Toxicological and nutritional status of trace elements in hair of women with in vitro fertilization (IVF)-induced pregnancy and their children

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Abstract
The objective of the present study was to assess toxic and nutritional trace element and mineral status in hair of women with IVF pregnancy and their children. Inductively-coupled plasma mass-spectrometry was used to assess hair trace element levels of 50 women with IVF pregnancy and 158 controls with spontaneous pregnancy and their children. Women with IVF pregnancy were characterized by significantly elevated hair As, Hg, Li, K, Na, and reduced Fe, Si, and Zn contents. Children from IVF pregnancy had significantly lower values of hair Cr, Fe, Mg, Sr, and Al content when compared to the control values, whereas hair Hg and Mo levels were higher. Hair trace element levels were associated with pregnancy complications and infertility, but not newborn characteristics. The results suggest the need for preconceptional monitoring and correction of the levels of trace elements in women in order to improve the course pregnancy and child development.

Toxicological and nutritional status of trace elements in hair of women with in vitro fertilization (IVF) pregnancy and their 9-month-old children

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Key words: in vitro fertilization; infertility; prenatal exposure; pregnancy

1. Introduction

Reproductive system is highly sensitive to various hazardous exposures and metabolic disturbances [1-3]. In particular, toxic metal exposure was found to be significantly associated with both male [4] and female [5] infertility. In females, cadmium (Cd), mercury (Hg), lead (Pb) and arsenic (As) significantly affect reproductive endocrine system due to their effect as endocrine disruptors [6]. The results of the LIFE Study have demonstrated that Cd and Pb exposure at environmentally relevant concentrations is associated with increased time to pregnancy [7].

In addition to toxic metal body burden, the level of essential trace elements has a significant effect on reproductive system [8]. In particular, female infertility has been linked to deficiency of zinc (Zn) [9], iron (Fe) [10], and selenium (Se) [11]. Se deficiency has also been linked to chronic miscarriage [12]. It has been suggested that the use of iodine (I), Se, and Fe supplementation may positively affect infertility treatment [13]. Moreover, essential trace elements may counteract toxic effects of heavy metals like Cd [14] or Hg [15].

Maternal toxic and essential trace element status affects offspring development and health [16]. In particular, prenatal Hg, Cd, Pb, As, and manganese (Mn) exposure is associated with adverse neurodevelopmental outcomes [17-19]. In utero As exposure is also associated with increased risk of cancer, lung, and cardiovascular disease [20]. An example of the link between essential trace elements levels and health of offspring is the finding that even marginal levels of maternal Zn deficiency may significantly affect fetal neurodevelopment [21].

Several studies have provided data on toxic and nutritional status of women undergoing in vitro fertilization (IVF). It has been demonstrated that women undergoing IVF are characterized by significantly reduced copper (Cu), Zn, and Se levels both in serum and follicular fluid [22]. Earlier research as part of the present longitudinal study showed

significantly lower levels of Cu, Fe, silicon (Si), Zn, calcium (Ca), and magnesium (Mg) in hair of women with IVF pregnancy, whereas hair As content significantly exceeded the control values [23]. Another study demonstrated that hair As content significantly correlated with follicular fluid As, Hg, and Pb levels [24]. In addition, metal exposure was found to be associated with negative outcome of in vitro fertilization [25-26], as well as problems with embryo development [27]. Hair toxic metal levels were also associated with in vitro fertilization outcomes [28].
Hair is widely used for monitoring of trace element status due to several advantages including easy and non-invasive sampling, high mineralization of the sample, as well as irreversible incorporation of trace elements into hair matrix [29]. In contrast to serum and urine, hair is indicative of long-term changes in trace element levels [29]. At the same time, certain limitations due to high variability of the results for using hair as a bioindicative matrix exist [30]. Moreover, the indicative capacity of hair varies for different trace elements [31].
The evidence is growing for altered levels of toxic and essential trace elements in women undergoing in vitro fertilization. However, very few studies to date addressed the links between the levels of toxic and essential trace elements in these women and such levels in their children. The primary objective of the present study was to assess toxic (aluminium (Al), As, Cd, Hg, lithium (Li), nickel (Ni), Pb, tin (Sn)) and essential (cobalt (Co), chromium (Cr), Cu, Fe, I, Mn, molybdenum (Mo), Se, Si, strontium (Sr), vanadium (V), Zn) elements and minerals (Ca, potassium (K), Mg, sodium (Na), phosphorus (P)) levels in hair of women with IVF pregnancy as well as its association with their children's hair element levels.

2. Materials and methods

Ethical approval
The protocol of the present study was approved by the Ethics Committee for Interdisciplinary Investigations (Tomsk State University). The study was performed in agreement with the principles of the Declaration of Helsinki and its later amendments (2013). All women took part in the present investigation on a voluntary basis and were informed about the experimental procedures. The informed consent was signed by all participants before the investigation. All sampling procedures involving children were performed in the presence of one of the parents.

Study design
The present study involved 208 pregnant women including 50 women with IVF pregnancy and 158 control cases with spontaneous pregnancy from the same location. The women lived in the Siberian Federal District of the Russian Federation. In order to prevent the influence of the side factors on hair trace element content, the following exclusion criteria were used: (i) the presence of metal implants (including dental amalgam fillings), (ii) occupational exposure to heavy metals, (iii) habitation near heavy metal emission sources (heavy industry); (v) smoking (both former and present); (vi) using infant formulas instead of breastfeeding.
Demographic information, data on the course of pregnancy and obstetric anamnesis were collected for all women in the maternity welfare clinics from the maternal anamnesis (Table 1). At delivery, newborn health status information (Table 2) was recorded in the maternity hospital. All women involved in the present study reported the use of vitamin/mineral supplements. Children were breastfed only till 6-7 months, with subsequent stage introduction of foods.

Hair sampling and preparation
Maternal hair samples were collected during the third trimester of pregnancy. Hair samples from their children were collected at the age of 9 months. Briefly, proximal parts of hair samples were collected from the occipital region using ethanol-precleaned stainless steel scissors (0.05–0.1 g). All examinees have washed their hair before sampling using usual commercial shampoos, that were shown not to affect hair trace element levels significantly if not enriched with a particular element [32].
In the laboratory hair samples were washed with acetone and subsequently rinsed three times with deionized water (18 MΩ · cm) prepared using DVS-M/1HA-1(2)-L electric distiller (Mediana-Filter, Podolsk, Russia). After drying on air at 60°C to a stable weight, 50 mg of hair samples were subjected to microwave degradation in the presence of concentrated nitric acid (Sigma-Aldrich Co., St. Louis, MO, USA) in Teflon containers using Berghof SW-4 DAP-40 (microwave frequency, 2.46 GHz; power, 1450 W) microwave system (Berghof Products + Instruments GmbH, 72800 Eningen, Germany) at 170–180°C for 20 minutes. The obtained solutions were added to a final volume of 15 ml with distilled deionized water and transferred into polypropylene test tubes for further analysis.

System settings and analysis

Assessment of the levels of toxic and essential trace elements and minerals was performed using inductively-coupled plasma mass-spectrometry in dynamic reaction cell mode (ICP-DRC-MS) at NexION 300D (PerkinElmer Inc., Shelton, CT, USA) equipped with 7-port FAST valve and ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA).
The system was calibrated via external calibration using standard solutions containing 0.5, 5, 10 and 50 μg/l of the studied elements prepared from Universal Data Acquisition Standards Kit (PerkinElmer Inc.). Internal online standardization with 10 μg/l yttrium-89 and rhodium-103 solutions was performed in order to adjust for incomplete acidity and viscosity between calibration and sample matrices. The solutions were prepared from Yttrium (Y) and Rhodium (Rh) Pure Single-Element Standard (PerkinElmer Inc. Shelton, CT, USA) on a matrix containing 8% 1-butanol (Merck KGaA, Gernsheim, Germany), 0.8% Triton X-100 (Sigma-Aldrich Co., St. Louis, MO, USA), 0.02% tetramethylammonium hydroxide (Alfa Aesar, Ward Hill, MA, USA) and 0.02% ethylenediaminetetraacetic acid (Sigma-Aldrich Co., St. Louis, MO, USA).
Laboratory quality control was performed using GBW09101 human hair certified reference material (Shanghai Institute of Nuclear Research, Shanghai, China). The recovery rates for all studied elements varied within the range of 88%-112%. The laboratory is also a participant of the Occupational and Environmental Laboratory Medicine External Quality Assessment Schemes (OELM EQAS).

Statistical analysis
Statistical analyses were performed using Statistica 10.0 (Statsoft, Tulsa, OK, USA). Data distribution was assessed using Shapiro-Wilk test and revealed non-Gaussian distribution for the levels of all toxic and essential elements in hair samples of the examinees. The median and 25–75 percentile boundaries (interquartile range, IQR) were used as descriptive statistics. Significance of group differences was assessed using the non-parametric Mann-Whitney U test for paired-group comparisons. Correlation analysis was performed using Spearman's rank correlation coefficient. Multiple regression analysis was used in order to assess the association between the period on infertility and hair levels of elements that were characterized by significant group difference. The level of significance of $p < 0.05$ was used for all statistical analyses.

3. Results

The results showed significant group differences in obstetric anamnesis (Table 1). In particular, women with IVF pregnancy were significantly older and had higher rates of extrauterine pregnancy, infertility in anamnesis, as well as significantly longer infertility period as compared to the control group. No significant group difference in the number of pregnancies, births, or spontaneous abortions was detected. Women with IVF pregnancy had significantly higher number of fetuses; but had higher risk of spontaneous abortions during the first, second, and third trimesters, as well as during the whole pregnancy in comparison to women with spontaneous pregnancy. No significant differences in the rate of arterial hypertension, colds, and anemia were detected between the groups. Women with IVF pregnancy had higher rate of thyroid pathology during the present pregnancy in comparison to the control group.
The type of pregnancy had a significant effect on birth outcome (Table 2). Although there were no significant group difference in Apgar 1 and 5 values, children from IVF pregnancy were characterized by 12%, 4%, and 4% lower values of newborn body weight, head and thoracic circumference, respectively.
Toxicological and nutritional status of trace elements in hair of women was also significantly associated with pregnancy type (Table 3). In particular, women with IVF pregnancy were characterized by significantly elevated hair levels of As, Hg, Li, K and Na, exceeding the control values by 50%, 53%, 38%, 76% and 49%, respectively. In turn, their hair contents of Fe, Si, and Zn were 41%, 21%, and 9% lower than respective control values.
The type of pregnancy had a significant effect on hair trace element levels in children (Table 4). Children from IVF pregnancy had 25%, 11%, 11%, 18%, and 31% lower values of hair Cr, Fe, Mg, Sr, and Al content than the comparison group of spontaneously conceived children. In turn, hair levels of Hg and Mo exceeded the control values by 46% and 28%, respectively. The 21% increase in hair As levels in children from IVF pregnancy was nearly significant.
Comparative analysis of maternal and children hair trace element levels demonstrated that the difference was nearly similar between the groups. Particularly, children from both groups were characterized by significantly higher hair levels of Al, As, Cd, Cr, I, K, Li, Mo, Na, Pb, Se, Sn, and V as compared to the respective maternal values. At the same time, in the control group maternal hair Ca, Co, Cu, Hg, Mg, Mn, Ni, P, Sr, and Zn exceeded the respective values from children, whereas no group difference was observed for hair Fe and Si contents. In the IVF group hair Fe, Si, Ni, and P levels in mothers did not differ significantly from those in their children.
Correlation analysis demonstrated positive associations between maternal and child hair trace element and mineral levels. In particular, significant correlations were detected for As ($r=0.154$; $p=0.031$), Co ($r=0.230$; $p=0.001$), Cu

(r=0.544; p<0.001), Hg (r=0.471; p<0.001), Mn (r=0.197; p=0.005), Na (r=0.175; p=0.014), and Se (r=0.767; p<0.001).

In addition, correlation analysis examined the associations between maternal obstetric anamnesis and both maternal and child hair trace element and mineral levels that were significantly different between the groups (Table 5). The results showed that both maternal and child hair Hg significantly correlated with maternal age and hypertension during pregnancy. Moreover, maternal hair Hg was associated with thyroid pathology during pregnancy. Maternal hair As levels were linked to the rate of acute respiratory infections during pregnancy as well as the period of infertility. The latter was also directly correlated with maternal hair K, Li, and Na. Maternal hair K levels were also associated with infertility in anamnesis and the number of fetuses in the present pregnancy. Both maternal hair Na and K were associated with the rate of colds in pregnancy. Children's hair Mo levels were positively correlated with infertility in anamnesis, the number of fetuses in the present pregnancy, and increased risk of spontaneous abortion during the whole period of pregnancy. Finally, children's hair Sr levels were associated with the number of pregnancies and thyroid pathology during pregnancy.

Multiple regression analysis (Table 6) was performed to evaluate the association between the period of infertility and trace element and mineral levels, as correlational analysis revealed the most significant associations with this parameter. Only maternal hair element levels were used as independent predictors, as no significant correlation between the length of infertility and children's hair metal levels was observed. The results showed that the crude model containing maternal hair As, K, Li, Na, Fe, Hg, Si, and Zn levels accounted for only 16% of the variability in the period of infertility, although the model was significant. After adjustment for obstetric anamnesis parameters (except "history of infertility") the model significantly accounted for 78% of the parameter variability. Hair As and K levels were directly associated with the period of infertility in the adjusted model.

In contrast to maternal parameters, children's parameters were weakly associated with hair trace element and mineral levels. In particular, only maternal hair K (r=-0.164; p=0.031) and Na (r=-0.155; p=0.042) were significantly inversely associated with newborn body weight. In turn, children's hair Fe content was weakly associated with Apgar 1 values (r=0.174; p=0.024).

4. Discussion

Overall, the results of the present study are consistent with the previous indications of increased levels of toxic metals and metalloids and reduced essential element levels in women undergoing IVF. Kim et al. (2013) proposed that elevated levels of Hg, As and other metals may be related to high seafood consumption in couples who underwent IVF [33]. However, this explanation is unlikely in the studied continental population, characterized by low rate of fish consumption. Certain lifestyle factors may also have a significant effect on hair trace element levels in pregnant women [34].

The results demonstrated that women with IVF pregnancy and their children had on average significantly increased hair Hg levels. These data correspond to an earlier finding of high frequency of Hg overload in women undergoing IVF [35]. The potential sources of increased body Hg burden may include industrial emissions, consumption of contaminated sea foods, as well as dental amalgams [36]. Elevated body Hg burden was shown to be associated with reduced pregnancy rate in IVF [26], although contradictory results also exist [37]. Hair Hg levels were also associated with reduced oocyte maturity [28]. A significant correlation between maternal and child hair Hg levels corresponds with data on transplacental Hg transport [38]. Particularly, it has been demonstrated that women exposed to Hg from amalgam fillings are characterized by significantly increased maternal and umbilical cord blood Hg levels [39]. Taking into account the role of prenatal Hg exposure in altered brain development, increased body Hg burden may not only increase the risk of infertility, but also predispose children to neurotoxicity. Although no effect of Hg on pregnancy outcome was revealed, hair Hg content significantly correlated with the prevalence of hypertension and thyroid pathology during pregnancy. Hypertensive effect may be associated with Hg-induced alteration of vascular reactivity and remodeling [40]. In turn, the relationship between Hg and thyroid pathology may be related to Hg-induced alteration in thyroid hormone production as well as stimulation of thyroid autoimmunity [41]. The association between both children and maternal hair Hg levels with maternal age is in agreement with our earlier data on age-related increase in hair Hg levels in adults [42].

Increased hair As levels in women with IVF pregnancy corresponds to our earlier data [23]. Elevated hair As content may be associated with the use of contaminated products and water, industrial emissions, smoking [43]. Hair As levels were found to correlate with follicular fluid metalloid levels [24]. It has been also demonstrated that blood As are significantly lower in women with positive outcome of IVF [44]. These data correspond to the observation of altered female fecundity under As exposure [45], being in agreement with positive correlation between hair As content and period of infertility, as well as overall reproductive and developmental toxicity of As [46]. Maternal As exposure was

also shown to have significant adverse effect on offspring health [47-48]. Although the correlation between maternal and children hair As levels was rather weak, the observed significance of the relationship is in agreement with earlier data on the association between maternal and newborn blood As levels [49]. Similar association between maternal exposure and children metal levels was also demonstrated for certain other metals including Cd [50], Mn [51], Se [52] corresponding to the results of the present study.

Significant elevation of hair Li levels in women with IVF pregnancy is of particular interest. Li is widely used for treatment of bipolar disorders including those in pregnancy [53]. Li imbalance was also shown to play a significant role in certain developmental brain disorders [44]. However, none of the women examined received specific Li therapy. Although no data regarding the interaction between Li and IVF exist, the metal may possess reproductive and developmental toxicity [45]. It has been demonstrated that Li exposure may be associated with altered oocyte maturation [46] and follicular atresia [47]. High umbilical cord blood Li levels may be also related to higher number of pregnancy complications as well as maternal blood Li concentrations [58].

The observed low levels of hair Fe in women with IVF pregnancy are in agreement with the earlier indications of reduced Fe status in female infertility [59], also corresponding to our earlier findings [23]. It has been also shown that higher Fe intake (first quartile) may reduce the risk of ovulatory infertility [10]. In addition, Fe deficiency was shown to have adverse effects on embryogenesis, development in general [60] and especially neural development [61].

Although Mo is an essential element and deficiency of Mo cofactors is associated with brain disorders [62], its overexposure may be toxic [63]. The potential sources of human Mo exposure may include industrial emissions (mining), as well as consumption of polluted water and bread [64], although their particular contribution to the elevated hair Mo in the examined children is unclear. Altered Mo status may be also associated with congenital disorders of Mo and Mo-dependent cofactor metabolism [64]. It is notable that we did not observe any significant group difference in hair Mo levels in pregnant women, whereas children from IVF pregnancy were characterized by higher Mo levels. In addition, only children's hair Mo levels significantly correlated with pregnancy complications including the history of infertility. These data correspond to the results of experimental studies demonstrating negative effects of MoO_3 nanoparticles on female reproductive hormone levels and ovarian function [65]. In vitro studies demonstrated that high-dose Mo exposure may significantly reduce embryo quality [66]. Increased hair Mo levels in children may be also of great importance in view of indications of the negative association between Mo exposure and infant neurodevelopment [67].

A significant increase in hair Na and K levels in women with IVF pregnancy is of particular interest. It has been demonstrated that high NaCl intake negatively affects follicle development through repression of cell proliferation and stimulation of apoptosis [68]. High salt intake in addition to fructose consumption resulted in a significant decrease in fertility [69]. Data from agricultural animals demonstrates that high K intake may also reduce female fertility [70], although human observations are absent. Hypothetically, altered Na and K handling may occur in the case of kidney pathology that is known to be associated with infertility [71].

Although no significant group difference in hair Se levels were detected the results showed significant correlation between maternal and children's values, consistent with the earlier indications of the positive association between maternal and fetal status [72], as well as the role of Se in pregnancy and development [12].

The present study has several potential limitations. First, maternal diet was not monitored during pregnancy and lactation. Second, serum trace element and mineral levels were not assessed using the study that could be beneficial to investigate the systemic changes of trace element status. Finally, the study included only children aged 9 months, whereas no hair analysis was performed at delivery, that does not exclude the potential influence of environmental exposure. Further investigations aimed at these points would be beneficial for assessment of perturbations of trace element and mineral metabolism in women with infertility and their children.

Overall, the results of this study suggest that women with IVF pregnancy on average have more alterations of toxicological and nutritional status of trace elements than naturally conceiving women. These alterations are associated with similar changes in their children. It is also notable that both maternal and children's hair trace element levels are associated with pregnancy complications, including infertility. The present study did not find significant correlations between maternal and children's trace element status and newborn parameters, including anthropometric values and Apgar scores. However, these associations may emerge later in development – which will be explored in this on-going longitudinal study. Multiple comparisons, performed in this study, increase the chance of false positives. In addition, the relatively small sample may be underpowered for establishing some weaker associations. Future research is needed to replicate the findings of this study in other samples. Although the results must be interpreted with caution, they indicate that preconceptional monitoring and correction of the levels of toxic and essential elements in women may reduce the rate of infertility and pregnancy complications, as well as improve children's health.

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Conflict of interest
The authors declare no conflict of interest

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Table 1. Obstetric anamnesis and pregnancy characteristics of the studied women

Parameter	Type of pregnancy		P value
	Spontaneous	IVF-induced	
Age, years	31.7±4.0	35.1±4.4	<0.001*
Pregnancy			
First	71/158 (45%)	26/50 (52%)	0.449
Second	50/158 (32%)	9/50 (18%)	
Third and more	37/158 (23%)	15/50 (30%)	
Childbirth			
First	98/158 (62%)	39/50 (78%)	0.106
Second	52/158 (33%)	10/50 (20%)	
Third and more	8/158 (5%)	1/50 (2%)	
Obstetric anamnesis			
History of spontaneous abortion, yes	48/158 (30%)	8/50 (16%)	0.124
History of extrauterine pregnancy, yes	16/158 (10%)	9/50 (18%)	0.023 *
History of infertility, yes	2/158 (1%)	50/50 (100%)	<0.001 *
Period of infertility, years	1.2±0.1	7.3±4.0	<0.001 *
Number of fetuses (this pregnancy)			
One	152/158 (96%)	37/50 (74%)	<0.001 *
Two or more	6/158 (4%)	13/50 (26%)	
Pregnancy complications			
Chronic hypoxia, yes	11/158 (7%)	6/50 (12%)	0.131
Risk of spontaneous abortion:			
1 trimester, yes	40/158 (25%)	17/50 (34%)	0.039 *
2 trimester, yes	38/158 (24%)	20/50 (40%)	0.001 *
3 trimester, yes	27/158 (17%)	19/50 (38%)	<0.001 *
Whole pregnancy, yes	20/158 (13%)	14/50 (28%)	0.001 *
Gestosis, yes	2/158 (1%)	4/50 (8%)	0.005 *
Arterial hypertension, yes	9/158 (6%)	5/50 (10%)	0.146
Acute respiratory infection, yes	17/158 (11%)	6/50 (12%)	0.506
Anemia, yes	83/158 (52%)	26/50 (52%)	0.169
Thyroid pathology, yes	14/158 (9%)	10/50 (20%)	0.006 *

The history of infertility (yes/no) was assessed as the number of women per group reporting infertility prior this pregnancy; The period of infertility was assessed in years in women who reported failure to achieve pregnancy after 12 months or more (> 1 year) of regular unprotected sexual intercourse;

Data expressed as mean ± SD or n (n is indicative of the number of women with particular characteristics from the total number of women in the group);

* Significant difference at $p < 0.05$ as assessed by the Mann-Whitney U test

Table 2. Characteristics of newborns (IVF-induced and spontaneous pregnancy)

Parameter	Type of pregnancy		P value
	Spontaneous	IVF-induced	
Apgar score 1, pts	8.1±0.7	8.2±0.8	0.709
Apgar score 5, pts	8.8±0.6	9.0±0.8	0.118
Body weight, g	3491±583	3127±698	0.003 *
Height, cm	52.7±4.9	51.6±4.4	0.080
Head circumference, cm	34.8±1.5	33.5±2.2	<0.001 *
Chest circumference, cm	34.2±1.9	33.0±3.1	0.022 *
Data expressed as mean ± SD; * Significant difference at $p < 0.05$ as assessed by the Mann-Whitney U test			

Table 3. Hair toxic and essential element content (μg/g) in hair of women with IVF-induced and spontaneous pregnancy

Element, μg/g	Type of pregnancy		P value
	Spontaneous	IVF	
Toxic and potentially toxic			
Al	3.485 (2.156-5.629)	3.737 (2.510-5.750)	0.530
As	0.010 (0.006-0.016)	0.015 (0.009-0.022)	0.005 *
Cd	0.009 (0.004-0.016)	0.010 (0.006-0.016)	0.490
Hg	0.306 (0.177-0.487)	0.468 (0.239-0.707)	0.008 *
Li	0.008 (0.004-0.013)	0.011 (0.006-0.020)	0.014 *
Ni	0.276 (0.180-0.418)	0.203 (0.149-0.367)	0.070
Pb	0.344 (0.205-0.600)	0.344 (0.190-0.707)	0.922
Sn	0.162 (0.086-0.507)	0.238 (0.105-0.548)	0.168
Essential and potentially essential			
Co	0.017 (0.009-0.044)	0.014 (0.007-0.073)	0.661
Cr	0.083 (0.044-0.162)	0.100 (0.073-0.163)	0.234
Cu	15.60 (10.89-26.54)	13.30 (10.30-17.78)	0.067
Fe	18.46 (11.32-30.18)	10.69 (7.97-21.31)	0.002 *
I	0.342 (0.224-0.516)	0.269 (0.177-0.659)	0.442
Mn	0.984 (0.579-1.840)	0.727 (0.524-1.579)	0.221
Mo	0.022 (0.016-0.027)	0.022 (0.019-0.026)	0.568
Se	0.404 (0.313-0.499)	0.411 (0.355-0.465)	0.863
Si	33.37 (19.42-47.50)	26.41 (17.20-32.93)	0.012 *
Sr	6.900 (4.035-11.327)	6.344 (4.085-9.856)	0.382
V	0.009 (0.005-0.018)	0.009 (0.004-0.026)	0.848
Zn	229.3 (192.3-303.6)	209.1 (174.8-253.1)	0.028 *
Minerals			
Ca	1941 (1144-2992)	1393 (930-2523)	0.082
K	100.4 (46.0-191.1)	176.0 (109.3-340.5)	0.001 *
Mg	127.7 (77.8-202.6)	101.4 (66.7-175.5)	0.085
Na	82.38 (47.02-143.00)	122.18 (64.54-250.51)	0.015 *
P	163.9 (143.0-185.5)	157.9 (142.5-176.7)	0.552
Data expressed as median (IQR); * - significant group difference as assessed by Mann-Whitney U-test at $p < 0.05$			

Table 4. Toxic and essential trace element and mineral levels in children (Spontaneous and IVF-induced pregnancy)

Element, µg/g	Type of pregnancy		P value
	Spontaneous	IVF-induced	
Toxic and potentially toxic			
Al	10.84 (6.19-18.58)	7.48 (4.89-11.77)	0.010 *
As	0.037 (0.023-0.062)	0.044 (0.032-0.060)	0.066
Cd	0.043 (0.021-0.072)	0.054 (0.023-0.065)	0.815
Hg	0.102 (0.057-0.188)	0.150 (0.064-0.281)	0.048 *
Ni	0.233 (0.141-0.309)	0.216 (0.155-0.288)	0.809
Pb	1.284 (0.677-2.161)	1.156 (0.609-1.866)	0.388
Sn	0.558 (0.319-0.934)	0.427 (0.270-0.846)	0.348
Essential and potentially essential			
Co	0.009 (0.006-0.018)	0.009 (0.007-0.016)	0.861
Cr	0.199 (0.134-0.333)	0.150 (0.105-0.234)	0.015 *
Cu	10.79 (8.51-13.67)	9.89 (8.07-11.51)	0.164
Fe	16.19 (11.71-24.15)	14.58 (8.89-18.62)	0.031 *
I	0.729 (0.437-1.488)	0.782 (0.526-1.340)	0.710
Li	0.019 (0.011-0.037)	0.021 (0.013-0.037)	0.670
Mn	0.351 (0.230-0.530)	0.308 (0.235-0.549)	0.980
Mo	0.043 (0.028-0.062)	0.056 (0.041-0.068)	0.009 *
Se	0.451 (0.379-0.527)	0.458 (0.411-0.502)	0.713
Si	26.85 (14.74-56.21)	30.79 (20.66-53.27)	0.419
Sr	0.985 (0.708-1.384)	0.810 (0.567-1.170)	0.014 *
V	0.020 (0.010-0.044)	0.023 (0.012-0.063)	0.452
Zn	75.34 (46.53-119.88)	65.17 (39.59-100.00)	0.260
Minerals			
Ca	351.2 (259.7-523.9)	309.7 (233.2-402.2)	0.087
K	987.7 (365.0-1621.1)	685.5 (268.4-1915.0)	0.732
Mg	22.47 (17.97-32.36)	19.94 (15.64-26.63)	0.038 *
Na	334.4 (163.1-607.3)	313.6 (109.3-644.4)	0.694
P	155.6 (130.9-175.7)	156.3 (127.5-168.6)	0.797
Data expressed as median (IQR); * - significant group difference as assessed by Mann-Whitney U-test at $p < 0.05$			

Table 5. Correlation between maternal and children's hair trace elements, obstetric anamnesis and pregnancy characteristics

Parameter		Women					Children		
		As	Hg	K	Li	Na	Hg	Mo	Sr
Age, years	r	-0.012	0.180	0.055	-0.016	0.006	0.172	-0.005	0.119
	p	0.867	0.009 *	0.429	0.824	0.938	0.013 *	0.943	0.089
Number of pregnancies	r	-0.02	-0.036	0.061	-0.043	0.044	0.066	0.023	0.166
	p	0.792	0.634	0.425	0.571	0.562	0.387	0.76	0.029 *
History of infertility	r	0.140	0.141	0.194	0.069	0.150	0.034	0.131	-0.075
	p	0.067	0.067	0.011 *	0.370	0.050 *	0.663	0.088	0.334
Period of infertility, years	r	0.281	0.172	0.390	0.198	0.267	0.028	0.125	-0.054
	p	0.003 *	0.067	< 0.001 *	0.034	0.004 *	0.771	0.183	0.568
Risk of spontaneous abortion during whole pregnancy	r	0.022	0.008	0.014	-0.082	0.015	-0.027	0.160	0.118
	p	0.772	0.999	0.857	0.283	0.847	0.728	0.036 *	0.125
Arterial hypertension	r	-0.021	0.229	0.039	0.02	0.005	0.366	-0.021	-0.012
	p	0.779	0.002 *	0.609	0.793	0.946	< 0.001 *	0.785	0.88
Acute respiratory infection	r	0.176	0.035	0.160	0.05	0.209	0.12	-0.045	0.002
	p	0.021 *	0.648	0.035 *	0.515	0.006 *	0.117	0.556	0.981
Thyroid pathology	r	0.026	0.150	0.014	0.057	0.015	0.053	0.097	0.17
	p	0.731	0.047 *	0.851	0.457	0.847	0.483	0.201	0.025 *

The history of infertility (yes/no) was assessed as the number of women per group reporting infertility prior this pregnancy; The period of infertility was assessed in years in women who reported failure to achieve pregnancy after 12 months or more (> 1 year) of regular unprotected sexual intercourse;

Only pairs with at least one significant correlation are presented; * - correlation is significant at $p < 0.05$

Table 6. Multiple regression analysis of the association between maternal hair trace element levels and period of infertility

Element	Model 1		Model 2	
	β	p^i	β	p
As	0.055	0.602	0.149	0.021 *
Fe	-0.073	0.418	0.001	0.980
Hg	0.087	0.341	-0.104	0.110
K	0.307	0.030 *	0.330	0.001 *
Li	0.087	0.368	0.023	0.711
Na	0.003	0.983	-0.123	0.183
Si	-0.089	0.338	0.038	0.534
Zn	-0.127	0.155	0.011	0.858
Multiple R	0.473		0.850	
Multiple R^2	0.224		0.723	
Adjusted R^2	0.165		0.650	
P for a model	<0.001 *		<0.001 *	

Data presented as regression coefficient (β) and individual p (p^i) value for every association and the overall p for a model; * - the association is significant at $p < 0.05$; Model 1 – crude model including only trace elements characterized by group difference; Model 2 – adjusted for obstetric anamnesis parameters (except “history of infertility”)