most researchers have paid more attention to adverse pregnancy outcomes, or the impact on mothers and fetuses. A previous study analyzed the relationship between the levels of various metals in urine and the risk of preterm delivery, these authors found that the levels of urinary Cu were positively correlated with the risk of preterm delivery and that Cu was the only metal selected from mixed exposure in elastic net (ENET) regularization analysis (Kim et al., 2018). Another study also reached a similar conclusion in serum samples, high serum levels of Cu were found to be associated with natural preterm delivery (Hao et al., 2019; Wilson et al., 2018). Cu can be transferred from mother to fetus through the placenta, a previous Chinese study found that high levels of Cu in umbilical cord serum led to lower birth weight, body length, and head circumference (Tang et al., 2016). Collectively, these studies show that Cu has a wide range of effects on the female reproductive system and that Cu could also influence ovarian function and female fertility.



		Model 1			Model 2		
Metal	**Quartile**	**OR(95%CI)**	**P-value**	**P-t**	**OR(95%CI)**	**P-value**	**P-t**
Ca	Q1	Ref		.689	Ref		.779
	Q2	1.43 (0.62-3.30)	.400		1.39 (0.57-3.40)	.472	
	Q3	1.43 (0.62-3.30)	.400		1.81 (0.73-4.59)	.203	
	Q4	0.84 (0.36-1.92)	.671		0.81 (0.33-1.99)	.652	
Cr	Q1	Ref		**.012**	Ref		**.043**
	Q2	0.91 (0.39-2.12)	.830		1.06 (0.43-2.61)	.900	
	Q3	0.58 (0.25-1.34)	.206		0.68 (0.28-1.64)	.394	
	Q4	0.37 (0.15-0.85)	**.022**		0.43 (0.17-1.05)	.067	
Co	Q1	Ref		.144	Ref		.076
	Q2	0.40 (0.17-0.93)	**.036**		0.38 (0.15-0.92)	**.035**	
	Q3	0.48 (0.20-1.12)	.092		0.49 (0.19-1.22)	.127	
	Q4	0.48 (0.20-1.12)	.092		0.38 (0.15-0.94)	**.039**	
Cu	Q1	Ref		**.017**	Ref		**.045**
	Q2	0.58 (0.25-1.34)	.205		0.64 (0.26-1.57)	.334	
	Q3	0.76 (0.32-1.76)	.521		0.80 (0.32-1.96)	.626	
	Q4	0.30 (0.13-0.71)	**.007**		0.33 (0.13-0.84)	**.022**	
Zn	Q1	Ref		.594	Ref		.471
	Q2	2.47 (1.07-5.86)	**.037**		2.96 (1.21-7.50)	**.019**	
	Q3	1.31 (0.57-3.05)	.524		1.53 (0.63-3.79)	.355	
	Q4	1.57 (0.68-3.65)	.291		1.79 (0.73-4.47)	.205	
Rb	Q1	Ref		**.017**	Ref		**.049**
	Q2	0.83 (0.36-1.93)	.670		0.95 (0.37-2.41)	.909	
	Q3	0.64 (0.27-1.46)	.291		0.73 (0.29-1.80)	.491	
	Q4	0.37 (0.15-0.85)	**.022**		0.42 (0.17-1.05)	.067	
Sr	Q1	Ref		.351	Ref		.366
	Q2	1.56 (0.68-3.63)	.292		1.94 (0.77-5.02)	.162	
	Q3	1.43 (0.62-3.31)	.399		1.97 (0.80-4.94)	.142	
	Q4	1.56 (0.68-3.63)	.292		1.54 (0.64-3.73)	.336	
Zr	<LOD	Ref			Ref		
	>LOD	1 (0.56-1.80)	.999		1.29 (0.68-2.46)	.439	

Fig. 3. Associations between 8 metals and fertility. Model 1: metals that were selected by LASSO were included in logistic regression models. Model 2: metals in Model 1 and adjusted for female age, age difference, occupation, education level, menarche age and pregnancy history.

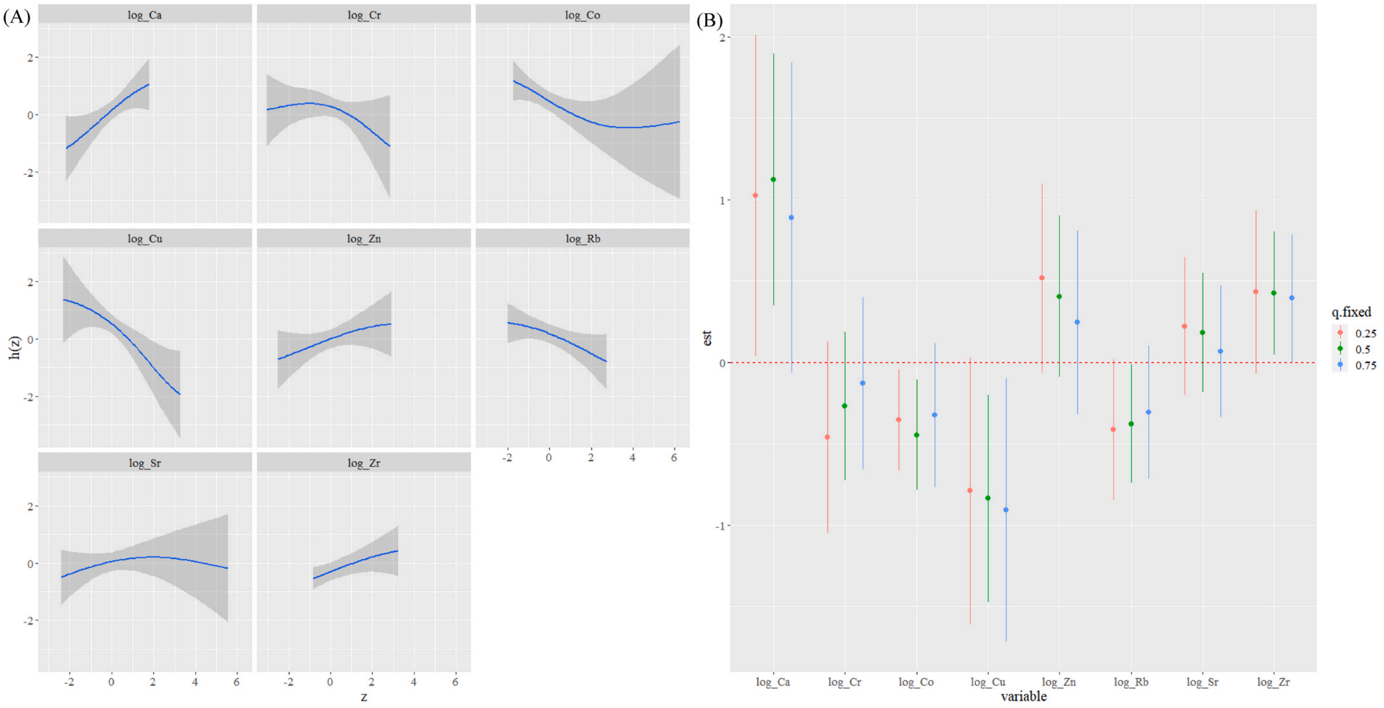


Fig. 4. Associations between 8 metals and fertility. Models were adjusted for female age, age difference, occupation, education level, menarche age and pregnancy history. (A) Univariate concentration-response functions with 95% confidence bands (shaded areas) for each metal with the other pollutants fixed at the median; (B) Plot showing the changes in fertility estimates with a 95% confidence interval in a single metal when all other metals were fixed at either the 25th, 50th, or 75th percentile.

Current knowledge indicates that Cu can cause damage to fertility by oxidative stress, pro-inflammatory reactions, and changes in blood lipids. Cu can promote oxidation by participating in free radical reactions, and high concentrations of Cu can promote the production of ROS/RNS (Serdar et al., 2006) via Cu-catalyzed Fenton-like reactions to form ROS (Valko et al., 2016), or by increasing Cu levels and reducing the activity of antioxidant glutathione to initiate oxidative stress (Jomova and Valko, 2011). Pregnancy itself is considered to be a

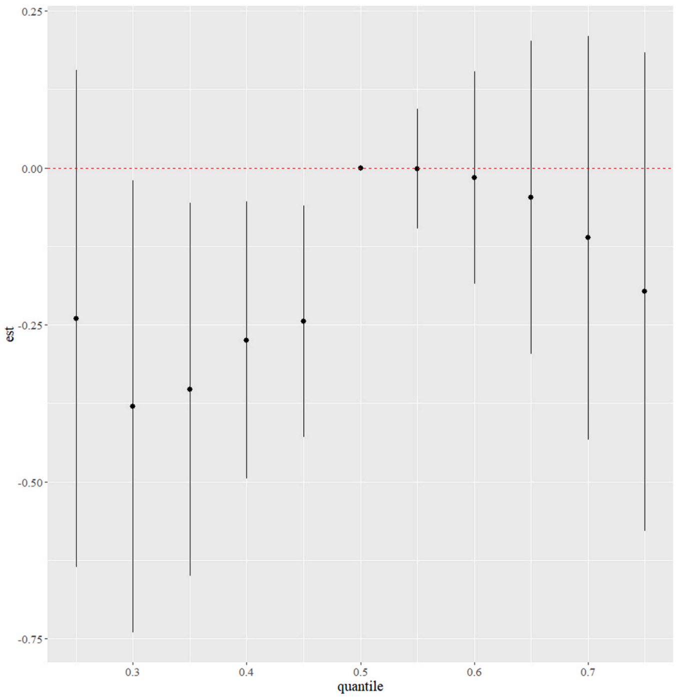


Fig. 5. Overall effects of eight metals with 95% confidence interval. This figure shows the estimated changes in fertility when eight metals were set at particular percentiles (ranging from 25th to 75th percentiles) compared to when all metals were at their 50th percentile.

pro-inflammatory state, especially during the peri-implantation period. A previous study reported that Cu may play a role in the inflammatory pathway (Wilson et al., 2018). An increase in Cu concentration can lead to an increase in total cholesterol (TC) and the concentration of triglycerides (TG) (Hao et al., 2019; Shoji et al., 2017), the mechanism responsible for changes in lipid levels is likely to be oxidative stress.

In our study, we also found that Cr caused damage to female fertility. Cr usually exists in the form of Cr (III) and Cr (VI) in the environment, both of these forms can be converted into each other (Sharma et al., 2008; Zhitkovich, 2011). Population epidemiological evidence shows that compared with women engaged in economic activities, the rate and proportion of spontaneous abortion among occupational women in Cr exposure metallurgy are significantly higher (Hemminki et al., 1983). An investigation of Cr poisoning in China also found that female workers exposed to occupational Cr had an increased risk of spontaneous abortion and threatened abortion (Yang et al., 2013). Similar results were observed in non-occupationally exposed populations, an assessment of the birth cohort in Hubei Province showed that maternal exposure to high levels of Cr (VI) during pregnancy can increase the risk of preterm delivery (Pan et al., 2017). In addition, animal experiments have found that Cr can lead to increased levels of oxidative stress biomarkers (e.g., H_20_2) and lipid peroxides (LPO) in the ovaries, this can lead to germ cell apoptosis and the induced rupture of germ cell cysts, advanced primordial follicle assembly, and primary follicle transformation (Sivakumar et al., 2014). Other animal experiments and cell experiments (H et al., 2019; Russo et al., 2005; Zhong et al., 2017) reported that the damage to fertility caused by Cr occurred via oxidative stress, thus suggesting that the damage caused by Cr exposure to female fertility may also be caused by oxidative stress. This could partly explain why we observed a certain interaction between Cu and Cr in this study, the possible mechanisms responsible for the damage caused by Cu and CR are predominantly ROS-mediated oxidative stress, consequently, cross-signal transduction and regulation may play a key role in the process of oxidative stress and may represent the cause of interaction (Valko et al., 2006).

In the human body, Co usually binds to albumin in the form of ions and then binds to vitamin B12 to exert functionality (Raeeszadeh et al., 2022), however, little is known about the precise total of Co. In a previous study, Li et al. (Li et al., 2019) found an association between low concentrations of Co in cord blood and an increased risk of preterm birth in a cohort study, in addition, a case of miscarriage was reported that was associated with increased levels of Co in the body caused by hip replacement (Grulli et al., 2021). Our literature searches showed that Co compounds may increase the risk of cancer occurrence or development, although this does not seem to be caused by the Co ion itself. In short, there is still a lack of sufficient evidence relating to the effect of Co on female fertility (Ćwiertnia et al., 2022).

Little is known about the biological effects and toxicity of Rb on the human body. A previous study found that the concentration of Rb in the urine of pregnant women was negatively correlated with the physical growth of newborns, especially in girls (Callan et al., 2016). In addition, other studies have found that low levels of urinary Rb have a protective effect on renal function (Yang et al., 2019) and that low levels of blood Rb are a protective factor for stroke (Xiao et al., 2019). However, the specific mechanisms underlying these observations have yet to be elucidated. Zn, as an essential trace element (micronutrient), was also shown to play a protective role. Zn plays a key role in the maintenance of homeostasis during pregnancy and fetal development (Mocchegiani et al., 2000). Zn deficiency in pregnant women is known to be related to adverse pregnancy outcomes, including abortion, premature delivery, and stillbirth (Lehti, 1993; Scholl et al., 1993). A cohort study in China found that Zn deficiency increased the risk of low birth weight and small for gestational age infants (Wang et al., 2015).

This study is the first to directly investigate the relationship between polymetallic exposure and fertility. Our study had several advantages. First, in addition to using a logistic regression model to fit the single metal model, we also used the BKMR method to investigate the effect of mixing between metals, the BKMR model is currently the more dominant model for mixture effects analysis (Frenoy et al., 2022; Tyagi et al., 2021; Valeri et al., 2017; Zhang et al., 2019). In addition, we fitted grouped BKMR models and WQS models for sensitivity analysis to verify the robustness of the results. Finally, all data and samples used in this nested case-control study were accurate and reliable, this depended on good quality control in the preliminary cohort.

This study also had some limitations that need to be considered. For example, plasma may not be the best biomarker for all metals. Furthermore, the distribution of each metal in the human body was not identical. Therefore, in the future, we should use other biological samples, including serum, urine, or blood cells for testing. This study only measured the concentration of metals in plasma samples collected at the time of enrollment, however, the metal load in the human body can be affected by various factors, including age, place of residence, and anthropometric status (Liu et al., 2017, pp. 2010–2012; O'Brien et al., 2000). Therefore, we need to collect more data to acquire long-term results relating to metal exposure levels. Finally, although we estimated the sample size in advance, our sample size was still insufficient, especially when exploring the interaction between multiple heavy metals. This study also has limitations in terms of confounding factors, as we were not able to obtain detailed data on factors such as dietary intake, physical activity, and frequency of couples' sexual activity, and we were not able to obtain data on men's sperm quality testing, which was mainly due to men's unwillingness to undergo sperm quality testing at the preconception stage, but we strictly screened the couples who were preparing for pregnancy and who reported that they had not been diagnosed with infertility, which could have prevented the men's sperm problem to some degree, and hopefully, we will be able to obtain more detailed information in the subsequent studies.

5. Conclusion

This was a nested and case-control study that explored the relationship between the plasma concentrations of metal elements and the fertility of pregnant women. We identified a negative correlation between Cu, Cr, Co, Rb, and fertilityfertility, and a positive correlation between Zn and fertility. We also found evidence of interaction between Cu and Cr. Further research is required to identify specific interactions between metals.

CRediT authorship contribution statement

Zhao Fanqi: Visualization, Project administration. **Wang Bei:** Supervision, Funding acquisition. **Wang Hao:** Writing – review & editing. **Ji Qian:** Writing – review & editing. **Ding Xiaoling:** Writing – review & editing. **Huang Lingling:** Validation, Investigation. **Yuan Jinhua:** Writing – review & editing, Formal analysis. **Hong Xiang:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Wang Wei:** Writing – original draft, Visualization, Software, Data curation.

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.

Data Availability

Data will be made available on request.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 82204057); Basic Research Program of Jiangsu Province (BK20220827); China Postdoctoral Science Foundation funded project (2022M720713); and Jiangsu Funding Program for Excellent Postdoctoral Talent.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116030.

References

Aitken, R.J., 2022. The changing tide of human fertility. Hum. Reprod. 37, 629–638. https://doi.org/10.1093/humrep/deac011.

Baxter, I., 2010. Ionomics: the functional genomics of elements. Brief. Funct. Genom. 9, 149–156. https://doi.org/10.1093/bfgp/elp055.

Bobb, J.F., Valeri, L., Claus Henn, B., Christiani, D.C., Wright, R.O., Mazumdar, M., Godleski, J.J., Coull, B.A., 2015. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics 16, 493–508. https://doi.org/10.1093/biostatistics/kxu058.

Bobb, J.F., Claus Henn, B., Valeri, L., Coull, B.A., 2018. Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. Environ. Health 17, 67. https://doi.org/10.1186/s12940-018-0413-y.

Callan, A.C., Hinwood, A.L., Heyworth, J., Phi, D.T., Odland, J.Ø., 2016. Sex specific influence on the relationship between maternal exposures to persistent chemicals and birth outcomes. Int. J. Hyg. Environ. Health 219, 734–741. https://doi.org/10.1016/j.ijheh.2016.09.018.

Canipari, R., De Santis, L., Cecconi, S., 2020. Female fertility and environmental pollution. Int J. Environ. Res. Public Health 17, 8802. https://doi.org/10.3390/ijerph17238802.

Carrico, C., Gennings, C., Wheeler, D.C., Factor-Litvak, P., 2015. Characterization of weighted quantile sum regression for highly correlated data in a risk analysis setting. J. Agric. Biol. Environ. Stat. 20, 100–120. https://doi.org/10.1007/s13253-014-0180-3.

Ćwiertnia, A., Kozłowski, M., Cymbaluk-Płoska, A., 2022. The role of iron and cobalt in gynecological diseases. Cells 12, 117. https://doi.org/10.3390/cells12010117.

Division of Analysis, Research, and Practice Integration, National Center for Injury Prevention and Control, U.S. Centers for Disease Control and Prevention, Atlanta, GA 30341, USA, Liu, Y., De, A., 2015. Multiple Imputation by Fully Conditional Specification for Dealing with Missing Data in a Large Epidemiologic Study. Int. J. Stats. Med. Res. 4, 287–295. https://doi.org/10.6000/1929–6029.2015.04.03.7.

Dutta, S., Gorain, B., Choudhury, H., Roychoudhury, S., Sengupta, P., 2022. Environmental and occupational exposure of metals and female reproductive health. Environ. Sci. Pollut. Res. Int. 29, 62067–62092. https://doi.org/10.1007/s11356-021-16581-9.

Frenoy, P., Perduca, V., Cano-Sancho, G., Antignac, J.-P., Severi, G., Mancini, F.R., 2022. Application of two statistical approaches (Bayesian Kernel Machine Regression and Principal Component Regression) to assess breast cancer risk in association to exposure to mixtures of brominated flame retardants and per- and polyfluorinated alkylated substances in the E3N cohort. Environ. Health 21, 27. https://doi.org/10.1186/s12940-022-00840-4.

GBD 2019 Demographics Collaborators, 2020. Global age-sex-specific fertility, mortality, healthy life expectancy (HALE), and population estimates in 204 countries and territories, 1950–2019: a comprehensive demographic analysis for the Global Burden of Disease Study 2019. Lancet 396, 1160–1203. https://doi.org/10.1016/S0140–6736(20)30977–6.

Grulli, F., Lonati, D., Ronchi, A., Perotti, F., Spinillo, A., Locatelli, C.A., 2021. Management of high concentrations of cobalt and chromium in blood due to metal-on-metal hip arthroplasty in a pregnant woman. Clin. Toxicol. (Philos.) 59, 72–73. https://doi.org/10.1080/15563650.2020.1757694.

Grzeszczak, K., Kwiatkowski, S., Kosik-Bogacka, D., 2020. The role of Fe, Zn, and Cu in pregnancy. Biomolecules 10, 1176. https://doi.org/10.3390/biom10081176.

H, G., Z, L., L, J., Q, L., C, G., X, Y., X, S., Y, L., J, C., 2019. Cr (VI) induces crosstalk between apoptosis and autophagy through endoplasmic reticulum stress in A549 cells. Chem. -Biol. Interact. 298 https://doi.org/10.1016/j.cbi.2018.10.024.

Handan Dökmeci, A., 2021. Environmental impacts of heavy metals and their bioremediation. In: Khaled Nazal, M., Zhao, H. (Eds.), Heavy Metals - Their Environmental Impacts and Mitigation. IntechOpen. https://doi.org/10.5772/intechopen.95103.

Hao, Y., Pang, Y., Yan, H., Zhang, Y., Liu, J., Jin, L., Yan, L., Chen, D., Hu, L., Liu, Q., Qin, Y., Wang, B., Li, Z., Ye, R., Zhang, L., Ren, A., 2019. Association of maternal serum copper during early pregnancy with the risk of spontaneous preterm birth: a nested case-control study in China. Environ. Int. 122, 237–243. https://doi.org/10.1016/j.envint.2018.11.009.

Hemminki, K., Kyyrönen, P., Niemi, M.L., Koskinen, K., Sallmén, M., Vainio, H., 1983. Spontaneous abortions in an industrialized community in Finland. Am. J. Public Health 73, 32–37. https://doi.org/10.2105/ajph.73.1.32.

Hong, X., Zhao, J., Yin, J., Zhao, F., Wang, W., Ding, X., Yu, H., Ma, X., Wang, B., 2022. The association between the pre-pregnancy vaginal microbiome and time-to-pregnancy: a Chinese pregnancy-planning cohort study. BMC Med 20, 246. https://doi.org/10.1186/s12916-022-02437-7.

Jomova, K., Valko, M., 2011. Advances in metal-induced oxidative stress and human disease. Toxicology 283, 65–87. https://doi.org/10.1016/j.tox.2011.03.001.

Kim, S.S., Meeker, J.D., Carroll, R., Zhao, S., Mourgas, M.J., Richards, M.J., Aung, M., Cantonwine, D.E., McElrath, T.F., Ferguson, K.K., 2018. Urinary trace metals individually and in mixtures in association with preterm birth. Environ. Int 121, 582–590. https://doi.org/10.1016/j.envint.2018.09.052.

Lehti, K.K., 1993. Stillbirth rates and folic acid and zinc status in low-socioeconomic pregnant women of Brazilian Amazon. Nutrition 9, 156–158.

Li, Z., Liang, C., Xia, X., Huang, K., Yan, S., Tao, R., Pan, W., Sheng, J., Tao, Y., Xiang, H., Hao, J., Wang, Q., Tong, S., Tao, F., 2019. Association between maternal and umbilical cord serum cobalt concentration during pregnancy and the risk of preterm birth: the Ma'anshan birth cohort (MABC) study. Chemosphere 218, 487–492. https://doi.org/10.1016/j.chemosphere.2018.11.122.

Lian, S., Zhang, T., Yu, Y., Zhang, B., 2021. Relationship of circulating copper level with gestational diabetes mellitus: a meta-analysis and systemic review. Biol. Trace Elem. Res. 199, 4396–4409. https://doi.org/10.1007/s12011-020-02566-1.

Liu, T., Zhang, M., Rahman, M.L., Wang, X., Hinkle, S.N., Zhang, C., Mueller, N.T., 2021. Exposure to heavy metals and trace minerals in first trimester and maternal blood pressure change over gestation. Environ. Int. 153, 106508 https://doi.org/10.1016/j.envint.2021.106508.

Liu, X., Piao, J., Huang, Z., Zhang, S.-Q., Li, W., Tian, Y., Yang, X., 2014. Determination of 16 selected trace elements in children plasma from China economical developed rural areas using high resolution magnetic sector inductively coupled mass spectrometry. J. Anal. Methods Chem. 2014, 1–6. https://doi.org/10.1155/2014/975820.

Liu, X., Zhang, Y., Piao, J., Mao, D., Li, Y., Li, W., Yang, L., Yang, X., 2017. Reference values of 14 serum trace elements for pregnant chinese women: a cross-sectional study in the China Nutrition and Health Survey 2010–2012. Nutrients 9, 309. https://doi.org/10.3390/nu9030309.

Mocchegiani, E., Muzzioli, M., Giacconi, R., 2000. Zinc, metallothioneins, immune responses, survival and ageing. Biogerontology 1, 133–143. https://doi.org/10.1023/a:1010095930854.

Nguyen, R.H.N., 2005. Terms in reproductive and perinatal epidemiology: I. Reproductive terms. J. Epidemiol. Community Health 59, 916–919. https://doi.org/10.1136/jech.2004.023457.

O'Brien, K.O., Zavaleta, N., Caulfield, L.E., Wen, J., Abrams, S.A., 2000. Prenatal iron supplements impair zinc absorption in pregnant Peruvian women. J. Nutr. 130, 2251–2255. https://doi.org/10.1093/jn/130.9.2251.

Ogura, S., Jakovljevic, M.M., 2018. Editorial: Global Population Aging - Health Care, Social and Economic Consequences. Front. Public Health 6, 335. https://doi.org/10.3389/fpubh.2018.00335.

Oura, P., 2021. Sociodemographic indicators of birth rate in a low fertility country–a nationwide study of 310 finnish municipalities covering > 5,000,000 inhabitants. Front. Public Health 9, 643561. https://doi.org/10.3389/fpubh.2021.643561.

Pan, X., Hu, J., Xia, W., Zhang, B., Liu, W., Zhang, C., Yang, J., Hu, C., Zhou, A., Chen, Z., Cao, J., Zhang, Y., Wang, Y., Huang, Z., Lv, B., Song, R., Zhang, J., Xu, S., Li, Y.,

2017. Prenatal chromium exposure and risk of preterm birth: a cohort study in Hubei, China. Sci. Rep. 7, 3048 https://doi.org/10.1038/s41598-017-03106-z.

Plunk, E.C., Richards, S.M., 2020. Endocrine-disrupting air pollutants and their effects on the hypothalamus-pituitary-gonadal axis. Int. J. Mol. Sci. 21, 9191. https://doi.org/10.3390/ijms21239191.

Raeeszadeh, M., Gravandi, H., Akbari, A., 2022. Determination of some heavy metals levels in the meat of animal species (sheep, beef, turkey, and ostrich) and carcinogenic health risk assessment in Kurdistan province in the west of Iran. Environ. Sci. Pollut. Res Int 29, 62248–62258. https://doi.org/10.1007/s11356-022-19589-x.

Russo, P., Catassi, A., Cesario, A., Imperatori, A., Rotolo, N., Fini, M., Granone, P., Dominioni, L., 2005. Molecular mechanisms of hexavalent chromium-induced apoptosis in human bronchoalveolar cells. Am. J. Respir. Cell Mol. Biol. 33 https://doi.org/10.1165/rcmb.2005-0213OC.

de Romaña, D.L., Olivares, M., Uauy, R., Araya, M., 2011. Risks and benefits of copper in light of new insights of copper homeostasis. J. Trace Elem. Med. Biol. 25, 3–13. https://doi.org/10.1016/j.jtemb.2010.11.004.

Salt, D.E., Baxter, I., Lahner, B., 2008. Ionomics and the study of the plant ionome. Annu. Rev. Plant Biol. 59, 709–733. https://doi.org/10.1146/annurev.arplant.59.032607.092942.

Scholl, T.O., Hediger, M.L., Schall, J.I., Fischer, R.L., Khoo, C.S., 1993. Low zinc intake during pregnancy: its association with preterm and very preterm delivery. Am. J. Epidemiol. 137, 1115–1124. https://doi.org/10.1093/oxfordjournals.aje.a116615.

Sengupta, P., Banerjee, R., Nath, S., Das, S., Banerjee, S., 2015. Metals and female reproductive toxicity. Hum. Exp. Toxicol. 34, 679–697. https://doi.org/10.1177/0960327114559611.

Serdar, Z., Gür, E., Develioğlu, O., 2006. Serum iron and copper status and oxidative stress in severe and mild preeclampsia. Cell Biochem Funct. 24, 209–215. https://doi.org/10.1002/cbf.1235.

Sharma, S.K., Petrusevski, B., Amy, G., 2008. Chromium removal from water: a review. J. Water Supply.: Res. Technol. -Aqua 57, 541–553. https://doi.org/10.2166/aqua.2008.080.

Shoji, H., Ikeda, N., Kojima, C., Kitamura, T., Suganuma, H., Hisata, K., Hirayama, S., Ueno, T., Miida, T., Shimizu, T., 2017. Relationship between copper and lipids and atherogenic indices soon after birth in Japanese preterm infants of 32-35 weeks. J. Dev. Orig. Health Dis. 8, 256–260. https://doi.org/10.1017/S2040174416000684.

Sivakumar, K.K., Stanley, J.A., Arosh, J.A., Pepling, M.E., Burghardt, R.C., Banu, S.K., 2014. Prenatal exposure to chromium induces early reproductive senescence by increasing germ cell apoptosis and advancing germ cell cyst breakdown in the F1 offspring. Dev. Biol. 388, 22–34. https://doi.org/10.1016/j.ydbio.2014.02.003.

Tang, M., Xu, C., Lin, N., Yin, S., Zhang, Y., Yu, X., Liu, W., 2016. Toxic effects of trace elements on newborns and their birth outcomes. Sci. Total Environ. 550, 73–79. https://doi.org/10.1016/j.scitotenv.2016.01.071.

Tyagi, P., James-Todd, T., Mínguez-Alarcón, L., Ford, J.B., Keller, M., Petrozza, J., Calafat, A.M., Hauser, R., Williams, P.L., Bellavia, A., EARTH study team, 2021. Identifying windows of susceptibility to endocrine disrupting chemicals in relation to gestational weight gain among pregnant women attending a fertility clinic. Environ. Res 194, 110638. https://doi.org/10.1016/j.envres.2020.110638.

Valeri, L., Mazumdar, M.M., Bobb, J.F., Claus Henn, B., Rodrigues, E., Sharif, O.I.A., Kile, M.L., Quamruzzaman, Q., Afroz, S., Golam, M., Amarasiriwardena, C., Bellinger, D.C., Christiani, D.C., Coull, B.A., Wright, R.O., 2017. The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20-40 months of age: evidence from rural Bangladesh. Environ. Health Perspect. 125, 067015 https://doi.org/10.1289/EHP614.

Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M., Mazur, M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Inter. 160, 1–40. https://doi.org/10.1016/j.cbi.2005.12.009.

Valko, M., Jomova, K., Rhodes, C.J., Kuča, K., Musílek, K., 2016. Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. Arch. Toxicol. 90, 1–37. https://doi.org/10.1007/s00204-015-1579-5.

Wang, H., Hu, Y.-F., Hao, J.-H., Chen, Y.-H., Su, P.-Y., Wang, Y., Yu, Z., Fu, L., Xu, Y.-Y., Zhang, C., Tao, F.-B., Xu, D.-X., 2015. Maternal zinc deficiency during pregnancy elevates the risks of fetal growth restriction: a population-based birth cohort study. Sci. Rep. 5, 11262 https://doi.org/10.1038/srep11262.

Wang, T., Lv, Z., Wen, Y., Zou, X., Zhou, G., Cheng, J., Zhong, D., Zhang, Y., Yu, S., Liu, N., Peng, C., Chen, G., Zheng, S., Huang, H., Liu, R., Huang, S., 2022. Associations of plasma multiple metals with risk of hyperuricemia: a cross-sectional study in a mid-aged and older population of China. Chemosphere 287, 132305. https://doi.org/10.1016/j.chemosphere.2021.132305.

Williams, M., Todd, G.D., Roney, N., Crawford, J., Coles, C., McClure, P.R., Garey, J.D., Zaccaria, K., Citra, M., 2012. Toxicological Profile for Manganese, Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles. Agency for Toxic Substances and Disease Registry (US), Atlanta (GA).

Wilson, R.L., Bianco-Miotto, T., Leemaqz, S.Y., Grzeskowiak, L.E., Dekker, G.A., Roberts, C.T., 2018. Early pregnancy maternal trace mineral status and the association with adverse pregnancy outcome in a cohort of Australian women. J. Trace Elem. Med. Biol. 46, 103–109. https://doi.org/10.1016/j.jtemb.2017.11.016.

World Fertility and Family Planning 2020: Highlights [WWW Document], n.d. URL https://webcache.googleusercontent.com/search?q=cache:9KFmSwuW_uAJ:https://www.un.org/en/development/desa/population/publications/pdf/family/World_Fertility_and_Family_Planning_2020_Highlights.pdf&cd=1&hl=zh-CN&ct=clnk&gl=hk (accessed 3.26.23).

Xiao, Y., Yuan, Y., Liu, Y., Yu, Y., Jia, N., Zhou, L., Wang, H., Huang, S., Zhang, Y., Yang, H., Li, X., Hu, F.B., Liang, L., Pan, A., Zhang, X., He, M., Cheng, J., Wu, T., 2019. Circulating multiple metals and incident stroke in Chinese adults. Stroke 50, 1661–1668. https://doi.org/10.1161/STROKEAHA.119.025060.

Yang, F., Yi, X., Guo, J., Xu, S., Xiao, Y., Huang, X., Duan, Y., Luo, D., Xiao, S., Huang, Z., Yuan, H., He, M., Shen, M., Chen, X., 2019. Association of plasma and urine metals levels with kidney function: A population-based cross-sectional study in China. Chemosphere 226, 321–328. https://doi.org/10.1016/j.chemosphere.2019.03.171.

Yang, Y., Liu, H., Xiang, X., Liu, F., 2013. Outline of occupational chromium poisoning in China. Bull. Environ. Contam. Toxicol. 90, 742–749. https://doi.org/10.1007/s00128-013-0998-3.

Zhang, Y., 2017. Trace elements and healthcare: a bioinformatics perspective. In: Shen, B. (Ed.), Translational Informatics in Smart Healthcare, Advances in Experimental Medicine and Biology. Springer Singapore, Singapore, pp. 63–98. https://doi.org/10.1007/978-981-10-5717-5_4.

Zhang, Y., Dong, T., Hu, W., Wang, Xu, Xu, B., Lin, Z., Hofer, T., Stefanoff, P., Chen, Y., Wang, Xinru, Xia, Y., 2019. Association between exposure to a mixture of phenols, pesticides, and phthalates and obesity: comparison of three statistical models. Environ. Int 123, 325–336. https://doi.org/10.1016/j.envint.2018.11.076.

Zhang, Y., Huang, B., Jin, J., Xiao, Y., Ying, H., 2022. Recent advances in the application of ionomics in metabolic diseases. Front Nutr. 9, 1111933 https://doi.org/10.3389/fnut.2022.1111933.

Zhitkovich, A., 2011. Chromium in drinking water: sources, metabolism, and cancer risks. Chem. Res. Toxicol. 24, 1617–1629. https://doi.org/10.1021/tx200251t.

Zhong, X., de Cássia da Silveira E Sá, R., Zhong, C., 2017. Mitochondrial biogenesis in response to chromium (VI) toxicity in human liver cells. Int. J. Mol. Sci. 18, 1877. https://doi.org/10.3390/ijms18091877.