Biological Trace Element Research (2025) 203:4439–4456
https://doi.org/10.1007/s12011-024-04507-8

RESEARCH

Associations of Essential and Non-Essential Trace Elements' Levels in the Blood, Serum, and Urine in Women with Premature Ovarian Insufficiency

Tina Kek[1] · Ksenija Geršak[2,3] · Nataša Karas Kuželički[4] · Dominika Celar Šturm[1] · Darja Mazej[5] · Janja Snoj Tratnik[5] · Ingrid Falnoga[5] · Milena Horvat[5] · Irma Virant-Klun[1]

Received: 25 October 2024 / Accepted: 27 December 2024 / Published online: 10 January 2025

Abstract

Premature ovarian insufficiency (POI) is poorly understood, with causes identified in only 25% of cases. Emerging evidence suggests links between trace elements (TEs) and POI. This study is the first to compare concentrations of manganese (Mn), copper (Cu), zinc (Zn), selenium (Se), molybdenum (Mo), arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) across urine, serum, and whole blood in women with POI compared to healthy controls (HC), aiming to explore their distribution and potential associations with POI. This cross-sectional-case-control study enrolled 81 participants (40 POI patients and 41 healthy controls) at the University Medical Centre Ljubljana, Slovenia. Blood and urine samples were collected to quantify basic biochemical parameters using standard clinical chemistry methods and concentrations of Mn, Cu, Zn, Se, Mo, As, Cd, Hg, and Pb using inductively coupled plasma-mass spectrometry (ICP-MS). Participants also completed questionnaires on socio-demographics, medical history, lifestyle, and nutrition. Data was analyzed using the Mann–Whitney U test, Student's t-tests, Fisher exact test, logistic regression models adjusted on body mass index (BMI), age, hematocrit, and Kendall's tau correlation. Women with POI had significantly higher BMI and red blood cell (RBC) indices, including hemoglobin, hematocrit, and red cell distribution width (RDW), compared to controls. A larger proportion of POI patients resided in rural agricultural areas. Liver and kidney function assessments showed no significant differences between the groups. Adjusted models revealed that POI patients had significantly lower urinary levels of Cu, Zn, Se, Mo, Cd, Hg, and Pb than controls, while whole blood Mn levels were higher. Serum Cu levels were significantly elevated in POI patients, whereas Pb, Cd, and Hg were lower. No significant differences were observed for As. Correlation analysis showed several strong to moderate associations among TEs across biofluids, but only weak correlations were found between TEs and demographic or biochemical factors. This study suggests potential associations between TEs and POI in women. Notably, most TEs (Zn, Se, Cu, Mo, Cd, Hg, Pb) were significantly lower in the urine of the POI group, while Cu, Cd, Hg, and Pb showed significant differences in both urine and serum.

Keywords Premature ovarian insufficiency · Essential · Non-essential trace elements · Biofluids · Reproductive health

✉ Tina Kek
tina.kek@kclj.si

1 Clinical Research Centre, University Medical Centre Ljubljana, Zaloška Cesta 2, 1000 Ljubljana, Slovenia

2 Division of Gynecology and Obstetrics, University Medical Centre Ljubljana, Šlajmarjeva 3, 1000 Ljubljana, Slovenia

3 Medical Faculty, University of Ljubljana, Vrazov Trg 2, 1000 Ljubljana, Slovenia

4 Faculty of Pharmacy, University of Ljubljana, Aškrčeva Cesta 7, 1000 Ljubljana, Slovenia

5 Department of Environmental Sciences, Institute Jožef Stefan, Jamova 39, 1000 Ljubljana, Slovenia

Background

Premature ovarian insufficiency (POI) is a multifaceted medical condition of young women that can significantly affect their overall quality of life. It refers to a cessation of ovarian function that occurs in women under the age of 40 years and may be associated with intermittent resumption of ovarian activity in over 25% of patients [1]. POI is characterized by hypergonadotropic hypoestrogenic oligo/amenorrhea, which arises from dysfunction or early depletion of the ovarian follicle pool [2–4]. POI is uncommon but not rare, with a generally estimated prevalence between 0.3 and 3.7%

in women under 40 years of age [3, 5]. According to the European Society of Human Reproduction and Embryology (ESHRE), the diagnostic criteria for POI are primary or secondary oligo/amenorrhea of more than 4 months duration, associated with an increase in serum levels of follicle-stimulating hormone (FSH) (> 25 IU/L), confirmed by a second measurement one month later, in women before the age of 40 [6]. Women with POI exhibit symptoms similar to those of natural menopause; however, these symptoms are accompanied by an earlier loss of fertility, which can be particularly distressing for young women [4]. Additionally, POI is associated with well-recognized long-term health sequelae, including osteoporosis, cardiovascular disease, dementia, and reduced life expectancy [1, 5]. These health issues may lead to increased stress levels, impairments in mental health, and diminished social functioning, all of which result in a significant decrease in quality of life [5, 7].

Etiologies of POI are still poorly understood, with only 25% of cases having an identified cause. Possible causes include genetic factors (related to the X chromosome and autosomal), autoimmune disorders, infectious, metabolic disorders, toxicant-related, and iatrogenic factors (such as chemotherapy, radiation, or surgery) [1, 3, 8]. In the majority of cases, the etiology remains unknown, leading to classification as idiopathic POI [9] highlighting the need to identify potential pathogenic factors, including environmental influences, that remain to be elucidated.

Almost three decades ago, a well-characterized cluster of POI in female workers exposed to environmental pollutant 2-bromopropane illustrated the plausibility of an environmental chemical contribution to this condition [3, 10]. Further studies suggested that environmental factors seem to be a significant determinant of ovarian reserve and are therefore suspected of contributing to the onset of POI [8, 11–14].

Trace elements (TEs) are defined as elements that are present at low concentrations (mg kg^{-1} or less) in most soils, plants, and living organisms [15]. Manganese (Mn), copper (Cu), zinc (Zn), selenium (Se), and molybdenum (Mo) are essential TEs, as they are vital for numerous biological processes at normal levels but can exert toxic effects at higher concentrations. Conversely, non-essential TEs arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) are not considered to have any essential or beneficial effects on organisms and may be toxic at any concentration [16].

The ovary contains various essential TEs such as Zn, Cu, Se, Mn and Mo, which are vital for oogenesis, oocyte maturation, ovulation as well as normal oocyte function [17, 18]. Their involvement in a broad range of biological processes, particularly cellular metabolism and antioxidant defenses, makes them especially relevant for the development of oocyte competence [17, 19]. Conversely, the accumulation of nonessential TEs and alterations in the ovarian content of essential TEs can damage the reserve of ovarian follicles, interfere with folliculogenesis, and disrupt ovarian steroidogenesis [16, 20]. An overview of the suggested pathological mechanisms of TEs reproductive toxicity, which may coexist, includes disruption of the endocrine and immune systems and the induction of oxidative stress [8].

Most studies investigating the impact of TEs exposure on human reproductive health have focused on infertile couples undergoing in vitro fertilization (IVF) cycles [16, 21–24]. Cumulative epidemiological and experimental data from human and animal studies suggest that TEs can accumulate in the ovaries [16], exerting detrimental effects on ovarian function and female fertility, even at relatively low exposure levels [3, 8, 14, 16, 20–27]. In our literature search, we identified nine studies that have analyzed levels of TEs in patients with POI to date, reporting inconclusive results [28–36] (Supplement 1).

Despite limited and incomplete investigations to date, associations between analyzed TEs and POI have been suggested. Previous studies have primarily focused on assessing Zn, Cu, and Se in a single biological sample. To our knowledge, this is the first study to evaluate differences in the concentrations of nine common TEs (Mn, Cu, Zn, As, Se, Mo, Hg, Cd, Pb) across urine, serum, and whole blood in patients with POI compared to a control group. The overall objective of this study was to investigate the concentrations and distribution of TEs in various biofluids (urine, whole blood, and serum) of women with POI compared to healthy controls (HC), and to explore the potential associations between these TEs and POI.

Methods

Study Population

The study was conducted at the University Medical Centre Ljubljana, Slovenia, from January 2021 to September 2023. A total of 81 participants, including 40 POI patients and 41 healthy controls, were enrolled in this cross-sectional case–control study. To ensure sufficient statistical power, we calculated the required sample size using the reported standard deviations (SDs) of TEs from previous studies on POI (33–41). However, significant inter-element variability in SDs prevented the determination of a reasonable unified sample size. As a result, we opted for a sample size that aligns with those used in prior studies.

The POI patients were recruited from the gynecological outpatient clinic of the University Medical Centre Ljubljana. The inclusion criteria for POI were based on the ESHRE diagnostic criteria (6), which include: (I) age below 40 years at the time of diagnosis, (II) oligo/amenorrhea for at least 4 months, and (III) elevated FSH levels (> 25 IU/L) on two occasions at least 4 weeks apart.Patients with known causes

of POI, such as a diagnosed abnormal karyotype or Fragile X syndrome / *FMR1*[1] gene premutation, as well as those with additional endocrine or chronic diseases, including polycystic ovary syndrome (PCOS), endometriosis, diabetes, Crohn's disease, celiac disease, cardiovascular diseases, liver diseases, kidney diseases, asthma, chronic obstructive pulmonary disease, musculoskeletal diseases, neurological diseases, cancer, thyroid disorders, and hyperprolactinemia, were excluded from the study. The control group consisted of healthy women with regular menstrual cycles, recruited from routine health checkups at the gynecological outpatient clinic in the Community Health Center Ljubljana. Both POI and control group participants met the following common inclusion criteria: a body mass index (BMI) between 18.5 and 29.9 kg/m^2 and an age range of 20 to 49 years. Common exclusion criteria for both groups included a history of reproductive, endocrine, or other chronic systemic disorders, as well as the use of any form of hormonal therapy. All study participants had resided permanently in Slovenia for at least 5 years prior to the date of study inclusion and denied any occupational exposure to analyzed TEs. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Republic of Slovenia National Medical Ethics Committee (No. 0120–158/2022/9). All participants gave their informed written consent to participate in the study.

Sampling and Chemical Analyses

Fasting morning blood and spot urine samples were collected concurrently at the Clinical Institute of Clinical Chemistry and Biochemistry (KIKKB) of the University Medical Centre Ljubljana, Slovenia, where a portion of each fresh sample was analyzed for basic biochemistry parameters (hemogram, urinalysis, liver, and kidney function) using standard enzymatic and colorimetric methods [37–40]. Samples from POI patients were collected at the time of recruitment, while samples from the control group were collected during the early follicular phase (from the second to the fifth day of the menstrual cycle), which reflects the baseline status of ovarian function. The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease (MDRD) [41, 42], and Chronic Kidney Disease Epidemiology Collaboration (CDK-EPI) formula [43], and liver function was evaluated using the Fibrosis-4 (FIB-4) index and the aspartate aminotransferase to platelet ratio index (APRI) [44]. The remaining aliquoted serum, whole blood, and urine samples were frozen at −80 °C and subsequently shipped on dry ice to the Institute Jožef Stefan, Department of Environmental Sciences in Ljubljana, Slovenia, where all analyses of TEs were conducted. TEs analysis was performed in whole blood, serum, and urine samples by validated analytical methods [45, 46]. About 0.3 g of whole blood/ serum sample or 0.5 mL of urine sample was weighed into pre-cleaned Teflon tubes. 0,5 mL of 65% HNO3 (suprapure) was added, and samples were subjected to a closed vessel microwave digestion (ULTRAWAVE, Single Reaction Chamber Microwave Digestion System, MILESTONE Srl, Sorisole, Italy). Digested solutions were transferred into measuring tubes and diluted to 5 mL with MilliQ water. The same procedure was applied for blank samples and reference materials. Determination of selected elements (Hg, Pb, Cd, As, Zn, Se, Cu and Mo) was performed using an Agilent 8800 triple quadrupole inductively coupled plasma mass spectrometer (ICP-QQQ, Agilent Technologies, Tokyo, Japan). Isotopes monitored were ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{75}As, ^{78}Se, ^{95,98}Mo, ^{111,114}Cd, ^{202}Hg, $^{206-208}$Pb. For the elimination of interferences collision, a reaction cell was used with helium, hydrogen, or oxygen gas. Internal standards (Y, Rh, Sc, and Gd) were added online. The concentrations were determined based on external calibration. Blank samples, control samples, and reference materials were tested together with the samples daily. The limits of detection (LOD) for all nine TEs in each biological sample, calculated as three times the standard deviation of the blank sample, are provided in Supplement 2. The quality of the results was checked via the regular use of the reference materials Seronorm Trace Elements Whole Blood Level 1 and Level 2, Seronorm Trace Elements Serum Level 1 and Level 2, Seronorm Trace element Urine Level 2 (SERO, Billingstad, Norway) and ClinChek® Urine Control, lyophil., for Trace Elements, Level I, (Receipe, Chemicals,&Instruments GmbH, Germany) and also by participation in the German External Quality Assessment Scheme (G-EQUAS, Erlangen -Nuremberg, Germany). The results of the analysis of the reference materials are combined in the supplement (Supplement 2). The results obtained were in good agreement with the reference values. Element concentrations in urine were adjusted for variations in diuresis among samples using creatinine concentration and specific gravity (SG). Since creatinine levels can vary based on factors such as age, body mass index, fat-free mass, and race/ethnicity [47], and due to systematic variation in urinary flow rates, there is a risk of underestimating exposure due to creatinine over-compensation, particularly for Cd and As [46, 48]. Therefore, we adjusted the measurements using SG, as it appears to be a more reliable alternative in the context of environmental exposures [46, 47, 49, 50]. For accurate exposure and nutritional status comparisons across individuals and within the study population, we present urine concentrations expressed per volume (µg/L) and normalized by creatinine [51] and SG [52].

[1] Fragile X Messenger Ribonucleoprotein 1 gene.

Questionnaire

Upon recruitment, participants completed a comprehensive questionnaire that included socio-demographic information, medical, gynecological, and reproductive history, current health status, as well as lifestyle and nutritional habits. The questionnaire was adapted from a previously validated questionnaire used in a national biomonitoring study of TEs in the Slovenian population [46].

Statistical Analysis

Descriptive statistics included the determination of mean and median values, standard deviation, and 95% confidence interval (CI) of the mean. The normality of the distribution of the variables was tested with the Shapiro–Wilk test. Measurements below the LOD were imputed using the SPSS multiple imputation procedure on natural logarithm-transformed data [53]. Subsequently, random values were imputed from the estimated distribution within the defined limits (0-corresponding LOD value). To compare levels of TEs between POI and the control group, we performed an independent samples t-test for normally distributed data and the Mann–Whitney test for non-normally distributed data. The Fisher's exact test was employed to compare categorical data between the study groups. To account for multiple comparisons and reduce the risk of false positives, a Bonferroni correction was applied to the bivariate analyses, adjusting the significance threshold to $p = 0.000115$. Kendall's tau correlation analyses were performed to evaluate correlations between TEs concentrations and statistically significant biochemical and demographic variables. This method was chosen due to the non-normal distribution of the data and the relatively small sample size. The associations between POI (primary outcome) and TE levels (primary exposure) were evaluated using multiple logistic regression models. TE levels were the primary exposure variable, and POI status was the binary outcome variable. Due to the small sample size, only statistically significant ($p < 0.05$) known POI risk factors with correlations below 0.8 ($T < 0.8$) were included as covariates. Age and BMI, established POI risk factors [54], were included in all models as confounders. Hematocrit was adjusted for in models analyzing whole blood TE concentrations to account for erythrocyte-bound TE normalization [55, 56]. In total, 27 logistic regression models were performed, each evaluating the association between a specific TE and POI. Further details of the models are provided in Supplement 8. All tests were two-sided, and a level of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS software (version 29, IBM, Chicago, IL, USA).

Results

In this cross-sectional-case–control study, demographic, biochemical, and environmental parameters, as well as levels of TE in urine, serum, and whole blood, were compared between POI patients and the control group. The demographic and biochemical characteristics of the POI and control groups are summarized in Table 1, Table 2, and Supplement 3. While POI patients were significantly older at enrolment compared to the control group, there was no significant difference between the age at diagnosis in the POI group and the age at enrolment in the control group ($p = 0.606$, Table 1). The median age at POI diagnosis was 32 years, ranging from 16 to 38 years. At the time of recruitment, the median (min–max) levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and anti-Müllerian hormone (AMH) in the POI group were 59.2 IU/L

Table 1 Distributions of statistically significant demographic and biochemical numerical variables

Non-normally distributed variables Median (min–max)	POI group ($n = 40$)	Control group ($n = 41$)	*p value*
Age at enrolment (years)	36 (21 – 47)	31 (20—39)	< 0.001[2]
Age at diagnosis (years)	32 (16 – 38)	/	0.606[2]
Time from diagnosis (years)	4.5 (0 – 12)	/	/
Haemoglobin (g/l)	136 (103 – 150)	131 (105 – 147)	0.007[2]
RDW (%)	13.60 (12.5 – 18.7)	12.6 (4.8 – 17.0)	< 0.001[2]
Frequency of weekly pasta intake (x times/week)	1 (0 – 7)	2 (0.5 – 5)	0.003[2]
Daily time spent driving car (hours)	1 (0 – 5)	1 (0 – 2.5)	0.030[2]
Normally distributed variables Mean ± SD	**POI group (n = 40)**	**Control group (n = 41)**	***p value***
Body mass index	23.3 ± 4.2	21.1 ± 1.8	0.005[1]
Haematocrit	0.405 ± 0.022	0.393 ± 0.024	0.019[1]

POI premature ovarian insufficiency, *SD* standard deviation, *RDW* red cell distribution width, 1 Student T test for independent samples, 2 Mann Whitney test

Table 2 Distributions of statistically significant categorical variables

Categorical variables		POI group ($n=40$)	Control group ($n=41$)	*p value*[3]
Medication use – nonhormonal (%)	Yes	42.9	15.2	0.023
	No	57.1	84.8	
Mineral supplementation (%)	Yes	39.3	78.8	0.003
	No	60.7	21.2	
Region of residence (%)	Osrednjeslovenska	45.5	80.5	0.006
	Gorenjska	6.1	2.4	
	Goriška	3.0	2.4	
	Jugovzhodna Slovenija	18.2	2.4	
	Obalno-Kraška	6.1	2.4	
	Podravska	3.0	2.4	
	Savinjska	9.1	2.4	
	Zasavska	9.1	0	
	Primorsko-Notranjska	0	2.4	
	Pomurska	0	2.4	
	Posavska	0	2.4	
Type of residence (%)	one family house	59.4	26.8	0.013
	multi-family house	18.8	24.4	
	apartment building	18.8	48.8	
	other	3.1	0	
Location of residence (%)	city center	25.0	51.2	0.029
	city suburb	31.3	36.6	
	industrial-craft zone	3.1	0	
	countryside, village	37.5	12.2	
	countryside, remote area	3.1	0	
Home surroundings agriculture (%)	Yes	68.8	34.2	0.008
	No	31.3	65.8	
Home surroundings vineyards (%)	Yes	21.9	2.4	0.011
	No	78.1	97.6	
Home surroundings fruit farming (%)	Yes	34.4	13.2	0.034
	No	65.6	86.8	
Is waste being burned in the neighborhood (%)	Yes	46.7	10.0	< 0.001
	No	53.3	90.0	
Employment status (%)	Employed	90.9	68.3	0.019
	Student	6.1	29.3	
	Other	3.0	2.4	
Alcohol consumption (%)	Never	34.4	2.5	< 0.001
	Sometimes	56.3	87.5	
	Regularly in small amounts	9.4	10.0	
Last remembered rice intake (%)	not in the last week	44.8	17.9	0.004
	in the last week	31.0	71.8	
	in the last 24 h	24.1	10.3	
Last remembered still mineral water intake (%)	not in the last week	92.6	60.5	0.009
	in the last week	7.4	23.7	
	in the last 24 h	0	15.8	
Last remembered fruit tea intake (%)	Not in the last week	53.6	26.3	0.034
	In the last 24 h	32.1	34.2	
	In the last week	14.3	39.5	

POI premature ovarian insufficiency, 3 Fisher exact test

(24–153 IU/L), 36 IU/L (18.9–87.3 IU/L), and 0.13 ng/L (0.01–1.85 ng/L), respectively. Hormonal levels were not analyzed in the control group, which consisted of healthy women with regular menstrual cycles. No significant differences were observed between the two groups regarding the age at menarche, parity, number of siblings, or liver and kidney function, as assessed by the FIB-4, APRI, MDRD, and CKD-EPI formulas, respectively. The POI group had a significantly higher BMI, with a 1.10-fold increase compared to the control group ($p = 0.005$), as well as elevated red blood cell (RBC) indices, including hemoglobin (1.04-fold, $p = 0.007$), hematocrit (1.03-fold, $p = 0.019$), and red cell distribution width (RDW) levels (1.08-fold, $p < 0.001$). However, after Bonferroni correction, statistical significance was retained only for age at enrolment and RDW levels.

Differences in Nutrition, Habits and Living Environment

Based on the questionnaire responses, no significant differences were observed between the two groups regarding the level of education, current workplace, smoking status, coffee consumption, number of amalgam fillings, or intake of fruits, seafood, vegetables, cereals, dairy products, and meat. In the group of former smokers, the number of responses regarding the duration since smoking cessation (POI $n = 3$; HC $n = 4$) was insufficient for further statistical analysis.

However, the control group reported significantly higher levels of alcohol consumption, showing a 1.55-fold increase compared to the POI group ($p < 0.001$) and more frequent pasta consumption (median: 2 times/week HC vs. 1 time/week POI, $p = 0.003$). The control group also had shorter time intervals since their last intake of rice (71.8% HC vs. 31.0% POI within the last week, $p = 0.004$), fruit tea (39.5% HC vs. 14.3% POI within the last week, $p = 0.034$), and still mineral water (23.7% HC vs. 7.4% POI within the last week, $p = 0.009$). Additionally, the control group showed a 2.01-fold higher frequency of mineral supplement use ($p = 0.003$) and reported non-hormonal medication use at 15.2%, compared to 42.9% in the POI group ($p = 0.023$).

The distribution of residential settings differed significantly between the groups. The POI group more frequently resided in agricultural areas, with 68.8% living near agricultural areas compared to 34.2% of the control group ($p = 0.008$), including vineyards (21.9% POI vs. 2.4% HC, $p = 0.011$) and fruit farms (34.4% POI vs. 13.2% HC, $p = 0.034$). The control group predominantly resided in the Osrednjeslovenska urban area, where their frequency was 76.9% higher than the POI group (80.5% HC vs. 45.5% POI, $p = 0.006$). Furthermore, the POI group reported significantly higher levels of waste burning in their neighborhoods (4.67-fold increase, $p < 0.001$) and spent more time driving daily (median: 1 h/day [0–5] POI vs. 1 h/day [0–2.5] HC, $p = 0.030$). After Bonferroni correction, statistical significance was maintained only for differences in alcohol consumption and the occurrence of waste burning in the vicinity of residences.

Kendall's tau correlation analyses evaluated the relationships among nine TEs and the statistically significant demographic and biochemical variables. The correlation coefficients and corresponding p-values for the elements in each biofluid are presented in Supplement 4.

Distributions of Trace Elements in Biofluids

The concentrations of TEs measured in this study were generally within the established reference intervals [38] for spot urine, whole blood, and serum specimens (Supplement 5) and were generally consistent with levels reported in women from various environmental studies (Supplement 6) [46, 57–75]. In both groups, most elements showed the highest concentrations in whole blood, except for Cu, Mo, and As, which were highest in serum and urine, respectively (Supplement 7).

Distributions of Trace Elements in Urine

The urinary concentrations (unadjusted and adjusted for creatinine and SG) and detection rates for healthy controls and POI patients are summarized in Supplement 5. Except for Mn, the TEs detection rates were higher than 80% in both groups. The detection rate for Mn was only 15% (POI group: 7.5%, control group: 22%); therefore, Mn was excluded from further analyses. After adjustment for SG, the median urinary levels of Cu, Zn, Se, Mo, Cd, Hg, and Pb were significantly higher in the control group than in the POI group. When adjusting for age and BMI, the difference in Pb levels was no longer significant, but the differences in Cu, Zn, Se, Mo, Cd, and Hg levels remained significant. However, the median As concentrations did not differ significantly between the two groups in either the unadjusted or adjusted models (Supplement 8) Figs. 1, 2, and 3.

Distributions of Trace Elements in Whole Blood

The whole blood concentrations and detection rates for healthy controls and POI patients are summarized in Supplement 5. The detection rate for all elements was 100%. Median blood levels of Cu, Zn, and Pb were significantly higher in the POI group compared to the control group. In contrast, the median blood level of Mo was significantly lower in the POI group. However, these differences were no longer statistically significant after adjusting for age, BMI, and hematocrit (for Zn, Cd, Pb, Hg, Mo). In the bivariate analysis, Mn levels did not differ significantly between the POI and control groups. However, after adjusting for age, BMI, and hematocrit (Ht), Mn levels

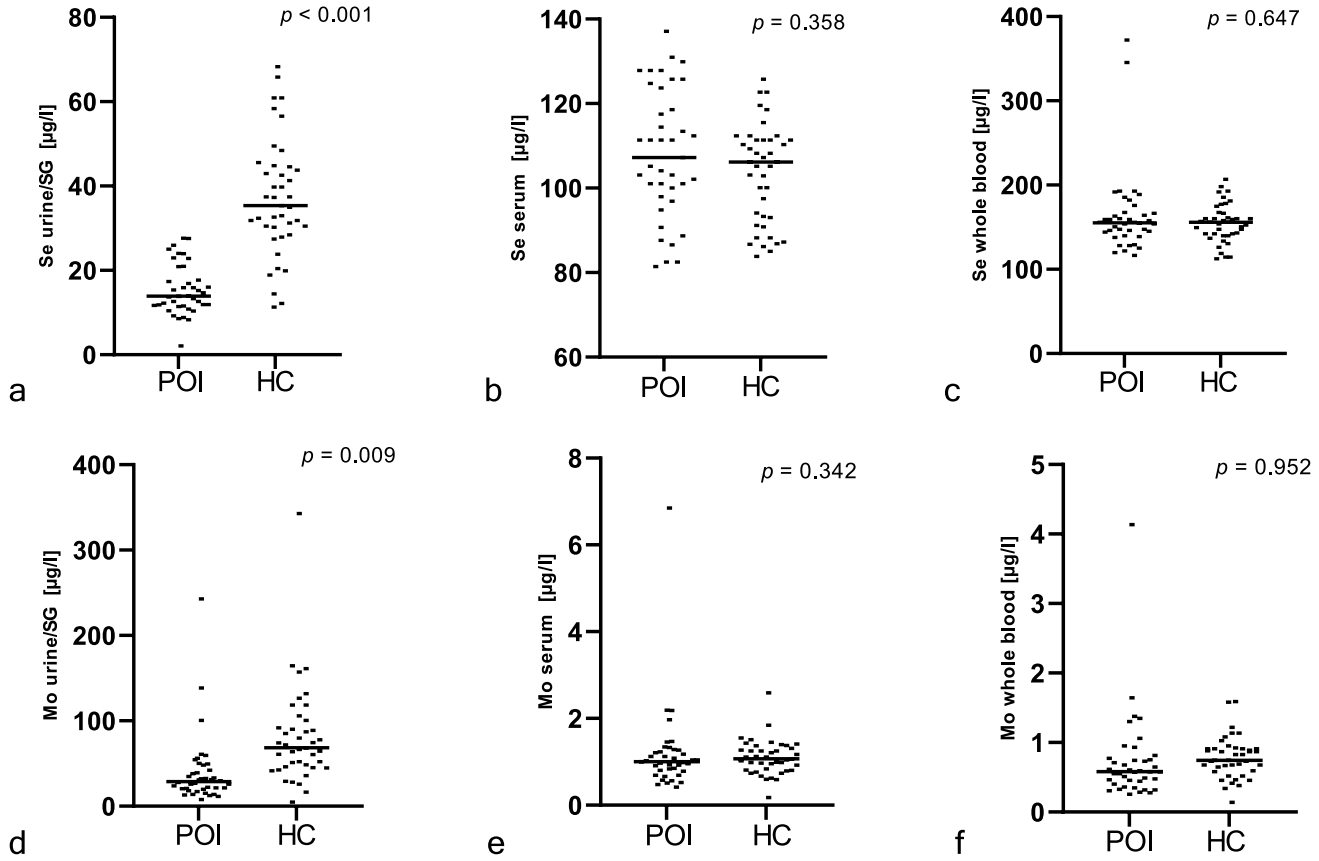

Fig. 1 Scatter box-and-whisker plots illustrating the distributions of essential TEs Se (Figures **a**, **b**, **c**) and Mo (Figures **d**, **e**, **f**) across different biofluids in the POI group and healthy controls (HC), along with their corresponding adjusted *p*-values

were significantly higher in the POI group. Subsequent analysis showed that Mn levels remained significant when age was included as a covariate, but this significance was lost when the model included either BMI or Ht individually or when both BMI and Ht were included together in the model (Supplement 8).

Distributions of Trace Elements in Serum

The serum concentrations and detection rates for healthy controls and POI patients are summarized in Supplement 5. In unadjusted models, the median serum levels of Mn and Cu were significantly higher, while the median serum levels of Cd, Hg, and Pb were significantly lower in the POI group compared to the control group. In adjusted models (accounting for age and BMI), the differences in element levels remained significant except for Mn, which was no longer significant (Supplement 8).

Correlations Between Trace Elements, Demographic and Biochemical Parameters

Kendall's tau correlation analyses were conducted to evaluate the relationships among nine TEs across different fluids, with the results detailed in Supplement 4. The analysis revealed several notable correlations. In both the overall study cohort and subgroup analyses, strong inter-biofluid correlations were observed between As, Mo, and Hg across different biofluids, with Kendall's tau coefficients ranging from 0.6 to 0.8. Additionally, Cu and Se showed moderate correlations between their serum and whole blood concentrations, with coefficients between 0.4 and 0.6. In urine, within the overall study cohort, a strong correlation was found between Cu and Se, with Kendall's tau coefficient of 0.625 ($p < 0.01$). Moderate correlations were observed between Cu and other elements such as Zn ($T = 0.447$, $p < 0.01$), Mo ($T = 0.453$, $p < 0.01$), and Cd ($T = 0.476$,

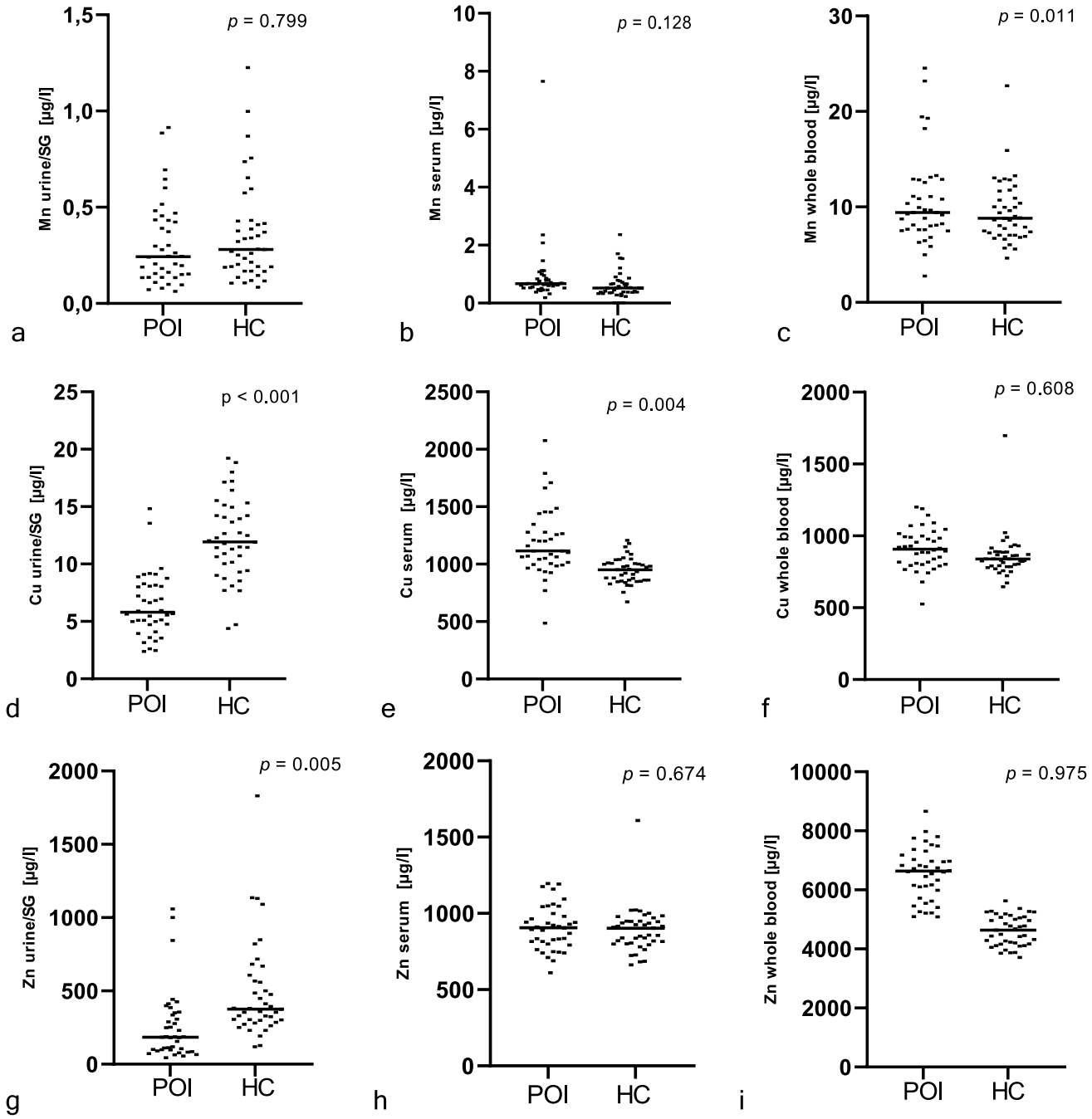

Fig. 2 Scatter box-and-whisker plots illustrating the distributions of essential TEs Mn (Figures **a**, **b**, **c**), Cu (Figures **d**, **e**, **f**), and Zn (Figures **g**, **h**, **i**) across different biofluids in the POI group and healthy controls (HC), along with their corresponding adjusted *p*-values

$p < 0.01$). Selenium also showed moderate correlations with Zn ($T = 0.433$, $p < 0.01$) and Mo ($T = 0.469$, $p < 0.01$) in urine. In the subgroup correlation analysis, a moderate correlation was observed between urinary Cu and Zn levels ($T = 0.497$, $p < 0.01$), as well as between Cu and Cd levels ($T = 0.472$, $p < 0.01$) in the POI group. In contrast, the control group showed generally weak correlations among urinary TEs ($T < 0.4$), with the exception of a moderate correlation between Cu and Se ($T = 0.436$, $p < 0.01$). In both subgroup and overall analyses, moderate correlations ($T = 0.4$—0.6) were also observed between whole blood Hg and As levels across urine, serum, and whole blood.

Only weak, statistically significant Kendall's tau correlations ($T < 0.4$) were observed between the TEs and significant demographic and biochemical parameters (Tables 1 and 2). These correlations were inconsistent, appearing in

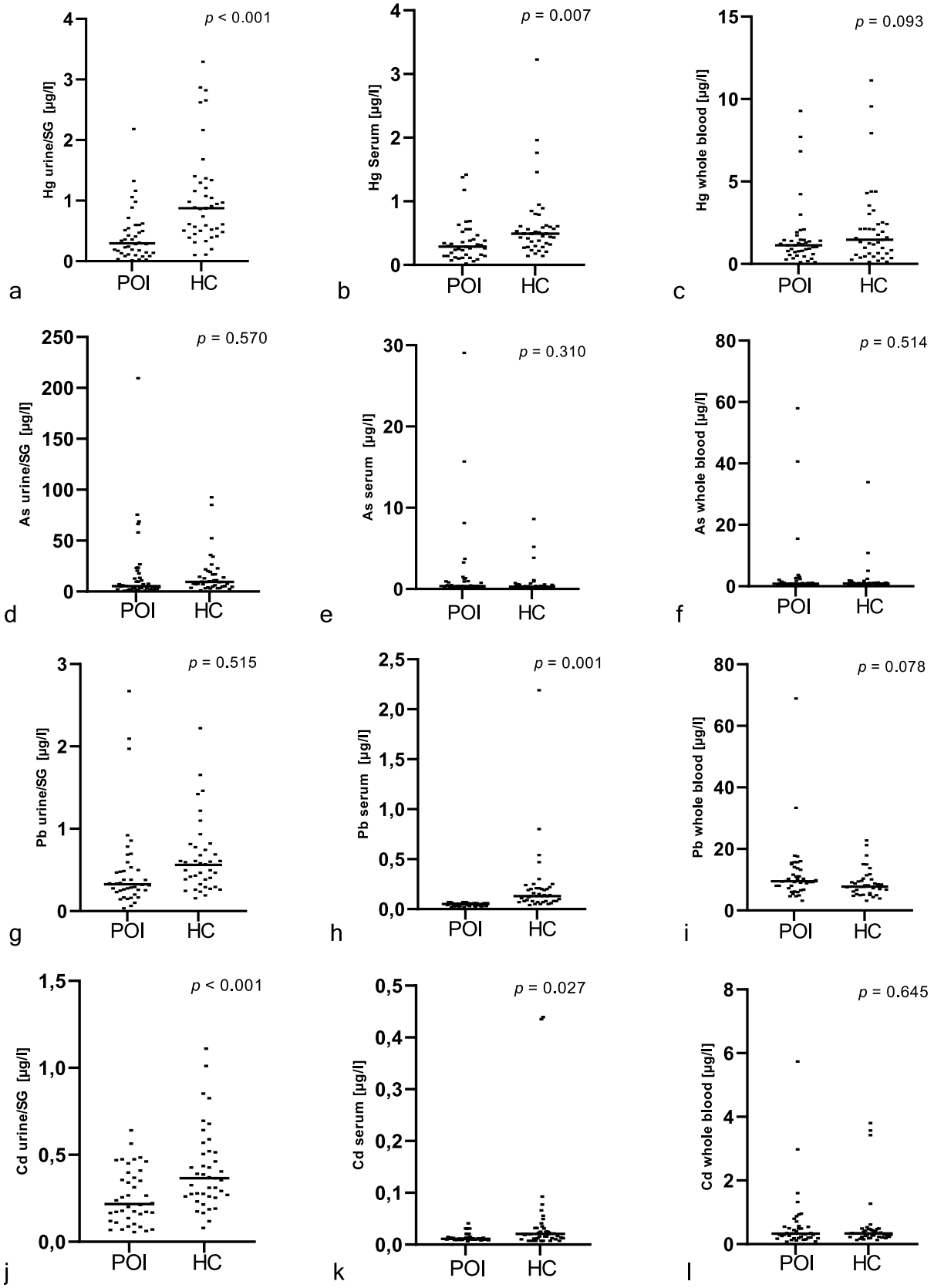

Fig. 3 Scatter box-and-whisker plots illustrating the distributions of non-essential TEs Hg (Figures **a**, **b**, **c**), As (Figures **d**, **e**, **f**), Pb (Figures **g**, **h**, **i**), and Cd (Figures **j**, **k**, **l**) across different biofluids in the POI group and healthy controls (HC), along with their corresponding adjusted *p*-values

the overall analyses but not consistently across subgroups, as detailed in Supplement 4. For instance, mineral supplementation was weakly negatively correlated with urinary Cu ($T=-0.311$, $p<0.01$) and Se ($T=-0.330$, $p<0.01$) levels in the overall analysis. However, this correlation was insignificant in the control group, which had significantly higher urinary Cu and Se levels and a higher reported intake of mineral supplements. In the POI group, mineral supplementation showed a weak negative correlation with whole blood As ($T=-0.350$, $p<0.01$), a TE that did not differ significantly between groups. Urinary Hg levels were weakly correlated with agricultural home surroundings in the overall analysis ($T=0.301$, $p<0.01$) and in the control group ($T=0.366$, $p<0.01$), but this association was not significant in the POI group, which had a higher proportion of individuals from rural, agricultural areas. Medication use was weakly negatively correlated with serum Se in the control group ($T=-0.312$, $p<0.01$) but was not significant in the overall or POI group analyses. Employment status, though not significant in the overall analysis, was weakly correlated with urinary Cu ($T=-0.369$, $p<0.01$) and whole blood Cu ($T=-0.339$, $p<0.01$) in the control group, as well as with urinary As ($T=-0.354$, $p<0.01$), whole blood As ($T=-0.363$, $p<0.01$), whole blood Pb ($T=0.338$, $p<0.01$), and urinary Mo ($T=-0.327$, $p<0.01$) in the POI group.

Discussion

The results of this study suggest that levels of certain TEs differ between POI patients and healthy controls. POI patients had significantly lower urinary levels of Cu, Zn, Se, Mo, Cd, Hg, and Pb compared to controls. In whole blood, Mn levels were significantly higher in POI patients after adjustment for age, hematocrit, and BMI. Serum Cu levels were significantly higher in POI patients, while Pb, Cd, and Hg levels were consistently lower. Only As levels showed no significant differences in any biological samples.

The TEs concentrations measured in our study align with those reported in women from various environmental health studies [46, 57–72], indicating a degree of consistency across different research settings. However, comprehensive comparisons of all analyzed elements in POI patients are limited. We identified nine studies comparing TE levels in POI patients to healthy controls [28–31, 35, 36] (Supplement 1). Our findings reveal both similarities and differences with these studies, with some TEs showing consistent patterns and others varying significantly. This variability highlights the complexity of evaluating TEs in POI and suggests influences such as geographical differences, dietary habits, and methodological variations.

Acknowledging the potential for exposure misclassification, we collected data on demographic, lifestyle, and dietary factors that may help to explain differences between the POI and control groups. However, our analyses showed only weak and inconsistent correlations between significant questionnaire variables (Table 1, Table 2) and measured TEs levels (Supplement 4). Additionally, kidney and liver function did not differ significantly between groups, excluding organ function as a confounding factor for TEs levels.

Our analysis of essential TEs showed the most notable differences in urinary concentrations, with Cu, Zn, Se, and Mo all being significantly lower in the POI group compared to healthy controls. Particularly, Cu emerged as a key element displaying significant differences in both urine and serum. Furthermore, Cu exhibited correlations with other TEs (Se, Zn, Mo, Cd) in urine, highlighting its central role in TEs metabolism and its potential involvement in the pathophysiology of POI.

Three previous studies have investigated Cu status in POI patients. Consistent with our findings, Kebapcilar et al. (2013) [28] reported significantly higher Cu levels in the serum of POI patients. Conversely, Verma et al. 2018 [30] and Li et al. (2021) [34] reported significantly lower serum and higher urinary levels of Cu in POI patients, respectively.

Studies on women undergoing in vitro fertilization highlight the essential role of Cu in ovarian function [17, 76, 77]. Disruptions in Cu homeostasis, as shown in animal studies, may accelerate ovarian aging through DNA damage, oxidative stress, and impaired steroidogenesis [18, 78–80], reducing follicle numbers across all developmental stages [79] hence affecting ovarian reserve.

Studies indicate that women generally have higher blood Cu levels than men [81–83], which was primarily attributed to the influence of estradiol (E2) [84]. In healthy, non-pregnant women not using hormonal therapies, serum Cu shows an inverse relationship with E2 levels [84, 85]. Furthermore, Ferdous et al. (2019) reported higher serum Cu levels in postmenopausal women compared to premenopausal women [86]. Cellular studies suggest that E2 increases Cu uptake by upregulating Cu transporters [87, 88], meeting higher metabolic demands during the reproductive cycle [87, 89]. This may lead to increased cellular Cu uptake, reduced serum Cu, and elevated urinary Cu excretion, explaining the lower serum and higher urinary Cu in controls compared to the opposite pattern in hypoestrogenic POI patients.

Beyond Cu, our analysis revealed that three additional essential TEs; Zn, Se, and Mo were also significantly lower in the urine of POI patients compared to controls, suggesting a broader pattern of altered TE metabolism and excretion in POI.

Urinary Zn levels were lower in the POI group compared to controls. However, Li et al. (2021) found no significant difference in urinary Zn levels but reported a negative correlation between urinary Zn and serum FSH in POI patients [34]. In contrast, two other studies reported lower serum Zn

levels in POI patients compared to healthy controls [28, 30]. Additionally, a large epidemiological study found significantly lower urinary Zn levels in postmenopausal women [90], suggesting altered Zn homeostasis under hypoestrogenic conditions. This finding supports evidence linking hormonal regulation to Zn homeostasis via Zn-specific transporter gene expression [91, 92]. Zn homeostasis is crucial in the female reproductive system, influencing oocyte maturation, quality, and functionality [93, 94]. Reduced Zn levels in POI patients have been associated with increased oxidative stress [30]. Since balanced levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are essential for follicular growth, angiogenesis, and sex hormone synthesis in ovarian tissue, an imbalance between Zn and ROS may contribute to the decline in oocyte quantity and quality [95]. However, further research is needed to elucidate the mechanisms underlying Zn metabolism in POI.

POI patients had significantly lower urinary Se levels compared to controls, contrasting with Li et al. (2021), who reported higher urinary Se levels in POI patients [45] (Supplement 1). Two previous studies reported decreased serum Se levels in POI patients [30, 31]. Se is essential for follicle development, steroid hormone synthesis, and embryonic development [18] and is found in granulosa cells of healthy follicles but is absent in atretic ones [22]. The lower urinary Se levels in POI patients may indicate increased Se utilization to counteract ROS associated with POI due to its antioxidant properties [96]. However, data on ceruloplasmin (Cu-binding glycoprotein) and Cu, Zn superoxide dismutase (SOD) activity are needed for a relevant conclusion.

Urinary Mo levels were significantly lower in POI patients compared to controls, contrasting with a previous study reporting higher serum Mo levels in POI patients linked to markers of vascular endothelial injury [35]. Mo is conserved at low intakes or higher metabolic demands but excreted rapidly at high levels. The effects of Mo on human reproductive health have been primarily studied in relation to male infertility, while its impact on female reproduction remains unclear [17]. Studies in women undergoing IVF have shown conflicting results: one found a positive correlation with oocyte retrieval [76], while another linked elevated urinary Mo to reduced implantation and pregnancy rates [17]. Animal studies suggest Mo may affect oocyte quality by modulating oxidative stress, vascular permeability, and androgen and estrogen receptor activity in a dose-dependent manner [35, 97–99].

Whole blood Mn levels did not significantly differ between the POI and control group in unadjusted analyses; however, after adjusting for age, BMI, and hematocrit, levels were significantly higher in the POI group. This significance was maintained with age as a covariate but lost when only BMI and hematocrit were included, suggesting age moderates the relationship between Mn levels and POI. Previous study found no link between urinary Mn levels and POI risk [34], and population studies show Mn levels stabilize in adulthood, with no direct age-related variability [100–103]. However, premenopausal women have higher Mn levels than postmenopausal women [102, 104, 105], possibly due to estrogen's role in upregulating intestinal Mn absorption [102, 106, 107]. In the ovary, Mn is essential for steroidogenesis, oocyte maturation, and corpus luteum function [18, 108], but both excess and deficiency have been linked to ovarian aging and impaired oocyte development in animal studies [18, 108–111]. Further research is needed to clarify Mn's role in hypoestrogenic conditions like POI and natural menopause [102]

The levels of nonessential TEs Cd, Hg, and Pb were significantly lower in the serum and urine of patients with POI compared to controls. Three previous studies have examined nonessential TEs in POI patients, with one study reporting no significant changes in serum levels of As, Hg, Cd, and Pb [29]. In contrast, Pan et al. (2020, 2021) found significantly higher urinary As and Cd levels in POI patients compared to healthy women [32, 33]. As summarized in Supplement 1, exposure levels in POI patients varied considerably between studies. For example, Pan et al. reported median As levels 4.7 times higher and geometric mean (GM) Cd levels 2.3 times higher in POI patients than those in our study. Although our study also found lower As and Cd levels in healthy controls, the difference was less pronounced. Overall, the levels of non-essential TEs observed in both POI patients and controls in our study were relatively low, suggesting a low level of exposure.

In environmental toxicology, it's generally accepted that the likelihood of an agent causing harm increases with higher doses or exposure levels, including for non-essential TEs [65]. While high doses of non-essential TEs are known to be harmful, low-dose exposure poses a subtle but significant threat [112, 113]. Animal and in vitro studies indicate that the toxic effects of Cd, Pb, and Hg on the ovaries are remarkably similar when each is administered individually. These elements can accumulate in the ovaries [23, 114–116], causing decreased follicular growth, an increased number of atretic follicles, degeneration of the corpus luteum, and prolonged and/or irregular cycles [115, 117–127]. These effects are linked to alterations in hormone levels, specifically FSH, LH, E2, P, AMH, and oxidative stress indices, such as increased levels of malondialdehyde (MDA) and decreased levels of antioxidants [128–134]. Even though detecting significant interactions or adverse effects on reproductive health may be challenging at low exposure levels, animal studies have demonstrated that even low doses of Cd, Pb, and Hg can accumulate in the ovaries [116, 122, 126, 134–140] leading to increased follicular apoptosis [116, 122, 135], the presence of atretic oocytes at various developmental stages [140] and disrupted steroidogenesis [116].

Low Cd and Pb exposure in healthy premenopausal women was linked to hormonal variations, with Pb increasing mean FSH levels while reducing its amplitude, and both Cd and Pb correlating with higher mean E2 levels [141], patterns suggesting early ovulatory resistance, resembling perimenopausal transitions where elevated FSH and E2 levels are needed for ovulation [141, 142]. These findings, combined with the high sensitivity of the applied methodology and the low rate of undetectable TEs values, suggest that the differences observed between the POI group and healthy controls are important, even at low exposure levels.

TEs concentrations in blood, serum, and urine primarily reflect exposure levels and may not accurately represent TEs accumulation in specific organs like the ovaries [143]. For example, Cd has been shown to accumulate in ovarian tissue over time, with concentrations increasing in women aged 30 to 65 years [144]. Higher TEs levels in healthy controls found in the present study may, besides differing exposure patterns, reflect lower cellular accumulation with more efficient detoxification and excretion processes. Further research is needed to elucidate the effects of low-level exposures and their potential interactions in the context of POI.

TE accumulation in the body is influenced not only by dietary intake and environmental exposure but also by genetic variations [143]. Genome-wide association studies have linked genetic variants in metal transporter genes to levels of Cu, Se, Zn, Mn, As, Cd, Pb, and Hg [143, 145–149]. Such genetic differences may increase susceptibility to TE accumulation and toxicity, affecting excretion or retention in hair, blood, erythrocytes, or urine [112]. However, little information is available on gene-TEs interactions in POI patients. A recent study by Mirinzehad et al. (2024) investigated the association between some POI-related genotypes and serum levels of Cu, Zn, calcium, phosphate, magnesium and vitamin D. The study reported that the C and G alleles of the rs4806660 polymorphism in the *TMEM150B*[2] gene and the rs244715 polymorphism in the *ZNF346*[3] gene, respectively, are independently associated with serum Cu levels in women with POI. In POI patients, the C allele of rs4806660 was linked to lower Cu levels compared to the TT genotype, while the GG genotype of rs244715 was associated with higher Cu levels than the AA genotype. Both *TMEM150B* and *ZNF346* encode proteins potentially involved in cell growth and survival [36].

According to the epidemiological studies carried out so far, a genetic predisposition to altered TEs homeostasis or vulnerability to non-essential TEs may help explain varying health impacts from environmental TEs exposure. However, further research is needed to clarify the genetic basis of susceptibility to TEs toxicity in POI pathogenesis [112].

Limitations and Strengths of the Study

Our study has several limitations that require cautious interpretation. The primary limitation is the small sample size, which reduces statistical power. However, participants were rigorously selected according to strict, internationally recognized criteria for POI [6], with the exclusion of individuals with known causes of POI, such as those with abnormal karyotypes or Fragile X syndrome/*FMR1*[4] gene premutation. Relevant biological samples were used for specific TEs. In clinical practice, the most commonly used biological specimens to assess exposure to non-essential TEs and the nutritional status of essential TEs are serum for Cu [57], Se [150], Zn [151], and Mo [152]; whole blood for Pb [153], Mn [154], and organic Hg species [155]; and urine for As [156], Cd [157], and inorganic Hg species [158]. This study thus comprehensively evaluates TE exposure and nutritional status by including all major biofluids. The cross-sectional design limits our ability to establish temporal relationships or causality between the analyzed elements and POI. Additionally, reliance on single time-point TE measurements may misclassify associations, as this approach does not capture relevant exposure windows, particularly since our study included patients with established ovarian failure. For a more accurate assessment of cumulative TEs exposure, alternative matrices like hair or nails, which better reflect long-term exposure, may be preferable. Finally, although we controlled for selected known confounders, residual confounding cannot be entirely ruled out due to the observational nature of the study. Addressing critical factors such as exposure timing, dosage, and susceptibility in relation to POI requires longitudinal studies. Such studies, with larger sample sizes and diverse populations, are necessary to clarify the role of TEs in POI and establish the directionality of these associations.

Conclusion

The development and diagnosis of POI in young women have profound physical and emotional consequences. POI significantly shortens a woman's fertile lifespan and causes distressing vasomotor and genitourinary symptoms associated with menopause. Additionally, it has widespread effects on general health, psychological and sexual well-being, and long-term bone, cardiovascular, and cognitive health [1]. Therefore, ovarian health should be viewed as an early indicator of premature somatic aging and reduced longevity, analogous to the 'canary in the coal mine' as a predictor of broader health issues [159]. Although the environmental etiology of POI is well established, the precise mechanisms involved are still not

[2] Transmembrane Protein 150B.

[3] Zinc Finger Protein 346.

[4] Fragile X Messenger Ribonucleoprotein 1 gene.

fully understood. Our study is the first to comprehensively analyze multiple essential and non-essential TEs across urine, whole blood, and serum in POI patients compared to healthy controls. These findings provide a foundation for future research into the mechanisms of POI and potential strategies for its management. Larger longitudinal studies are needed to confirm these results and investigate the therapeutic potential of TEs modulation in POI.

Abbreviations *AMH*: Anti-Müllerian Hormone; *APRI*: Aspartate Aminotransferase to Platelet Ratio Index; *As*: Arsenic; *BMI*: Body Mass Index; *Cd*: Cadmium; *CI*: Confidence Interval; *CDK-EPI*: Chronic Kidney Disease Epidemiology Collaboration Formula; *Cu*: Copper; *E2*: Estradiol; *ESHRE*: European Society of Human Reproduction and Embryology; *FIB-4*: Fibrosis-4 Index; *FMR1*: Fragile X Messenger Ribonucleoprotein 1 gene; *FSH*: Follicle-Stimulating Hormone; *GFR*: Glomerular Filtration Rate; *GM*: Geometric Mean; *GPX*: Selenium-Dependent Glutathione Peroxidase; *HC*: Healthy Controls; *Hg*: Mercury; *Ht*: Hematocrit; *IVF*: In Vitro Fertilization; *LH*: Luteinizing Hormone; *LOD*: Limits of Detection; *LR*: Binary Logistic Regression; *MDA*: Malondialdehyde; *MDRD*: Modification of Diet in Renal Disease; *Mn*: Manganese; *Mo*: Molybdenum; *P*: Progesterone; *PCOS*: Polycystic Ovary Syndrome; *Pb*: Lead; *POI*: Premature Ovarian Insufficiency; *RBC*: Red Blood Cell; *RDW*: Red Cell Distribution Width; *SDs*: Standard Deviations; *Se*: Selenium; *SG*: Specific Gravity; *SOD*: Cu, Zn Superoxide Dismutase; *TEs*: Trace Elements; *TMEM150B*: Transmembrane Protein 150B gene; *ZNF346*: Zinc Finger Protein 346 gene; *Zn*: Zinc

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12011-024-04507-8.

Acknowledgements Acknowledgments We would like to express our sincere gratitude to Ms. Tinkara Srnovršnik, M.D., and Prof. Dr. Bojana Pinter M.D. for their invaluable assistance in recruiting participants for the control group. Our thanks extend to Mrs. Vera Troha, Prof. Dr. Joško Osredkar, and Dr. Urška Čegovnik Primožič from the Institute of Clinical Chemistry and Biochemistry at the University Medical Centre Ljubljana for their outstanding professionalism and dedication in the collection and analysis of laboratory samples. Special thanks are also extended to Dr. Marta Jagodic Hudobivnik and Polona Klemenčič for their contributions to the analysis of TEs in biofluids. Furthermore, we acknowledge the financial support provided by the Slovenian Research and Innovation Agency, which made this study possible. Finally, we sincerely thank all our study participants for their involvement in the study.

Author Contributions Author Contributions Conceptualization: KG, IVK; data curation: KG, IVK, TK, DCŠ; formal analysis: DM, MH, NKK, TK; writing—original draft: TK; reviewing original draft: IVK, NKK, KG, MH, DM, JST, IF; funding acquisition: IVK. All authors read and approved the final manuscript.

Funding This work was funded by the Slovenian Research and Innovation Agency (AIRS) as part of the research project J3-2530 titled "The Impact of Endocrine Disruptors (Bisphenols, Parabens, Triclosan) and Potentially Toxic and Essential Chemical Elements on Birth, Infertility, and Ovarian Cancer in Slovenia," as well as the program P3-0124 titled "Metabolic and Congenital Factors of Reproductive Health, Birth III.".

Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval and Consent to Participate Ethical approval was obtained from the Medical Ethics Committee of the Republic of Slovenia (0120–158/2022/9, 19.7.2022). Informed consent was obtained from all subjects involved in the study.

Consent for Publication All authors have read and agreed to the published version of the manuscript.

Competing Interests The authors declare no competing interests.

References

1. Panay N, Anderson RA, Nappi RE, Vincent AJ, Vujovic S, Webber L, Wolfman W (2020) Premature ovarian insufficiency: an international menopause society white paper. Climacteric 23:426–446. https://doi.org/10.1080/13697137.2020.1804547
2. Allouch A, Al-Barazenji T, Al-Shafai M, Abdallah AM (2024) The landscape of genetic variations in non-syndromic primary ovarian insufficiency in the MENA region: a systematic review. Front Endocrinol 14. https://doi.org/10.3389/fendo.2023.1289333
3. Béranger R, Hoffmann P, Christin-Maitre S, Bonneterre V (2012) Occupational exposures to chemicals as a possible etiology in premature ovarian failure: a critical analysis of the literature. Reprod Toxicol 33:269–279. https://doi.org/10.1016/j.reprotox.2012.01.002
4. Shelling AN, Ahmed Nasef N (2023) The role of lifestyle and dietary factors in the development of premature ovarian insufficiency. Antioxidants (Basel) 12:1601. https://doi.org/10.3390/antiox12081601
5. Singer D, Mann E, Hunter MS, Pitkin J, Panay N (2011) The silent grief: psychosocial aspects of premature ovarian failure. Climacteric 14:428–437. https://doi.org/10.3109/13697137.2011.571320
6. The ESHRE Guideline Group on POI, Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, Cifkova R, de Muinck Keizer-Schrama S, Hogervorst E, Janse F, Liao L, Vlaisavljevic V, Zillikens C, N. Vermeulen (2016) ESHRE Guideline: management of women with premature ovarian insufficiency†. Human Reproduction 31:926–937. https://doi.org/10.1093/humrep/dew027
7. Land KL, Miller FG, Fugate AC, Hannon PR (2022) The effects of endocrine-disrupting chemicals on ovarian- and ovulation-related fertility outcomes. Mol Reprod Dev 89:608–631. https://doi.org/10.1002/mrd.23652

8. Vabre P, Gatimel N, Moreau J, Gayrard V, Picard-Hagen N, Parinaud J, Leandri RD (2017) Environmental pollutants, a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data. Environ Health 16:37. https://doi.org/10.1186/s12940-017-0242-4
9. Rudnicka E, Kruszewska J, Klicka K, Kowalczyk J, Grymowicz M, Skórska J, Pięta W, Smolarczyk R (2018) Premature ovarian insufficiency - aetiopathology, epidemiology, and diagnostic evaluation. Prz Menopauzalny 17:105–108. https://doi.org/10.5114/pm.2018.78550
10. Koh JM, Kim CH, Hong SK, Lee KU, Kim YT, Kim OJ, Kim GS (1998) Primary ovarian failure caused by a solvent containing 2-bromopropane. Eur J Endocrinol 138:554–556. https://doi.org/10.1530/eje.0.1380554
11. Pan J, Liu P, Yu X, Zhang Z, Liu J (2024) The adverse role of endocrine disrupting chemicals in the reproductive system. Front Endocrinol (Lausanne) 14:1324993. https://doi.org/10.3389/fendo.2023.1324993
12. Li C, Cao M, Ma L, Ye X, Song Y, Pan W, Xu Z, Ma X, Lan Y, Chen P, Liu W, Liu J, Zhou J (2018) Pyrethroid pesticide exposure and risk of primary ovarian insufficiency in chinese women. Environ Sci Technol 52:3240–3248. https://doi.org/10.1021/acs.est.7b06689
13. Zhang S, Tan R, Pan R, Xiong J, Tian Y, Wu J, Chen L (2018) Association of perfluoroalkyl and polyfluoroalkyl substances with premature ovarian insufficiency in chinese women. J Clin Endocrinol Metab 103:2543–2551. https://doi.org/10.1210/jc.2017-02783
14. Zhu X, Liu M, Dong R, Gao L, Hu J, Zhang X, Wu X, Fan B, Chen C, Xu W (2024) mechanism exploration of environmental pollutants on premature ovarian insufficiency: a systematic review and meta-analysis. Reprod Sci 31:99–106. https://doi.org/10.1007/s43032-023-01326-5
15. He ZL, Yang XE, Stoffella PJ (2005) Trace elements in agroecosystems and impacts on the environment. J Trace Elem Med Biol 19:125–140. https://doi.org/10.1016/j.jtemb.2005.02.010
16. Forte G, Ariu F, Bocca B, Solinas G, Leoni GG, Podda A, Madeddu R, Bogliolo L (2023) Heavy metal(loid) accumulation in the ovarian tissue of free-ranging queens and bitches inhabiting highly polluted urban environments. Animals (Basel) 13:650. https://doi.org/10.3390/ani13040650
17. Gonzalez-Martin R, Palomar A, Quiñonero A, Pellicer N, Fernandez-Saavedra R, Conde-Vilda E, Quejido AJ, Whitehead C, Scott RT, Dominguez F (2023) The impact of essential trace elements on ovarian response and reproductive outcomes following single euploid embryo transfer. Int J Mol Sci 24:10968. https://doi.org/10.3390/ijms241310968
18. Tang W, Zhu X, Chen Y, Yang S, Wu C, Chen D, Xue L, Guo Y, Dai Y, Wei S, Wu M, Wu M, Wang S (2024) Towards prolonging ovarian reproductive life: Insights into trace elements homeostasis. Ageing Res Rev 97:102311. https://doi.org/10.1016/j.arr.2024.102311
19. Roth Z (2018) Symposium review: reduction in oocyte developmental competence by stress is associated with alterations in mitochondrial function. J Dairy Sci 101:3642–3654. https://doi.org/10.3168/jds.2017-13389
20. Priya K, Setty M, Babu UV, Pai KSR (2021) Implications of environmental toxicants on ovarian follicles: how it can adversely affect the female fertility? Environ Sci Pollut Res Int 28:67925–67939. https://doi.org/10.1007/s11356-021-16489-4
21. Cavallini A, Lippolis C, Vacca M, Nardelli C, Castegna A, Arnesano F, Carella N, Depalo R (2016) The effects of chronic lifelong activation of the AHR pathway by industrial chemical pollutants on female human reproduction. PLoS ONE 11:e0152181. https://doi.org/10.1371/journal.pone.0152181
22. Ceko MJ, Hummitzsch K, Hatzirodos N, Bonner WM, Aitken JB, Russell DL, Lane M, Rodgers RJ, Harris HH (2015) X-Ray fluorescence imaging and other analyses identify selenium and GPX1 as important in female reproductive function. Metallomics 7:71–82. https://doi.org/10.1039/c4mt00228h
23. Paksy K, Gáti I, Náray M, Rajczy K (2001) Lead accumulation in human ovarian follicular fluid, and in vitro effect of lead on progesterone production by cultured human ovarian granulosa cells. J Toxicol Environ Health A 62:359–366. https://doi.org/10.1080/152873901300018093
24. Obasi CN, Frazzoli C, Orisakwe OE (2022) Heavy metals and metalloids exposure and in vitro fertilization: Critical concerns in human reproductive medicine. Front Reprod Health 4:1037379. https://doi.org/10.3389/frph.2022.1037379
25. Rzymski P, Tomczyk K, Rzymski P, Poniedziałek B, Opala T, Wilczak M (2015) Impact of heavy metals on the female reproductive system. Ann Agric Environ Med 22:259–264. https://doi.org/10.5604/12321966.1152077
26. Massányi P, Massányi M, Madeddu R, Stawarz R, Lukáč N (2020) Effects of cadmium lead, and mercury on the structure and function of reproductive organs. Toxics 8:94. https://doi.org/10.3390/toxics8040094
27. McClam M, Liu J, Fan Y, Zhan T, Zhang Q, Porter DE, Scott GI, Xiao S (2023) Associations between exposure to cadmium, lead, mercury and mixtures and women's infertility and long-term amenorrhea. Arch Public Health 81:161. https://doi.org/10.1186/s13690-023-01172-6
28. Kebapcilar AG, Kulaksizoglu M, Kebapcilar L, Gonen MS, Unlü A, Topcu A, Demirci F, Taner CE (2013) Is there a link between premature ovarian failure and serum concentrations of vitamin D, zinc, and copper? Menopause 20:94–99. https://doi.org/10.1097/gme.0b013e31826015ca
29. Özel Ş, Aktulay A, Aykut O, Uncu H, Celik H, Soysal S, Çınar M, Ustun Y (2017) Evaluation of serum heavy metal levels on primary ovarian insufficiency. Medicine Science 6:1. https://doi.org/10.5455/medscience.2017.06.8666
30. Verma P, Sharma AK, Shankar H, Sharma A, Rao DN (2018) role of trace elements, oxidative stress and immune system: a triad in premature ovarian failure. Biol Trace Elem Res 184:325–333. https://doi.org/10.1007/s12011-017-1197-6
31. Delkhorrami M, Farshbaf-Khalili A, Mirghafourvand M, Oskouei BS (2020) Low serum selenium levels in iranian women with idiopathic primary ovarian insufficiency: a case-control study. J Biochem Tech, Special Issue (1):71–78
32. Pan W, Ye X, Zhu Z, Li C, Zhou J, Liu J (2020) A case-control study of arsenic exposure with the risk of primary ovarian insufficiency in women. Environ Sci Pollut Res Int 27:25220–25229. https://doi.org/10.1007/s11356-020-08806-0
33. Pan W, Ye X, Zhu Z, Li C, Zhou J, Liu J (2021) Urinary cadmium concentrations and risk of primary ovarian insufficiency in women: a case-control study. Environ Geochem Health 43:2025–2035. https://doi.org/10.1007/s10653-020-00775-0
34. Li C, Ma L, Qi T, Pan W, Huang Y, Luo J, Ye X, Lan Y, Liu J, Zhou W, Ruan F, Zhou J (2021) Urinary trace elements in association with premature ovarian insufficiency and reproductive hormones in a Chinese population. Ecotoxicol Environ Saf 225:112731. https://doi.org/10.1016/j.ecoenv.2021.112731
35. Wang Q, Wang L, Sun J, Huang Y, Zhang Q, Wei L, Lai D (2022) excessive plasma molybdenum induces ovarian dysfunction through increasing vascular permeability,. https://papers.ssrn.com/abstract=4252969. Accessed 16 Jul 2024
36. Mirinezhad MR, Aghsizadeh M, Ghazizadeh H, Ghoflchi S, Bidary MZ, Naghipour A, Ferns GA, Hamzehloei T, Pasdar A, Ghayour-Mobarhan M (2024) Micronutrients intake and genetic variants associated with premature ovarian insufficiency. MASHAD cohort study, BMC Womens Health 24:91. https://doi.org/10.1186/s12905-023-02865-4

37 Bishop ML, Fody EP, Schoeff LE (eds) (2018) Clinical chemistry: principles, techniques, and correlations, Enhanced, 8th edn. Jones & Bartlett Learning, Burlington, MA

38. Laboratorijski vodnik UKC Ljubljana (n.d.) https://lab.biarti.si/?klinika=KIKKB. Accessed 19 Oct 2024
39. Cleophas TJ, Zwinderman AH, Cleophas TF, Cleophas EP eds (2009) Summary of validation procedures for diagnostic tests, in: statistics applied to clinical trials, Springer Netherlands, Dordrecht, pp 433–447. https://doi.org/10.1007/978-1-4020-9523-8_37
40. Burkhardt D (1998) Laboratorijski izvidi: razumeti medicino; pregled normalnih vrednosti; kako dosežemo trajno ugodne rezultate. Mavrica, Celje
41. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation modification of diet in renal disease study group. Ann Intern Med 130:461–470. https://doi.org/10.7326/0003-4819-130-6-199903160-00002
42. Pöge U, Gerhardta T, Palmedo H, Klehr H-U, Sauerbruch T, Woitas RP (2005) MDRD equations for estimation of GFR in renal transplant recipients. Am J Transplant 5:1306–1311. https://doi.org/10.1111/j.1600-6143.2005.00861.x
43. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, Crews DC, Doria A, Estrella MM, Froissart M, Grams ME, Greene T, Grubb A, Gudnason V, Gutiérrez OM, Kalil R, Karger AB, Mauer M, Navis G, Nelson RG, Poggio ED, Rodby R, Rossing P, Rule AD, Selvin E, Seegmiller JC, Shlipak MG, Torres VE, Yang W, Ballew SH, Couture SJ, Powe NR, Levey AS (2021) New creatinine- and cystatin C-based equations to estimate GFR without race. N Engl J Med 385:1737–1749. https://doi.org/10.1056/NEJMoa2102953
44. Berzigotti A, Tsochatzis E, Boursier J, Castera L, Cazzagon N, Friedrich-Rust M, Petta S, Thiele M (2021) EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis - 2021 update. J Hepatol 75:659–689. https://doi.org/10.1016/j.jhep.2021.05.025
45. Stajnko A, Tuhvatshin R, Suranova G, Mazej D, Šlejkovec Z, Falnoga I, Krušič Ž, Lespukh E, Stegnar P (2020) Trace elements and ALAD gene polymorphisms in general population from three uranium legacy sites - A case study in Kyrgyzstan. Sci Total Environ 719:134427. https://doi.org/10.1016/j.scitotenv.2019.134427
46. Snoj Tratnik J, Falnoga I, Mazej D, Kocman D, Fajon V, Jagodic M, Stajnko A, Trdin A, Šlejkovec Z, Jeran Z, Osredkar J, Sešek-Briški A, Krsnik M, Kobal AB, Kononenko L, Horvat M (2019) Results of the first national human biomonitoring in Slovenia: Trace elements in men and lactating women, predictors of exposure and reference values. Int J Hygiene Environ Health 222:563–582. https://doi.org/10.1016/j.ijheh.2019.02.008
47. Hoet P, Deumer G, Bernard A, Lison D, Haufroid V (2016) Urinary trace element concentrations in environmental settings: is there a value for systematic creatinine adjustment or do we introduce a bias? J Expo Sci Environ Epidemiol 26:296–302. https://doi.org/10.1038/jes.2015.23
48. Hays SM, Aylward LL, Blount BC (2015) Variation in urinary flow rates according to demographic characteristics and body mass index in NHANES: potential confounding of associations between health outcomes and urinary biomarker concentrations. Environ Health Perspect 123:293–300. https://doi.org/10.1289/ehp.1408944
49. Stajnko A, Falnoga I, Tratnik JS, Mazej D, Jagodic M, Krsnik M, Kobal AB, Prezelj M, Kononenko L, Horvat M (2017) Low cadmium exposure in males and lactating females-estimation of biomarkers. Environ Res 152:109–119. https://doi.org/10.1016/j.envres.2016.09.025
50. Moore RET, Rehkämper M, Kreissig K, Strekopytov S, Larner F (2018) Determination of major and trace element variability in healthy human urine by ICP-QMS and specific gravity normalisation. RSC Adv 8:38022–38035. https://doi.org/10.1039/C8RA06794E
51. Cone EJ, Caplan YH, Moser F, Robert T, Shelby MK, Black DL (2009) Normalization of urinary drug concentrations with specific gravity and creatinine. J Anal Toxicol 33:1–7. https://doi.org/10.1093/jat/33.1.1
52. Suwazono Y, Akesson A, Alfvén T, Järup L, Vahter M (2005) Creatinine versus specific gravity-adjusted urinary cadmium concentrations. Biomarkers 10:117–126. https://doi.org/10.1080/13547500500159001
53. van Buuren S (2018) Flexible Imputation of Missing Data, Second Edition, 2nd ed, Chapman and Hall/CRC, New York. https://doi.org/10.1201/9780429492259
54. Giri R, Vincent AJ (2020) Prevalence and risk factors of premature ovarian insufficiency/early menopause. Semin Reprod Med 38:237–246. https://doi.org/10.1055/s-0040-1722317
55. Wai KM, Sawada K, Kumagai M, Itai K, Tokuda I, Murashita K, Nakaji S, Ihara K (2020) Relationship between selected trace elements and hematological parameters among japanese community dwellers. Nutrients 12:1615. https://doi.org/10.3390/nu12061615
56. Bornhorst J, Mcmillin G (2006) Trace and toxic elemental testing in the clinical laboratory. Labmedicine 37:690–695. https://doi.org/10.1309/XN6B-LHL1-Q14V-XG9M
57. Toxicological Profile for Copper, Toxicological Profiles | ATSDR (2024) https://www.atsdr.cdc.gov/toxprofiledocs/index.html. Accessed 15 Oct 2024
58. Canada H (2021) Sixth report on human biomonitoring of environmental chemicals in Canada,. https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/environmental-contaminants/sixth-report-human-biomonitoring.html. Accessed 2 July 2024
59. Osredkar J (2013) Biomonitoring of selected trace elements in women, men and children from Slovenia, E3S Web of Conferences. https://www.academia.edu/105172058/Biomonitoring_of_selected_trace_elements_in_women_men_and_children_from_Slovenia. Accessed 3 Jul 2024
60. Toxicological Profile For Arsenic (2007) Agency for Toxic Substances and Disease Registry (US), Atlanta (GA),. http://www.ncbi.nlm.nih.gov/books/NBK591633/. Accessed 3 Jul 2024
61. Heitland P, Köster HD (2021) Human biomonitoring of 73 elements in blood, serum, erythrocytes and urine. J Trace Elem Med Biol 64:126706. https://doi.org/10.1016/j.jtemb.2020.126706
62. Nisse C, Tagne-Fotso R, Howsam M (2017) members of health examination centres of the nord – Pas-de-Calais region network, C. Richeval, L. Labat, A. Leroyer, blood and urinary levels of metals and metalloids in the general adult population of Northern France: The IMEPOGE study, 2008–2010. Int J Hyg Environ Health 220:341–363. https://doi.org/10.1016/j.ijheh.2016.09.020

63 Soylemez E, Ozcagli E, Goktas EF, Kurekci F, Omurtag GZ (2023) Human Biomonitoring of Trace Elements (Zn, Cu, Mn, Cd and As) Level in a Healthy Urban Population in Türkiye. Jrp 27(5):2087–2094. https://doi.org/10.29228/jrp.487

64. Alimonti A, Bocca B, Mattei D, Pino A (2011) Programme for biomonitoring the Italian population exposure (PROBE): internal dose of metals, Rapp. ISTISAN. https://www.iss.it/documents/20126/45616/11_9_web.pdf/f6611d7b-b4a1-f494-a727-8096259632e1?t=1581098758230. Accessed 3 Jul 2024
65. Syversen T, Evje L, Wolf S, Flaten T, Lierhagen S, Simic A (2021) Trace elements in the large population-based HUNT3 survey, biological trace element research 199. https://doi.org/10.1007/s12011-020-02376-5
66. Haines DA, Saravanabhavan G, Werry K, Khoury C (2017) An overview of human biomonitoring of environmental chemicals in the Canadian Health Measures Survey: 2007–2019. Int J Hyg Environ Health 220:13–28. https://doi.org/10.1016/j.ijheh.2016.08.002

67. Zhang L-L, Lu L, Pan Y-J, Ding C-G, Xu D-Y, Huang C-F, Pan X-F, Zheng W (2015) Baseline blood levels of manganese, lead, cadmium, copper, and zinc in residents of Beijing suburb. Environ Res 140:10–17. https://doi.org/10.1016/j.envres.2015.03.008
68. Burguera JL, Burguera M (2007) Molybdenum in human whole blood of adult residents of the Merida State (Venezuela). J Trace Elem Med Biol 21:178–183. https://doi.org/10.1016/j.jtemb.2007.03.005
69. Cui Y, Zhong Q, Hu M, Sheng J, Yang Y, Liang L, Wang X, Yang Y, Zhou M, Huang F (2017) Human biomonitoring of eight trace elements in urine of residents living in rural areas along the Yangtze River, China. Environ Sci Pollut Res 24:1–11. https://doi.org/10.1007/s11356-017-0414-3
70. Phiri FP, Ander EL, Lark RM, Joy EJM, Kalimbira AA, Suchdev PS, Gondwe J, Hamilton EM, Watts MJ, Broadley MR (2021) Spatial analysis of urine zinc (Zn) concentration for women of reproductive age and school age children in Malawi. Environ Geochem Health 43:259–271. https://doi.org/10.1007/s10653-020-00700-5
71. Gallagher CM, Moonga BS, Kovach JS (2010) Cadmium, follicle-stimulating hormone, and effects on bone in women age 42–60 years, NHANES III. Environ Res 110:105–111. https://doi.org/10.1016/j.envres.2009.09.012
72. Toxicological Profile for Selenium (2003) Agency for Toxic Substances and Disease Registry (US), Atlanta (GA). http://www.ncbi.nlm.nih.gov/books/NBK600367/. Accessed 3 Jul 2024
73. Simić A, Hansen AF, Syversen T, Lierhagen S, Ciesielski TM, Romundstad PR, Midthjell K, Åsvold BO, Flaten TP (2022) Trace elements in whole blood in the general population in Trøndelag County, Norway: The HUNT3 Survey. Sci Total Environ 806:150875. https://doi.org/10.1016/j.scitotenv.2021.150875
74. Toxicological Profiles | ATSDR (2024). https://www.atsdr.cdc.gov/toxprofiledocs/index.html. Accessed 15 Oct 2024
75. France Štiglic A, Falnoga I, Briški AS, Žavbi M, Osredkar J, Skitek M, Marc J (2024) Reference intervals of 24 trace elements in blood, plasma and erythrocytes for the Slovenian adult population. Clin Chem Lab Med 62:946–957. https://doi.org/10.1515/cclm-2023-0731
76. Ingle ME, Bloom MS, Parsons PJ, Steuerwald AJ, Kruger P, Fujimoto VY (2017) Associations between IVF outcomes and essential trace elements measured in follicular fluid and urine: a pilot study. J Assist Reprod Genet 34:253–261. https://doi.org/10.1007/s10815-016-0853-7
77. Wdowiak A, Wdowiak E, Bojar I (2017) Evaluation of trace metals in follicular fluid in ICSI-treated patients. Ann Agric Environ Med 25:213–218. https://doi.org/10.26444/aaem/75422
78. Desaulniers D, Zhou G, Stalker A, Cummings-Lorbetskie C (2023) Effects of copper or zinc organometallics on cytotoxicity, DNA damage and epigenetic changes in the HC-04 human liver cell line. Int J Mol Sci 24:15580. https://doi.org/10.3390/ijms242115580
79. Babaei H, Roshangar L, Sakhaee E, Abshenas J, Kheirandish R, Dehghani R (2012) Ultrastructural and morphometrical changes of mice ovaries following experimentally induced copper poisoning. Iran Red Crescent Med J 14:558–568. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3482328/. Accessed 8 Jul 2024
80. Zhang L-H, Luo Z, Song Y-F, Shi X, Pan Y-X, Fan Y-F, Xu Y-H (2016) Effects and mechanisms of waterborne copper exposure influencing ovary development and related hormones secretion in yellow catfish *Pelteobagrus fulvidraco*. Aquat Toxicol 178:88–98. https://doi.org/10.1016/j.aquatox.2016.07.014
81. Letzel S, Roßbach B, Drexler H, Hartwig A (2018) M a. K. Commission, Copper and its inorganic compounds [BAT Value Documentation, 2017], in: The MAK-Collection for Occupational Health and Safety, John Wiley & Sons, Ltd,. Pp 244–257. https://doi.org/10.1002/3527600418.bb744050e2318.
82. Recommendations of the SCOEL - ECHA (n.d.). https://echa.europa.eu/recommendations-of-the-scoel. Accessed 8 Jul 2024
83. Bornhorst J, Kipp AP, Haase H, Meyer S, Schwerdtle T (2018) The crux of inept biomarkers for risks and benefits of trace elements. TrAC, Trends Anal Chem 104:183–190. https://doi.org/10.1016/j.trac.2017.11.007
84. Michos C, Kalfakakou V, Karkabounas S, Kiortsis D, Evangelou A (2010) Changes in copper and zinc plasma concentrations during the normal menstrual cycle in women. Gynecol Endocrinol 26:250–255. https://doi.org/10.3109/09513590903247857
85. Liang Q (2022) Association between Serum Copper, Selenium, Zinc, and Serum Estradiol in Women, Int. J Endocrinol 2022:8747693. https://doi.org/10.1155/2022/8747693
86. Ferdous N, Mishu FA, Shamsunnahar -, Tanjilul Haque SM, Rahman Khan AA, Siddique S (2019) Estimation of serum copper in post-menopausal women attending in a tertiary care hospital, Bangladesh. Anwer Khan Mod Med Coll J 10:110–113. https://doi.org/10.3329/akmmcj.v10i2.44118
87. Arredondo M, Núñez H, López G, Pizarro F, Ayala M, Araya M (2010) Influence of estrogens on copper indicators: in vivo and in vitro studies. Biol Trace Elem Res 134:252–264. https://doi.org/10.1007/s12011-009-8475-x
88. Di Domenico M, Castoria G, Bilancio A, Migliaccio A, Auricchio F (1996) Estradiol activation of human colon carcinoma-derived Caco-2 cell growth. Cancer Res 56:4516–4521
89. Kuo SM, Leavitt PS (1999) Genistein increases metallothionein expression in human intestinal cells, Caco-2. Biochem Cell Biol 77:79–88. https://doi.org/10.1139/o99-012
90. Ikeda M, Ezaki T, Moriguchi J (2007) Levels of calcium, magnesium and zinc in urine among adult women in relation to age with special reference to menopause. J Nutr Health Aging 11:394–401
91. Pascua AM, Nikoloff N, Carranza AC, Anchordoquy JP, Quintana S, Barbisán G, Díaz S, Anchordoquy JM, Furnus CC (2020) Reproductive hormones influence zinc homeostasis in the bovine cumulus-oocyte complex: Impact on intracellular zinc concentration and transporters gene expression. Theriogenology 146:48–57. https://doi.org/10.1016/j.theriogenology.2020.01.054
92. Cousins RJ, Liuzzi JP, Lichten LA (2006) Mammalian zinc transport, trafficking, and signals. J Biol Chem 281:24085–24089. https://doi.org/10.1074/jbc.R600011200
93. Garner TB, Hester JM, Carothers A, Diaz FJ (2021) Role of zinc in female reproduction. Biol Reprod 104:976–994. https://doi.org/10.1093/biolre/ioab023
94. Nutrients (n.d.) | Free Full-Text | Minerals and the Menstrual Cycle: Impacts on Ovulation and Endometrial Health,. https://www.mdpi.com/2072-6643/16/7/1008. Accessed 11 Jul 2024
95. Shi Y-Q, Zhu X-T, Zhang S-N, Ma Y-F, Han Y-H, Jiang Y, Zhang Y-H (2023) Premature ovarian insufficiency: a review on the role of oxidative stress and the application of antioxidants. Front Endocrinol (Lausanne) 14:1172481. https://doi.org/10.3389/fendo.2023.1172481
96. Qazi IH, Angel C, Yang H, Pan B, Zoidis E, Zeng C-J, Han H, Zhou G-B (2018) Selenium, selenoproteins, and female reproduction: a review. Molecules 23:3053. https://doi.org/10.3390/molecules23123053
97. Dahmer MK, Housley PR, Pratt WB (1984) Effects of molybdate and endogenous inhibitors on steroid-receptor inactivation, transformation, and translocation. Annu Rev Physiol 46:67–81. https://doi.org/10.1146/annurev.ph.46.030184.000435
98. Zhang Y-L, Liu F-J, Chen X-L, Zhang Z-Q, Shu R-Z, Yu X-L, Zhai X-W, Jin L-J, Ma X-G, Qi Q, Liu Z-J (2013) Dual effects of molybdenum on mouse oocyte quality and ovarian oxidative stress. Systems Biology in Reproductive Medicine 59:312–318. https://doi.org/10.3109/19396368.2013.826296
99. Fungwe TV, Buddingh F, Demick DS, Lox CD, Yang MT, Yang SP (1990) The role of dietary molybdenum on estrous activity, fertility, reproduction and molybdenum and copper enzyme activities of female rats. Nutr Res 10:515–524. https://doi.org/10.1016/S0271-5317(05)80061-2

100. Kim Y, Lee B-K (2011) Iron deficiency increases blood manganese level in the Korean general population according to KNHANES 2008. Neurotoxicology 32:247–254. https://doi.org/10.1016/j.neuro.2010.12.006
101. Rükgauer M, Klein J, Kruse-Jarres JD (1997) Reference values for the trace elements copper, manganese, selenium, and zinc in the serum/plasma of children, adolescents, and adults. J Trace Elem Med Biol 11:92–98. https://doi.org/10.1016/S0946-672X(97)80032-6
102. Kim Y (2018) Sex, pregnancy, and age-specific differences of blood manganese levels in relation to iron status; what does it mean? Toxicol Rep 5:28–30. https://doi.org/10.1016/j.toxrep.2017.12.003
103 Oulhote Y, Mergler D, Bouchard MF (2014) Sex- and age-differences in blood manganese levels in the U.S. general population: national health and nutrition examination survey 2011–2012. Environmental Health 13:87. https://doi.org/10.1186/1476-069X-13-87
104. Kim Y, Lobdell DT, Wright CW, Gocheva VV, Hudgens E, Bowler RM (2015) Blood metal concentrations of manganese, lead, and cadmium in relation to serum ferritin levels in Ohio residents. Biol Trace Elem Res 165:1–9. https://doi.org/10.1007/s12011-014-0223-1
105. Lee B-K, Kim Y (2012) Effects of menopause on blood manganese levels in women: analysis of 2008–2009 Korean national health and nutrition examination survey data. Neurotoxicology 33:401–405. https://doi.org/10.1016/j.neuro.2012.04.015
106. Davis CD, Wolf TL, Greger JL (1992) Varying levels of manganese and iron affect absorption and gut endogenous losses of manganese by rats. J Nutr 122:1300–1308. https://doi.org/10.1093/jn/122.6.1300
107. Finley JW (1999) Manganese absorption and retention by young women is associated with serum ferritin concentration. Am J Clin Nutr 70:37–43. https://doi.org/10.1093/ajcn/70.1.37
108. Studer JM, Schweer WP, Gabler NK, Ross JW (2022) Functions of manganese in reproduction. Anim Reprod Sci 238:106924. https://doi.org/10.1016/j.anireprosci.2022.106924
109. Anchordoquy J, Anchordoquy J, Sirini M, Mattioli G, Picco S, Furnus C (2013) Effect of different manganese concentrations during in vitro maturation of bovine oocytes on DNA integrity of cumulus cells and subsequent embryo development. Reprod Domest Anim 48:905–911. https://doi.org/10.1111/rda.12184
110. Anchordoquy JP, Anchordoquy JM, Sirini MA, Testa JA, Peral-García P, Furnus CC (2016) The importance of manganese in the cytoplasmic maturation of cattle oocytes: blastocyst production improvement regardless of cumulus cells presence during *in vitro* maturation. Zygote 24:139–148. https://doi.org/10.1017/S0967199414000823
111. Hidiroglou M, Ho SK, Ivan M, Shearer DA (1978) Manganese status of pasturing ewes, of pregnant ewes and doe rabbits on low manganese diets and of dairy cows with cystic ovaries. Can J Comp Med 42:100–107
112. Andreoli V, Sprovieri F (2017) genetic aspects of susceptibility to mercury toxicity: an overview. Int J Environ Res Public Health 14:93. https://doi.org/10.3390/ijerph14010093
113. Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M (2021) Toxic mechanisms of five heavy metals: mercury lead, chromium, cadmium, and arsenic. Front Pharmacol 12:643972. https://doi.org/10.3389/fphar.2021.643972
114. Massányi P, Bárdos L, Oppel K, Hluchý S, Kovácik J, Csicsai G, Toman R (1999) Distribution of cadmium in selected organs of mice: effects of cadmium on organ contents of retinoids and beta-carotene. Acta Physiol Hung 86:99–104
115. Al-Saleh I, Shinwari N, Al-Amodi M (2009) Accumulation of mercury in ovaries of mice after the application of skin-lightening creams. Biol Trace Elem Res 131:43–54. https://doi.org/10.1007/s12011-009-8341-x
116. He Y, Wang L, Li X, Zhao H (2020) The effects of chronic lead exposure on the ovaries of female juvenile Japanese quails (*Coturnix japonica*): Developmental delay, histopathological alterations, hormone release disruption and gene expression disorder. Ecotoxicol Environ Saf 205:111338. https://doi.org/10.1016/j.ecoenv.2020.111338
117 Massányi P, Uhrín V, Toman R, Kovácik J, Bíro D (1999) Histological changes in the oviduct of rabbits afteradministration of cadmium. J Anim Feed Sci 8:255–261. https://doi.org/10.22358/jafs/68933/1999
118. MP, TR, VM, CP (1995)Evaluation of selected parameters of a metabolic profile and levels of cadmium in reproductive organs of rabbits after an experimental administration, Acta Physiologica Hungarica 83. https://pubmed.ncbi.nlm.nih.gov/8852652/. Accessed 15 Oct 2024
119. Dhir V, Dhand P (2010) Toxicological approach in chronic exposure to lead on reproductive functions in female rats *(Rattus norvegicus)*. Toxicol Int 17:1. https://doi.org/10.4103/0971-6580.68340
120. Altunkaynak BZ, Akgül N, Yahyazadeh A, Altunkaynak ME, Turkmen AP, Akgül HM, Ünal B (2016) Effect of mercury vapor inhalation on rat ovary: Stereology and histopathology. J Obstet Gynaecol Res 42:410–416. https://doi.org/10.1111/jog.12911
121. Massányi P, Lukác N, Uhrín V, Toman R, Pivko J, Rafay J, Forgács Z, Somosy Z (2007) Female reproductive toxicology of cadmium. Acta Biol Hung 58:287–299. https://doi.org/10.1556/ABiol.58.2007.3.5
122. Dumitrescu E, Chiurciu V, Muselin F, Popescu R, Brezovan D, Cristina RT (2015) Effects of long-term exposure of female rats to low levels of lead: ovary and uterus histological architecture changes, Turk. J Biol 39:284–289. https://doi.org/10.3906/biy-1407-6
123. Ma Y, Zhu M, Miao L, Zhang X, Dong X, Zou X (2018) Mercuric chloride induced ovarian oxidative stress by suppressing Nrf2-Keap1 signal pathway and its downstream genes in laying hens. Biol Trace Elem Res 185:185–196. https://doi.org/10.1007/s12011-018-1244-y
124. Khan A (2004) Effects of inorganic mercury on reproductive performance of mice. Food Chem Toxicol 42:571–577. https://doi.org/10.1016/j.fct.2003.10.018
125. Bjørklund G, Chirumbolo S, Dadar M, Pivina L, Lindh U, Butnariu M, Aaseth J (2019) Mercury exposure and its effects on fertility and pregnancy outcome. Basic Clin Pharmacol Toxicol 125:317–327. https://doi.org/10.1111/bcpt.13264
126. Osowski A, Fedoniuk L, Bilyk Y, Fedchyshyn O, Sas M, Kramar S, Lomakina Y, Fik V, Chorniy S, Wojtkiewicz J (2023) Lead exposure assessment and its impact on the structural organization and morphological peculiarities of rat ovaries. Toxics 11:769. https://doi.org/10.3390/toxics11090769
127. da Costa CS, Oliveira TF, Freitas-Lima LC, Padilha AS, Krause M, Carneiro MTWD, Salgado BS, Graceli JB (2021) Subacute cadmium exposure disrupts the hypothalamic-pituitary-gonadal axis, leading to polycystic ovarian syndrome and premature ovarian failure features in female rats. Environ Pollut 269:116154. https://doi.org/10.1016/j.envpol.2020.116154
128. Lee YM, Chung HW, Jeong K, Sung Y-A, Lee H, Ye S, Ha E-H (2018) Association between cadmium and anti-Mullerian hormone in premenopausal women at particular ages. Ann Occup Environ Med 30:44. https://doi.org/10.1186/s40557-018-0255-7
129. Paksy K, Varga B, Náray M, Olajos F, Folly G (1992) Altered ovarian progesterone secretion induced by cadmium fails to interfere with embryo transport in the oviduct of the rat. Reprod Toxicol 6:77–83. https://doi.org/10.1016/0890-6238(92)90024-N
130. Ruslee SS, Zaid SSM, Bakrin IH, Goh YM, Mustapha NM (2020) Protective effect of Tualang honey against cadmium-induced morphological abnormalities and oxidative stress in the ovary of rats. BMC Complement Med Ther 20:160. https://doi.org/10.1186/s12906-020-02960-1

131. Nna VU, Usman UZ, Ofutet EO, Owu DU (2017) Quercetin exerts preventive, ameliorative and prophylactic effects on cadmium chloride - induced oxidative stress in the uterus and ovaries of female Wistar rats. Food Chem Toxicol 102:143–155. https://doi.org/10.1016/j.fct.2017.02.010
132. Naicker N, Norris SA, Mathee A, Becker P, Richter L (2010) Lead exposure is associated with a delay in the onset of puberty in South African adolescent females: findings from the Birth to Twenty cohort. Sci Total Environ 408:4949–4954. https://doi.org/10.1016/j.scitotenv.2010.07.037
133. Nkomo P, Richter LM, Kagura J, Mathee A, Naicker N, Norris SA (2018) Environmental lead exposure and pubertal trajectory classes in South African adolescent males and females. Sci Total Environ 628–629:1437–1445. https://doi.org/10.1016/j.scitotenv.2018.02.150
134. Davis BJ, Price HC, O'Connor RW, Fernando R, Rowland AS, Morgan DL (2001) Mercury vapor and female reproductive toxicity. Toxicol Sci 59:291–296. https://doi.org/10.1093/toxsci/59.2.291
135. Yang S, Zhang Z, He J, Li J, Zhang J, Xing H, Xu S (2012) Ovarian toxicity induced by dietary cadmium in Hen. Biol Trace Elem Res 148:53–60. https://doi.org/10.1007/s12011-012-9343-7
136. Takiguchi M, Yoshihara S (2006) New aspects of cadmium as endocrine disruptor. Environ Sci 13:107–116
137. Henriques MC, Loureiro S, Fardilha M, Herdeiro MT (2019) Exposure to mercury and human reproductive health: a systematic review. Reprod Toxicol 85:93–103. https://doi.org/10.1016/j.reprotox.2019.02.012
138. Maretta M, Marettová E (2022) Toxic effects of cadmium on the female reproductive organs a review, folia. Veterinaria 66:56–66. https://doi.org/10.2478/fv-2022-0038
139. Leoni G, Bogliolo L, Deiana G, Berlinguer F, Rosati I, Pintus PP, Ledda S, Naitana S (2002) Influence of cadmium exposure on in vitro ovine gamete dysfunction. Reprod Toxicol 16:371–377. https://doi.org/10.1016/s0890-6238(02)00040-0
140. Zhang Q-F, Li Y-W, Liu Z-H, Chen Q-L (2016) Reproductive toxicity of inorganic mercury exposure in adult zebrafish: Histological damage, oxidative stress, and alterations of sex hormone and gene expression in the hypothalamic-pituitary-gonadal axis. Aquat Toxicol 177:417–424. https://doi.org/10.1016/j.aquatox.2016.06.018
141. Pollack AZ, Schisterman EF, Goldman LR, Mumford SL, Albert PS, Jones RL, Wactawski-Wende J (2011) Cadmium, lead, and mercury in relation to reproductive hormones and anovulation in premenopausal women. Environ Health Perspect 119:1156–1161. https://doi.org/10.1289/ehp.1003284
142. Santoro N, Brown JR, Adel T, Skurnick JH (1996) Characterization of reproductive hormonal dynamics in the perimenopause. J Clin Endocrinol Metab 81:1495–1501. https://doi.org/10.1210/jcem.81.4.8636357
143. Moksnes MR, Hansen AF, Wolford BN, Thomas LF, Rasheed H, Simić A, Bhatta L, Brantsæter AL, Surakka I, Zhou W, Magnus P, Njølstad PR, Andreassen OA, Syversen T, Zheng J, Fritsche LG, Evans DM, Warrington NM, Nøst TH, Åsvold BO, Flaten TP, Willer CJ, Hveem K, Brumpton BM (2024) A genome-wide association study provides insights into the genetic etiology of 57 essential and non-essential trace elements in humans. Commun Biol 7:1–14. https://doi.org/10.1038/s42003-024-06101-z
144 Varga B, Zsolnai B, Paksy K, Náray M, Ungváry GY (1993) Age dependent accumulation of cadmium in the human ovary. Reprodu Toxicol 7:225–228. https://doi.org/10.1016/0890-6238(93)90228-Y
145. Whitfield JB, Dy V, McQuilty R, Zhu G, Heath AC, Montgomery GW, Martin NG (2010) Genetic effects on toxic and essential elements in humans: arsenic, cadmium, copper, lead, mercury, selenium, and zinc in erythrocytes. Environ Health Perspect 118:776–782. https://doi.org/10.1289/ehp.0901541
146. Whitfield JB, Dy V, McQuilty R, Zhu G, Montgomery GW, Ferreira MAR, Duffy DL, Neale MC, Heijmans BT, Heath AC, Martin NG (2007) Evidence of genetic effects on blood lead concentration. Environ Health Perspect 115:1224–1230. https://doi.org/10.1289/ehp.8847
147. Evans DM, Zhu G, Dy V, Heath AC, Madden PAF, Kemp JP, McMahon G, St Pourcain B, Timpson NJ, Golding J, Lawlor DA, Steer C, Montgomery GW, Martin NG, Smith GD, Whitfield JB (2013) Genome-wide association study identifies loci affecting blood copper, selenium and zinc. Hum Mol Genet 22:3998–4006. https://doi.org/10.1093/hmg/ddt239
148. Ng E, Lind PM, Lindgren C, Ingelsson E, Mahajan A, Morris A, Lind L (2015) Genome-wide association study of toxic metals and trace elements reveals novel associations. Hum Mol Genet 24:4739–4745. https://doi.org/10.1093/hmg/ddv190
149. Yang W, Li L, Feng X, Cheng H, Ge X, Bao Y, Huang L, Wang F, Liu C, Chen X, Mo Z, Yang X (2022) Genome-wide association and Mendelian randomization study of blood copper levels and 213 deep phenotypes in humans. Commun Biol 5:405. https://doi.org/10.1038/s42003-022-03351-7
150. Combs GF (2015) Biomarkers of selenium status. Nutrients 7:2209–2236. https://doi.org/10.3390/nu7042209
151. Hall AG, King JC, McDonald CM (2022) comparison of serum, plasma, and liver zinc measurements by AAS, ICP-OES, and ICP-MS in diverse laboratory settings. Biol Trace Elem Res 200:2606–2613. https://doi.org/10.1007/s12011-021-02883-z
152. Oskarsson A, Kippler M (2023) Molybdenum – a scoping review for nordic nutrition recommendations 2023. Food Nutr Res 67. https://doi.org/10.29219/fnr.v67.10326. https://doi.org/10.29219/fnr.v67.10326
153. Barbosa F, Tanus-Santos JE, Gerlach RF, Parsons PJ (2005) A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ Health Perspect 113:1669–1674. https://doi.org/10.1289/ehp.7917
154. Karyakina NA, Shilnikova N, Farhat N, Ramoju S, Cline B, Momoli F, Mattison D, Jensen N, Terrell R, Krewski D (2022) Biomarkers for occupational manganese exposure. Crit Rev Toxicol 52:636–663. https://doi.org/10.1080/10408444.2022.2128718
155. Kershaw TG, Clarkson TW, Dhahir PH (1980) The relationship between blood levels and dose of methylmercury in man. Arch Environ Health 35:28–36. https://doi.org/10.1080/00039896.1980.10667458
156. Hulle MV, Zhang C, Schotte B, Mees L, Vanhaecke F, Vanholder R, Zhang XR, Cornelis R (2004) Identification of some arsenic species in human urine and blood after ingestion of Chinese seaweed Laminaria. J Anal At Spectrom 19:58–64. https://doi.org/10.1039/B307457A
157. Faroon O, Ashizawa A, Wright S, Tucker P, Jenkins K, Ingerman L, Rudisill C (2012) toxicological profile for cadmium, agency for toxic substances and disease registry (US), Atlanta (GA). http://www.ncbi.nlm.nih.gov/books/NBK158838/. Accessed 14 Sept 2024
158. Clarkson TW, Magos L (2006) The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 36:609–662. https://doi.org/10.1080/10408440600845619
159. Nabi D, Bosi D, Gupta N, Thaker N, Fissore R, Brayboy LM (2023) Multidrug resistance transporter-1 dysfunction perturbs meiosis and Ca2+ homeostasis in oocytes. Reproduction 165:79–91. https://doi.org/10.1530/REP-22-0192