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Toxic Metal and Trace Element Concentrations in Blood and Outcome of In Vitro Fertilization in Women

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Abstract

The aim of this study was to investigate the association of trace element and toxic metal concentrations in blood and the outcome of in vitro fertilization (IVF). The study included 104 consecutive patients that underwent assisted reproductive technology (ART) procedures. The following parameters were determined: cadmium (Cd), mercury (Hg), arsenic (As), and lead (Pb); and copper (Cu), zinc (Zn), selenium (Se), and magnesium (Mg). Serum samples were obtained before commencing stimulation. Patients with smoking habit had significantly higher Pb concentrations ($P = 0.022$), as well as higher concentrations of As and Hg but not significantly. All subjects were divided into groups of pregnant and nonpregnant patients. Pregnant patients had lower mean values of Mg ($P = 0.009$), As ($P < 0.05$), and Pb ($P = 0.034$), compared to nonpregnant, and a significant correlation between pregnancy outcome and concentrations of Mg, Cd, and Pb was found. Women who had had delivered had lower Mg ($P = 0.009$) and Cd ($P = 0.014$) concentrations. There was a significant correlation of the negative outcome of IVF procedure with higher concentrations of Pb ($P = 0.046$) and Cd ($P = 0.012$). In conclusion, our results suggest that there is a difference in Mg, Pb, and Cd concentrations between pregnant and nonpregnant women. There was no association between toxic metals and number and quality of oocytes and embryos, while there was with fertilization rate. Concerning trace elements, we did not find the correlation of trace elements with oocyte number and quality, nor with a number of fertilized oocytes, except for Cu. Patients who were pregnant had lower concentrations of Mg.

Keywords In vitro fertilization · Pregnancy · Toxic metals · Trace elements

Abbreviations

IVF   In vitro fertilization
ART   Assisted reproductive techniques
ICSI Intracytoplasmic sperm injection
BMI   Body mass index
GnRH Gonadotropin-releasing hormone
GT   Gonadotropin

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Introduction

Although pregnancy rates in in vitro fertilization (IVF) cycles are increasing, different factors that may influence the success of IVF procedures are still being investigated. Infertility has a multifactorial etiology with many genetic and environmental factors included [1]. Environmental and lifestyle factors are of particular interest, because unlike genetic causes, we can influence them in terms of treatment or prevention, which is significant in the treatment of infertility [1].

Toxic metals are widespread in the human environment, food, water, air, cigarettes, and alcoholic beverages and are often taken in trace concentrations over time through gastrointestinal system, respiratory system, and skin [2]. The studies suggest the toxic effects on the reproductive function, among other organic systems, acting directly on specific reproductive organs or indirectly to the neuroendocrine system [3]. Exposure to toxic metals can affect hormonal changes, ovulation, maturation of the oocyte, and

the reduction or loss of fertility, and hence the achievement of pregnancy in women [4, 5]. Also, some of them, such as cadmium and lead, increase the rate of spontaneous abortion and affect fetal development [6]. Reproductive toxicity resulting from long-term exposure to in trace concentrations of toxic metals can have an impact on the success of IVF procedures [7]. In addition, studies demonstrated that deficiency of trace elements may have effect on reproductive health too [8]. Zinc and selenium play a role in sexual development, ovulation, and menstrual cycle [1]. There is an association between these nutrients and their derivatives and spontaneous abortions and congenital malformations [1]. Some studies found also a lack of Cu, Se, and Zn in IVF patients [1, 9, 10]. There are not many studies on serum Mg levels in IVF patients, but in one, it was suggested that there is a relationship between magnesium and sex steroid hormones [11]. Concentrations of toxic metals and trace elements are usually measured in the blood, urine, and/or follicular fluid in IVF studies, and the latest two studies used hair for assessment [12, 13]. In the study that made a comparison between blood and follicular fluid element concentrations, in IVF patients, the average blood concentrations were similar to those in follicular fluid of large follicles [9]; therefore, we decided to determine concentrations of heavy metals and trace elements in the blood only.

The aim of our study was to investigate the association of toxic metals and trace metals, in blood and IVF outcome, as well as its influence on the number of received oocytes, number of mature oocytes, embryo quality, fertilization, and pregnancy.

Materials and Methods

Study Subjects

The study included 104 consecutive female patients that underwent ART procedures as infertility treatment at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade. All patients agreed to participate in the study and signed an informed consent for all the undertaken procedures. The study was approved by The Ethics Committee of the Faculty of Medicine, University of Belgrade.

Participant recruitment and clinical protocols were previously described in detail [14]. Briefly, including criteria for patients were as follows: age 18–40 years; body mass index (BMI) from 18 to 30 kg/m^2; regular menstrual cycles from 25 to 32 days; and without any medical disease, endocrinology disease, hydrosalpinx, or endometriosis stages III and IV. Infertility cause was categorized as male, tubal, ovarian, unknown, or combined. For all

patients, age, BMI, years of treating infertility, smoking status (smoker/nonsmoker), and the cause of infertility were determined before commencing IVF procedure. In respect of supplements, all women were advised to use folic acid 400 μg per day, from 1 to 3 months before procedure. We divided patients into two groups, based on the outcome of IVF, those who failed to achieve pregnancy (nonpregnant) women and those who achieved pregnancy (pregnant) after IVF. Depending on age, ovarian reserve, and previous IVF cycle, patients were submitted to different protocols: short gonadotropin-releasing hormone (GnRH) antagonist protocol with contraceptive pretreatment ($n = 19$), or without it ($n = 60$), and long GnRH agonist protocol ($n = 25$). Methods of insemination were IVF, intracytoplasmic sperm injection (ICSI), or a combined method (IVF/ICSI). In assessing the quality of embryos, the Istanbul consensus clinical embryologist criteria were used as the reference frame [15]. Embryo transfer was performed on day 2 or 3 after the oocyte retrieval. For luteal support phase, patients received intramuscular progesterone, until the 12th week of gestation in cases of pregnancies. Pregnancy was diagnosed by positive serum β-hCG (> 25 MIU/mL) 14 days after the embryo transfer. Clinical pregnancies were confirmed by transvaginal ultrasound findings: gestational sac with a vital embryo and the 6-week gestation. Finally, we registered whether the examined women managed to maintain pregnancy and have a term delivery of a healthy child or they had a miscarriage.

Sample Collection and Analysis

Serum and whole blood specimens were obtained from each patient between the second and fourth day of the menstrual cycle, before commencing of stimulation, when the basal hormonal status was determined. The laboratory was blinded to clinical data and IVF procedure. After preparing a whole blood specimen, trace elements copper (Cu), zinc (Zn), selenium (Se), and magnesium (Mg) as well as toxic metals cadmium (Cd), mercury (Hg), arsenic (As), and lead (Pb) were determined. Blood samples for determining the content of trace elements and toxic metals (about 1 mL) were weighed in a Teflon vessel of microwave digestion apparatus (START D, Milestone, Sorisole, Italy) and then 8 mL conc. HNO$_3$ for digestion mineralized by the microwave oven mineralization process. The conditions for the mineralization of the sample tested were set by adjusting the parameters of the digestion program, which was to achieve a temperature of 170 °C for 10 min, which was maintained for the next 10 min, then maintaining the ventilation for 15 min. The samples prepared in the manner described above were quantitatively

transferred to normal vessels, and then in these solutions, the concentration of metals was determined by the inductively coupled plasma mass spectrometry (ICP-MS) (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany).

All methods for determining the metal concentration are validated, which covered linearity for the given range of concentrations, LOD and LOQ derivation, coefficient of variation, accuracy, and recovery. For the purpose of testing the method of determining metals by ICP-MS, the certified reference material (*Seronorm*™ Trace Elements Whole Blood, Serro AS, Billingstad, Norway) was used. Recovery rates from certified reference material analysis were 100.5, 99.6, 99.9, 98.7, 101.4, 100.2, 101.8, and 98.9% for Mg, Cu, Zn, As, Se, Cd, Hg, and Pb, respectively.

Statistical Analysis

Results were presented as arithmetic mean ± standard deviation for variables with normal distribution and as median and interquartile range for variables whose distribution is not normal. Testing of distribution was carried out by Kolmogorov-Smirnov analysis. Categorical variables are presented as relative or absolute frequency. Analysis of categorical values was performed using the chi-square test. Comparison of the mean values of independent groups of data was performed by ANOVA analysis. For parameters without normal distribution, test of significance between groups was performed using the Kruskal-Wallis test. The association of selected variables with outcome was assessed with the univariate and multivariate logistics regression analyses. A significance of 0.05 was required for a variable to be included into the multivariate model, whereas 0.1 was the cutoff value for exclusion. Odd ratios with the corresponding 95% confidence intervals were estimated. The sensitivity and specificity of identified parameters Mg and Pb blood concentrations for outcome prediction were evaluated with receiver operating characteristic curves. All analysis were performed using the Statistical Package for the Social Sciences (SPSS) 22, and differences were considered statistically significant at probability level less than 0.05.

Results

Demographic variables of 104 patients included in the study, as well as causes and duration of infertility, stimulation protocols, the method of insemination, and outcome of IVF, are shown in Table 1. Significantly, more patients who achieved pregnancy were less than 35 years old ($P = 0.004$), and most pregnant patients had had a delivery of a healthy baby ($P = 0.000$). Among pregnant patients, the most common cause of infertility was the male and unknown factor, and the ovarian cause was least presented ($P = 0.031$). Mean values with standard deviations and minimum and maximum values of clinical and cycle characteristics were shown in Table 2. Pregnant patients had significantly more oocytes retrieved ($P = 0.026$), as well as more fertilized oocytes ($P = 0.032$). Patients who achieved pregnancy had shorter duration of infertility ($P = 0.050$).

Patients who had positive outcome of IVF procedure had significantly lower Mg concentrations (94.4%), as well as lower concentrations of Cd (97.6%) and Pb (80.6%). We also compared mean concentrations of toxic metals and trace elements to IVF outcome (Table 3). In patients who achieved pregnancy, we found significantly lower mean concentrations of Mg ($P = 0.009$), as well as As ($P = 0.050$) and Pb ($P = 0.034$). Mean concentrations of Cd were higher in patients who failed to achieve pregnancy.

In Table 4, correlation of toxic metals and trace elements with cycle characteristics are shown. Analyzing interval of values and characteristics of IVF outcome, we found direct significant Pearson's correlation of higher Cu concentrations and higher GT dose ($P = 0.039$), but also of lower Pb concentrations and higher GT dose ($P = 0.008$). There was a significant correlation between higher Cu concentrations and a higher number of fertilized oocytes ($P = 0.041$). Spearman correlation of toxic metal and trace element concentrations with IVF outcome is shown in Table 5, and there was a significant correlation between the positive outcome of IVF procedure and the lower concentrations of Mg ($P = 0.010$). In correlation with negative outcome were higher concentrations of Pb ($P = 0.046$) and Cd ($P = 0.012$). When embryo quality was correlated with the outcome of IVF, we found a significant correlation of pregnancy and better quality of embryos ($P = 0.013$), as well as with higher gonadotropin dose ($P = 0.045$).

Concerning the outcome of pregnancy, patients who had delivery had the correlation with lower Mg ($P = 0.009$) and Cd ($P = 0.029$) concentrations, while there was a significant correlation between miscarriages and higher concentrations of Pb ($P = 0.029$) (Table 6).

In univariate and multivariate logistic regression analysis, all the variables that were examined were included in the regression model. The parameters such as BMI, duration of infertility, insemination method, and fertilization rate did not show significant predictive power in the univariate logistic regression analysis in our investigation. In our study, patients older than 35 years indicate a 69% decrease of the positive outcome ($P = 0.005$). Unknown cause of infertility was a positive predictor for pregnancy ($P = 0.037$), while ovarian cause indicates 87.7% significantly

Table 1 Distribution of patients depending on demographic variables, infertility cause, stimulation protocols, method of insemination, and the outcome of IVF

	Total	%	Nonpregnant		Pregnant		P
	$N = 104$		$N = 63$	%	$N = 41$	%	
Age							
< 35 years	48	46	22	35	26	63	
35 and more	56	54	41	65	15	37	0.004
Total	104	100	63	100	41	100	
BMI							
Underweight	5	5	2	3	3	7	0.530
Normal weight	81	78	51	81	30	73	
Overweight	18	17	10	16	8	20	
Smoking							
Smokers	28	27	21	33	7	17	0.068
Nonsmokers	76	73	42	67	34	83	
Infertility duration							
< 3 years	27	26	15	24	12	29	0.537
3 years and more	77	74	48	76	29	71	
Method of insemination							
Cancelation of procedure	9	9	9	14	0	0	
IVF	24	23	13	21	11	27	0.083
ICSI	28	27	17	27	11	27	
Combined	43	41	24	38	19	46	
Cause of infertility							
Male factor	33	32	20	32	13	32	
Combined	5	5	2	3	3	7	0.031
Unknown	22	21	9	14	13	32	
Ovarian	22	21	19	30	3	7	
Tubal	22	21	13	21	9	22	
Stimulation protocol							
Short antagonist	60	58	42	67	18	44	0.057
Short antagonist with OC	19	18	8	13	11	27	
Long agonist	25	24	13	21	12	29	
Pregnancy outcome							
Nonpregnant	63	61	63	100	0	0	
Delivery	29	28	0	0	29	71	
Miscarriage	9	9	0	0	9	22	0.000
Biochemical pregnancy	3	3	0	0	3	7	

χ^2 test

decrease in the chance for a positive outcome ($P = 0.010$). Higher gonadotropin dose ($P = 0.041$), as well as higher number of fertilized oocytes ($P = 0.040$), were positive predictors of pregnancy. Lower embryo quality indicates a 79.4% decrease in the chance of positive outcome ($P = 0.021$). Higher Mg concentrations ($P = 0.020$), as well as higher Pb concentrations ($P = 0.050$), are associated with an 83.9 and 62.7% increased chance of negative outcome. Higher Cd concentrations indicate an 89.4% decreased chance for the positive outcome ($P = 0.035$) (Table 7). The results of multivariate regression analysis showed a decrease in chances of pregnancy in patients older than 35 years for 83.1% ($P = 0.007$). Higher gonadotropin dose is a predictor of positive outcome ($P = 0.015$). There was a decrease in chances of pregnancy in patients with lower embryo quality for 86% ($P = 0.049$). Increased Mg concentrations ($P = 0.032$), as well as Pb concentrations ($P = 0.026$), were associated with 88.3 and 77.6% chance for a negative outcome (Table 7).

Receiver operating characteristic (ROC) curves were constructed for Mg and area under the curve (AUC) for the pregnancy. This relationship was confirmed (AUC of 0.651, $P = 0.015$). The optimal cutoff point for Mg was found to be 3.3 mg/dL resulting in a sensitivity of 94%

Table 2 Demographic variables and cycle characteristics

	Nonpregnant		Pregnant		P
	Mean ± SD	Min.-Max.	Mean ± SD	Min.-Max.	
Age (years)	35.11 ± 4.08	21–41	33.95 ± 3.71	25–40	0.145
BMI	22.32 ± 2.83	16.5–29.4	21.65 ± 2.82	16.8–27.5	0.243
Duration of infertility (years)	5.83 ± 3.43	2–18	4.63 ± 1.71	2–9	[‡]0.05
Gonadotropin dose (IU)	2194.5 ± 636.8	900–4275	2335.5 ± 537.6	1200–3375	[‡]0.589
Oocyte (number)	6.49 ± 6.84	0–31	8.32 ± 4.83	1–21	[‡]0.026
MII (number)	4.67 ± 5.09	0–24	6.83 ± 4.02	1–16	[‡]0.063
Fertilized oocyte (number)	2.93 ± 2.90	0–13	4.63 ± 2.88	1–11	[‡]0.032
Class A	2.05 ± 1.32	1–6	2.82 ± 2.25	1–11	[‡]0.777
Class B	1.88 ± 1.15	1–6	1.68 ± 0.82	1–4	[‡]0.999
Class C	1.00 ± 0.00	1–1	1.80 ± 1.10	1–3	[‡]0.586
Fertilization rate (%)	67.64 ± 28.57	4.17–100	73.80 ± 25.60	10–100	[‡]0.429

Min. minimum, *Max.* maximum, *Mean ± SD* mean ± standard deviation, ANOVA *F* test, [‡] K-W test

and a specificity of 30% for positive status (Fig. 1). The AUC was 0.632 for the pregnancy. The cutoff point for Pb was 0.96 μg/dL resulting in a sensitivity of 92% and a specificity of 40% for pregnancy (Fig. 2) (*P* = 0.034) Using the Pb (μg/dL) cutoff value from the initial ROC curve, lower values Pb predicted pregnancy with a specificity of 92% and sensitivity of 40% (*P* = 0.034).

Discussion

Humans are exposed to toxic metals in trace concentrations through dietary sources or airborne pollution [16], and when presented in the blood or follicular fluid, they can have an effect on the reproductive health of women and consequently on the outcome of IVF procedures [2]. Although the data are controversial and for certain elements scarce, it is known that fertility is impaired in women professionally exposed to Hg, Cd, and Pb [17–19].

However, data from studies that examined the influence of exposure to those toxic metals in trace concentrations on reproductive function in women are not consistent [20–23].

In our study we wanted to find out if there was any effect of toxic metals and trace elements on any step in IVF procedure including on the outcome itself. We did not find correlation of toxic metal blood concentration either with oocyte number or with number of mature (MII) and fertilized oocytes. The finding is consistent with the study of Bloom et al. [24] in which there was no correlation of heavy metals with mature oocytes, while in the previous study [25], they found lower fertilization rate when Pb in follicular fluid was increased. In the study of Al-Selah et al. [26], concentrations of Pb, Cd, and Hg were determined in blood and follicular fluid in 619 women underwent IVF procedure. They also found a reverse proportional relationship between Pb concentrations and fertilization rate. But, interestingly, they found a positive

Table 3 Mean concentrations of trace elements and toxic metals in pregnant and nonpregnant patients

	Nonpregnant		Pregnant		P
	Mean ± SD	Min.-Max.	Mean ± SD	Min.-Max.	
Mg (mg/dL)	3.11 ± 0.28	2.26–3.69	2.95 ± 0.29	2.22–3.48	0.009
Cu (μg/dL)	116.48 ± 20.59	55–177	121.14 ± 19.14	88–177	0.280
Zn (mmol/L)	88.98 ± 12.58	65.46–110.43	84.81 ± 12.45	43.90–109.51	0.123
As (μg/L)	1.29 ± 1.31	0.50–6.90	0.85 ± 0.44	0.50–2.44	[‡]0.050
Se (μg/L)	78.38–30.40	49–170	74.43–29.02	49–150	0.936
Cd (μg/L)	1.83 ± 5.44	0.49–31.14	0.51 ± 0.13	0.49–1.24	[‡]0.012
Hg (μg/L)	1.27 ± 0.86	0.90–5.27	2.07 ± 3.37	0.90–17.14	[‡]0.966
Pb (μg/dL)	1.02 ± 0.66	0.39–4.30	0.72 ± 0.38	0.10–2.23	[‡]0.034

Mean ± SD mean ± standard deviation, *Min.* minimum, *Max.* maximum, ANOVA *F* test, [‡] K-W test

Table 4 Correlation of trace elements and toxic metals with cycle characteristics

Pearson correlation (r)	Mg (mg/dL)	Cu (µg/dL)	Zn (mmol/L)	As (µg/L)	Se (µg/L)	Cd (µg/L)	Hg (µg/L)	Pb (µg/dL)
Gonadotropin dose	0.109	0.218*	0.073	0.207	− 0.020	0.092	0.024	− 0.279**
MII oocyte number	− 0.154	0.131	− 0.004	− 0.122	− 0.004	− 0.16	0.006	− 0.095
Fertilized oocytes	− 0.127	0.221*	− 0.066	− 0.115	0.011	− 0.154	0.064	− 0.091
Number of fertilized oocyte	0.016	0.164	0.001	− 0.029	0.031	− 0.197	0.12	− 0.048
Fertilization rate (%)	− 0.134	0.16	− 0.004	0.029	− 0.069	0.144	− 0.034	− 0.03

*$P < 0.05$; **$P < 0.01$

correlation between Cd in follicular fluid and fertilization rate, which is hard to interpret. It could be supported by Henson and Chedrese [27] suggestion that Cd might have paradoxical effect on steroidogenesis, acting on ovarian and reproductive tract morphology, with extremely low dosages reported to stimulate ovarian luteal progesterone biosynthesis and high dosages inhibiting it.

Our data suggest that pregnant patients had lower concentrations of As and Pb, while nonpregnant patients had higher concentrations of Pb and Cd. Cd levels were lower in patients who had had delivery. Aforementioned toxic metals were predictors for pregnancy. Bloom et al. [24] found in their multivariable model 35 and 33% lower probability of clinical and biochemical pregnancies in correlation with Hg blood concentration, while there was no correlation with long-term exposure to Pb and Cd in female population. When they analyzed Cd concentrations in blood, they found 94 and 82% lower probability for clinical and biochemical pregnancies. Nonpregnant women had higher mean values of Hg and higher concentrations of Cd in blood; similar was found in our study too, while for Pb concentrations, results were reversed. Tolunay and et al. [2] determined concentrations of Cd, Pb, Hg, and As as well as of Cu, Zn, and Fe in blood and follicular fluid and they had two groups of patients, those

with clinical pregnancies and those with miscarriages and biochemical pregnancies. Statistically significant negative correlation between concentrations of Pb in blood and MII oocytes, implantation rates, and clinical pregnancies was found. The result concerning pregnancies corresponds to our finding. Longitudinal study of infertility and environment (LIFE) did not found correlation between Hg levels in blood and biochemical pregnancies in 500 women [28], neither between concentrations of Pb and pregnancy rate, as well as in some other studies [7, 29]. The effect of As on female fertility has not been sufficiently investigated and is not clear. Pb and Cd have been shown to increase free radical species [30], demonstrate physiological estrogenic effect [31, 32], and alter progesterone synthesis [33] which could have deleterious effect with respect to pregnancy achievement. We should also mention that albeit higher Cd and Pb concentrations were found in nonpregnant patients, there was decreased chance for pregnancy in patients older than 35 years, longer duration of infertility, and ovarian cause of infertility that were more represented in nonpregnant group, indicating that many factors can influence the outcome of IVF procedure.

In our study, we did not find the correlation of either trace element concentrations with oocyte number nor with a number of fertilized oocytes, except higher concentrations of

Table 5 Spearman correlation of trace elements and toxic metals with characteristics and outcome of IVF

		*Embryos	Method of insemination	**GT dose	Stimulation protocol	Mg	Cu
Outcome of IVF nonpregnant/-pregnant	r	− 0.263*	0.127	0.197*	0.200*	− 0.267*	0.168
Outcome of pregnancy	r	0.251*	0.110	0.175	0.203*	0.227*	− 0.174
		Zn	As	Se	Cd	Hg	Pb
Outcome of IVF nonpregnant/-pregnant	r	0.071	− 0.126	− 0.090	− 0.245*	− 0.044	− 0.208*
Outcome of pregnancy	r	− 0.085	0.108	0.116	0.218*	0.047	0.245*

*Embryos—high (A and B) quality or low (C and D) quality embryos,**GT dose—< 2100 IU, > 2100 IU, cutoff values from our results, based of which intervals were derived: Cu < 98=> µg/dL, Mg < 3.3≥ mg/dL, Zn <(70–100)=> mmol/l, As < 1>= µg/L, Se < 50>= µg/L, Cd < 0.5=> µg/L, Hg < 1=> µg/L, Pb < 0.96=> µg/dL, *$P < 0.05$; **$P < 0.01$

Table 6 Spearman correlation of trace elements and toxic metals with pregnancy outcome

Spearman correlation (r)		Mg	Zn	Cu	As	Se	Cd	Hg	Pb
Deliveries	r	− 0.283**	− 0.141	0.095	− 0.162	− 0.026	− 0.252*	− 0.046	− 0.040
Miscarriages	r	− 0.052	− 0.078	0.102	0.057	− 0.060	− 0.123	0.018	− 0.238*

Cutoff values from our results, based of which intervals were derived: Mg <3.3≥ mg/dL, Zn <(70–100)=> mmol/L, Cu < 98=> μg/dL, As <1>= μg/L, Se < 50>= μg/L, Cd < 0.5 => μg/L, Hg < 1>= μg/L, Pb < 0.96=> μg/dL *P < 0.05; **P < 0.01

Cu, were in direct correlation with a higher number of fertilized oocytes. Similar finding had one recent study by Ingle et al. [29] that examined correlation between trace elements in follicular fluid and urine and IVF outcome in 58 patients. In that study, mean number of retrieved oocytes was in correlation with higher concentrations of Cu in urine. Mg in follicular fluid was in negative correlation with MII oocytes, while Mg in urine was in positive correlation.

While in Ingle et al. [29] study Zn in follicular fluid was in reversed correlation with mean fertilization rate, and there was no correlation with implantation rate, pregnancy, and life birth rate. As an interesting finding, we detected lower concentrations of Mg in blood of pregnant patients, while mean concentrations of Cu were slightly higher and mean concentrations of Zn and Se were slightly lower in this group. Patients that had had delivered had lower concentrations of Mg and Cu in blood compared to those with miscarriages. Also both in univariate and multivariate regression analysis, predictors for positive outcome were lower Mg concentrations. Mg binds to cell membranes and stabilizes them, as well as proteins and nucleic acids [34]; also, it protects lipoproteins from reactive oxygen species [35], but there are no data in literature of its association with IVF outcome. In the recent study [13], it was shown that women who conceived after IVF procedure had lower levels of trace elements including Mg, compared to women with natural pregnancy. All that correspond partially to our results, but in our study, lower concentrations of Mg did not affect the pregnancy rate neither the delivery rate. Grossi et al. found the slight decline of total serum magnesium levels in women during controlled ovarian stimulation [36]. These findings point out that screening of infertile patients for magnesium levels before the start of IVF procedure and supplementation of Mg would be beneficial as probably the dietary intake is lower and requirements are higher during ovarian stimulation and pregnancy. In the study of Bloom et al. [37], Mg concentrations were higher in women with positive pregnancy test compared with those who had negative test results, while Zn concentrations were lower in women with a positive pregnancy test, but not significantly. Zinc has recently been recognized as an important

Table 7 Univariate and Multivariate regression analysis of the outcome of IVF

	Univariate				Multivariate			
	OR	P	95% C.I. for OR		OR	P	95% C.I. for OR	
			Lower	Upper			Lower	Upper
Age	0.310	0.005	0.136	0.703	0.169	0.007	0.047	0.611
Smoking	2.429	0.072	0.923	6.391				
Unknown cause	2.786	0.037	1.062	7.310				
Ovarian cause	0.183	0.010	0.050	0.666				
Gonadotropin dose (IU)	2.276	0.041	1.035	5.005	4.229	0.015	1.330	13.444
Number of fertilized oocytes	2.391	0.040	1.039	5.502				
Embryo class	0.206	0.021	0.054	0.787	0.140	0.049	0.020	0.993
Cd (μg/L)	0.106	0.035	0.013	0.852				
Mg (mg/dL)	0.161	0.020	0.034	0.752	0.117	0.032	0.016	0.816
Pb (μg/dL)	0.373	0.050	0.139	0.998	0.224	0.026	0.060	0.835

Cutoff values from our results, based of which intervals were derived: age < 35 years>, smoking/no smoking, GT dose—< 2100 IU, Embryos—high (A and B) quality or low (C and D) quality embryos, Mg < 3.3≥ mg/dL, Cd < 0.5=> μg/L, Pb < 0.96=> μg/dL

Fig. 1 Receiver operating characteristic (ROC) curves for pregnancy and Mg
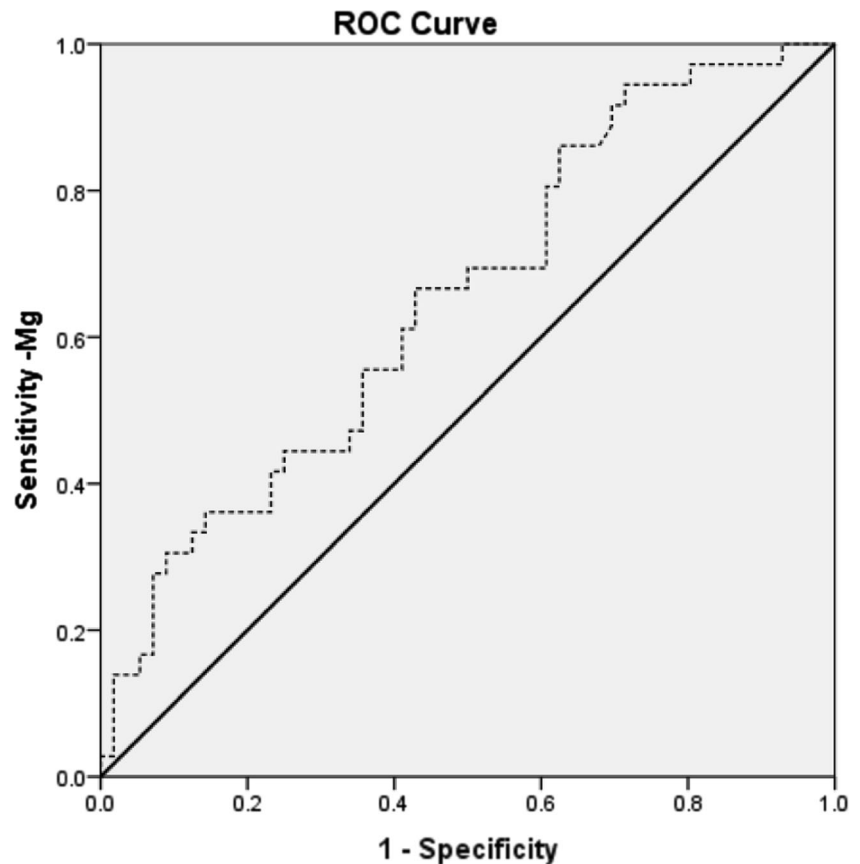
Diagonal segments are produced by ties.

factor in the completion of meiosis and oocyte activation in vitro [38–41], as well as in follicular rupture and completion of meiosis in vivo [41]. Tolunay et al. [2] determined concentrations of Cd, Pb, Hg, and As as well as of Cu, Zn, and Fe in blood and follicular fluid, and they found a lower pregnancy rate in patients with higher concentrations of Cu in follicular fluid. In our study, patients with higher concentrations of Cu had more fertilized oocytes and more pregnancies, but those who had had delivered had lower Cu concentrations compared with women that had miscarriage. Cu is involved in normal reproduction and it is necessary for different metabolic processes and enzymatic reactions [42], but possible negative effect on follicular maturation and embryo development was suggested when the exposure to Cu is chronic [2]. Since our results show Cu affecting positively fertility, more studies are needed to set the cutoffs. In our study, we did not find any significant difference in Se concentrations between pregnant and nonpregnant women. Data on Se in female fertility are scarce. It was reported that IVF patients had lower levels of Se than the control group [43, 44] and lower concentrations of Se were reported in

women who experienced miscarriages compared to women with clinical pregnancies [45, 46].

Limitations and Strengths

Although the results are interesting, it would be more relevant if we had a larger number of patients. Also, our patients used folic acid supplementation, but they did not report use of other supplements and we also did not know their eating habits. Besides, reproduction requires a couple, and our analysis included the female partner only. It is known that toxic metals and trace elements impact semen parameters [47–49], so the future assessment will require incorporation of toxic metal and trace element concentrations from male partners too. This research is significant because there are time-specific vulnerable windows of human development when environmental factors, even small exposures, can alter developmental programming signals and trigger adverse health consequences that can manifest across the lifespan of individuals and generations [50–53]. There is

Fig. 2 Receiver operating characteristic (ROC) curves for pregnancy and Pb
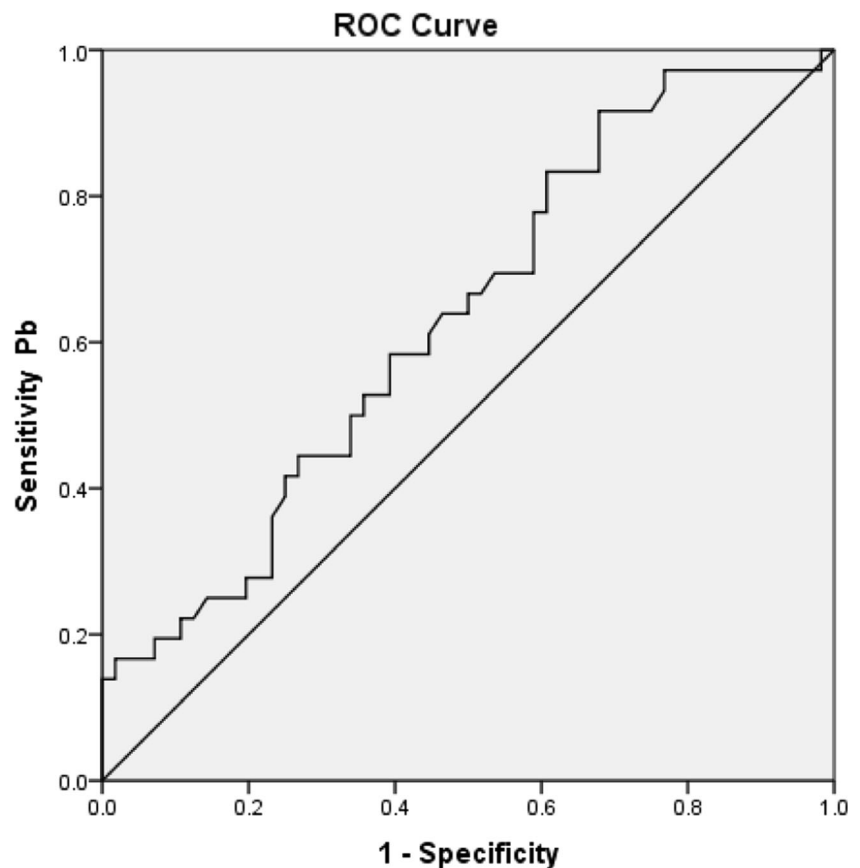
a need for population-based, multidisciplinary research and also implementation of prevention.

Conclusion

Our results suggest that there is a difference in some toxic metal and trace element concentrations between pregnant and non-pregnant women. There was no association between toxic metals and the number and quality of oocytes and embryos. Patients who were pregnant had lower concentrations of As and Pb, while nonpregnant women had higher concentrations of Pb and Cd, indicating a possible negative impact of toxic metals on IVF outcome. Concerning trace elements, we did not find the correlation of trace elements with oocyte number and quality, nor with a number of fertilized oocytes, except higher concentrations of Cu, was in direct correlation with a higher number of fertilized oocytes. Patients who were pregnant had lower concentrations of Mg. Larger studies in IVF population are required in order to find the association between the outcomes of the procedure and trace elements and toxic metals.

Compliance with Ethical Standards

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of Interest The authors declare that they have no conflict of interests.

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