

# Hair Trace Element and Electrolyte Content in Women with Natural and In Vitro Fertilization-Induced Pregnancy

Anatoly V. Skalny<sup>1,2,3,4</sup> · Alexey A. Tinkov<sup>2,3,4,5</sup> · Irina Voronina<sup>6,7</sup> · Olga Terekhina<sup>6</sup> · Margarita G. Skalnaya<sup>4</sup> · Yulia Kovas<sup>6,8</sup>

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**Abstract** The objective of the present study was to perform comparative analysis of hair trace element content in women with natural and in vitro fertilization (IVF)-induced pregnancy. Hair trace element content in 33 women with IVF-induced pregnancy and 99 age- and body mass index-matched control pregnant women (natural pregnancy) was assessed using inductively coupled plasma mass spectrometry. The results demonstrated that IVF-pregnant women are characterized by significantly lower hair levels of Cu, Fe, Si, Zn, Ca, Mg, and Ba at  $p < 0.05$  or lower. Comparison of the individual levels with the national reference values demonstrated higher incidence of Fe and Cu deficiency in IVF-pregnant women in comparison to that of the controls. IVF pregnancy was also associated with higher hair As levels ( $p < 0.05$ ). Multiple regression analysis revealed a significant interrelation between IVF pregnancy and hair Cu, Fe, Si, and As content. Hair Cu levels were also influenced by vitamin/mineral supplementation and the number of pregnancies, whereas hair Zn content was dependent on prepregnancy anthropometric

parameters. In turn, planning of pregnancy had a significant impact on Mg levels in scalp hair. Generally, the obtained data demonstrate an elevated risk of copper, iron, zinc, calcium, and magnesium deficiency and arsenic overload in women with IVF-induced pregnancy. The obtained data indicate the necessity of regular monitoring of micronutrient status in IVF-pregnant women in order to prevent potential deleterious effects of altered mineral homeostasis.

**Keywords** In vitro fertilization · Iron · Copper · Deficiency · Arsenic

## Introduction

Multiple studies demonstrated that dietary factors, including vitamins [1] and trace elements [2], may have a significant effect on reproductive health. Deficiency of essential trace elements has been shown to be associated with impaired fertility [2]. In particular, it has been suggested that women with recurrent miscarriages have more selenium deficiency in comparison to healthy controls [3]. Experimental studies with animals demonstrated that dietary Zn deficiency is associated with impaired embryogenesis in animals conceived through in vitro fertilization (IVF) [4].

Correspondingly, adequate micronutrient intake may play a role in prevention of female infertility [5]. Vitamin D deficiency was observed to be rather common in infertile couples requiring assisted reproduction technologies [6]. Women undergoing IVF were also characterized by lower serum and follicle fluid selenium and zinc concentrations [7]. Increased vitamin C, E, and A intake has been associated with shorter time to pregnancy in couples being treated for unexplained infertility [8]. Dietary non-heme iron intake including iron supplements has been shown to reduce the risk of ovulatory

✉ Anatoly V. Skalny  
skalny3@gmail.com

<sup>1</sup> All-Russian Research Institute of Medicinal and Aromatic Plants, Moscow, Russia

<sup>2</sup> Orenburg State University, Orenburg, Russia

<sup>3</sup> Yaroslavl State University, Yaroslavl, Russia

<sup>4</sup> RUDN University, Moscow, Russia

<sup>5</sup> Orenburg State Medical University, Orenburg, Russia

<sup>6</sup> Tomsk State University, Tomsk, Russia

<sup>7</sup> Psychological Institute of Russian Academy of Education, Moscow, Russia

<sup>8</sup> Goldsmiths, University of London, London, UK

62 infertility [9]. At the same time, a review of the supplementa-  
 63 tion trials demonstrated that the effect of micronutrient sup-  
 64 plementation on female fertility is rather unclear [10].

65 Micronutrient status has also been shown to contribute to  
 66 the efficiency of assisted reproductive technologies. For exam-  
 67 ple, a positive association between blood Zn and Mg con-  
 68 centrations with the probability of pregnancy has been dem-  
 69 onstrated [11]. The normal level of folic acid is associated with  
 70 successive IVF [12]. Higher folate intake has also been asso-  
 71 ciated with higher live birth rates in women undergoing  
 72 assisted reproduction [13]. However, data on essential trace  
 73 element status in women undergoing IVF are insufficient and  
 74 somewhat contradictory.

75 Toxic metal exposure (including occupational) also has a  
 76 significant effect on reproductive system functioning [14]. In  
 77 particular, the existing data indicate a significant negative in-  
 78 fluence of Pb exposure on female fertility [15]. Multiple stud-  
 79 ies have demonstrated that the effect of cadmium on ovaries,  
 80 oogenesis, and embryogenesis (both in pre- and  
 81 post-implantation periods) is mediated by Cd-induced oxida-  
 82 tive stress, apoptosis, altered cell adhesion, interference with  
 83 essential trace element metabolism, and DNA damage [16]. In  
 84 addition, certain toxic metals including Cd, Hg, Pb, and As act  
 85 as endocrine disruptors affecting endocrine and reproductive  
 86 endocrine system signaling [17]. Moreover, it has been dem-  
 87 onstrated that increased blood toxic trace elements (Pb, Hg,  
 88 and Pb) levels may affect the outcome of IVF [11].

89 Therefore, the existing data demonstrate that monitoring of  
 90 trace element status of women with reproductive problems is  
 91 of particular importance in order to reveal deficiency of the  
 92 essential trace elements and possible excess of the toxic ones.  
 93 Moreover, simultaneous assessment of trace element status is  
 94 also required as the interaction of essential and toxic trace  
 95 elements may have a significant impact on fertility [18].

96 Hair is widely used for trace element status assessment due  
 97 to non-invasiveness of sampling, simplicity of storage, irre-  
 98 versible binding of trace elements into the hair matrix, and  
 99 high degree of mineralization [19]. Therefore, hair trace ele-  
 100 ment content may be indicative of the nutritional status of the  
 101 organism for a period of time, whereas blood, serum, and  
 102 urinary trace element levels reflect current physiological state  
 103 of the organism due to homeostatic regulation [20]. Hair may  
 104 be also used for assessment of environmental exposure to  
 105 trace elements [21]. At the same time, hair trace element con-  
 106 tent may vary in response to a number of factors including  
 107 gender, age, geographical location, ethnicity, and living and  
 108 dietary habits, as well as physiological state of the organism  
 109 [22]. Therefore, appropriate reference values should be used  
 110 in order to improve interpretation of the obtained hair trace  
 111 element data [23].

112 Earlier studies demonstrated the dynamics of hair trace ele-  
 113 ment content in pregnancy [24]. Our previous studies demon-  
 114 strated that trace element levels in pregnant women may respond

115 to certain lifestyle factors, such as alcohol consumption [25, 26].  
 116 Moreover, hair trace element analysis in pregnant women may be  
 117 indicative of certain perinatal pathologies [27].

118 In vitro fertilization is the one of the most effective assisted  
 119 reproductive technologies today. Briefly, it includes ovarian  
 120 hyperstimulation for optimization of follicle development and  
 121 egg production, subsequent egg retrieval, and in vitro fertili-  
 122 zation by co-cultivation of eggs and sperms, embryo culture  
 123 for 3–5 days, and, finally, transfer of the embryo into the  
 124 uterus [28].

125 As the use of reproductive technologies is growing, it is  
 126 important to identify factors of risk that may be characteristic  
 127 of women undergoing IVF treatment. Therefore, the primary  
 128 objective of the present study was to perform comparative  
 129 analysis of hair trace element content in women with natural  
 130 and IVF-induced pregnancy.

## 131 Materials and Methods

132 A total of 33 women with IVF-induced pregnancy were en-  
 133 rolled in the present investigation. The control group included  
 134 99 women with natural pregnancy who were matched to the  
 135 cases for age, anthropometric parameters (weight, height, and  
 136 body mass index (BMI)), and the place of habitation. The IVF  
 137 and control groups consisted of women living in the Siberian  
 138 Federal District of the Russian Federation (Tomsk, Novosibirsk,  
 139 and Barnaul) in similar proportions. Only cases of normal  
 140 pregnancy were included in the present study. In order to prevent  
 141 the influence of the side factors on hair trace element status,  
 142 the following exclusion criteria were used: (i) the presence of  
 143 metal implants (including dental amalgam fillings), (ii) occupa-  
 144 tional exposure to heavy metals, (iii) the use of hormonal  
 145 replacement therapy, (iv) smoking (both before and during  
 146 pregnancy).

147 All pregnant women had filled in a questionnaire and pro-  
 148 vided personal information on age at menarche, age at first  
 149 sex, marital status (and years married), and education. They  
 150 have also specified whether the present pregnancy is the first  
 151 one and planned. Information about the use of vitamin/mineral  
 152 supplements, iron supplements, and the period of iron supple-  
 153 mentation was also collected using the questionnaire.

154 Prepregnancy anthropometric parameters (height and  
 155 weight) were registered. Prepregnancy BMI was calculated  
 156 using the values of body height (m) and weight (kg) using  
 157 the standard formula ( $BMI (kg/m^2) = \text{body weight}/\text{height}$ ).

158 Table 1 provides a summary of anthropometric and personal  
 159 data of the examined women with natural and IVF-induced  
 160 pregnancy.

161 Scalp hair samples were collected from the occipital region  
 162 using ethanol-precleaned stainless steel scissors (0.05–0.1 g)  
 163 in the third trimester of pregnancy from women with both  
 164 normal and IVF-induced pregnancy. Only proximal parts of

**Table 1** Population description

Parameter	Natural pregnancy ( <i>n</i> = 99)	IVF pregnancy ( <i>n</i> = 33)	<i>p</i> value
Age, years	30.6 ± 3.7	31.8 ± 4.5	0.094
Prepregnancy height, cm	165.5 ± 6.3	166.2 ± 4.8	0.849
Prepregnancy weight, kg	63.1 ± 13.7	64.0 ± 14.3	0.722
Prepregnancy BMI	23.0 ± 4.6	23.1 ± 4.8	0.930
Age of menarche, years	13.2 ± 1.4	12.6 ± 1.2	0.065
Age of first sex, years	18.2 ± 2.4	18.4 ± 3.0	0.770
Marital status			
Married, <i>n</i>	85/99	30/33	0.458
Cohabiting, <i>n</i>	12/99	2/33	0.132
Single, <i>n</i>	2/99	1/33	0.745
Years married	4.2 ± 3.5	5.5 ± 4.9	0.185
Education (highest)			
Secondary school	2/99	1/33	0.744
College	12/99	3/33	0.543
University	78/99	28/33	0.476
PhD	–	2/33	–
Other (not specified)	7/99	–	–
Pregnancy			
First pregnancy, <i>n</i>	30/9	19/33	0.005*
Planned pregnancy, <i>n</i>	80/99	33/33	0.025*
Use of vitamin/mineral supplements, <i>n</i>	92/99	24/33	0.002*
Use of Fe supplements, <i>n</i>	41/96	13/33	0.743
Fe supplementation, days	65 ± 70	100 ± 101	0.291

Data expressed as mean ± SD or *n* (*n* is indicative of the number of women with a 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

165 the collected hair strands were used for chemical analysis. All  
 166 women have washed their hair before sampling using usual  
 167 commercial shampoos. It has been shown that the use of dif-  
 168 ferent shampoos does not significantly affect hair mineral con-  
 169 tent [29].

170 The obtained hair samples were washed with acetone and  
 171 rinsed thrice with distilled deionized water (18 MΩ cm) with  
 172 subsequent drying on air at 60 °C till air-dry condition [30].  
 173 The deionized water was obtained by an electric distiller with  
 174 combined membrane set DVS-M/1 HA-1(2)-L  
 175 (Mediana-Filter, Podolsk, Russia). Acetone as a washing  
 176 agent removes mechanical contamination (dirt, dust) but does  
 177 not alter the level of trace elements externally bound to hair  
 178 matrix [31]. After drying, 0.05 g of hair was introduced into  
 179 Teflon tubes containing concentrated nitric acid (HNO<sub>3</sub>)  
 180 (Fluka, Sigma-Aldrich, Co.). Microwave digestion of the sam-  
 181 ples was performed in BerghofSW-4 DAP-40 (Berghof  
 182 Products & Instruments, Germany) system at 170–180 °C  
 183 for 20 min. After cooling the system, the obtained solutions  
 184 were transferred into polypropylene test tubes. The liners were  
 185 rinsed thrice by distilled deionized water, and the rinses trans-  
 186 ferred into the correspondent test tubes. Afterwards, distilled

deionized water was added to the samples to a total volume of 187  
 15 ml and vigorously mixed manually. The obtained solution 188  
 was used for chemical analysis. 189

190 Analysis of hair for trace elements was performed by in-  
 191 ductively coupled plasma mass spectrometry (ICP-MS) at  
 192 NexION 300D (PerkinElmer Inc., Shelton, CT 06484, USA)  
 193 equipped with the 7-port FAST valve and ESI SC DX4  
 194 autosampler (Elemental Scientific Inc., Omaha, NE 68122,  
 195 USA). The use of Dynamic Reaction Cell (DRC) technology  
 196 allowed to remove the majority of interferences. The system  
 197 was calibrated using standard solutions prior to the analysis.  
 198 Briefly, trace element solutions with a final concentration of  
 199 0.5, 5, 10, and 50 ng/l were prepared from Universal Data  
 200 Acquisition Standards Kit (PerkinElmer Inc., Shelton, CT  
 201 06484, USA) by dilution with distilled deionized water and  
 202 acidification with 1% HNO<sub>3</sub>. Internal standards containing  
 203 10 µg/l yttrium-89 and rhodium-103 were used. The standards  
 204 were prepared from Yttrium (Y) Pure Single-Element  
 205 Standard (PerkinElmer Inc., Shelton, CT 06484, USA) and  
 206 Rhodium (Rh) Pure Single-Element Standard (PerkinElmer  
 207 Inc., Shelton, CT 06484, USA) on a matrix containing 8%  
 208 1-butanol (Merck KGaA), 0.8% Triton X-100

209 (Sigma-Aldrich, Co.), 0.02% tetramethylammonium hydrox- 258  
 210 ide (Alfa-Aesar, Ward Hill, MA 01835 USA), and 0.02% 259  
 211 ethylenediaminetetraacetic acid (Sigma-Aldrich, Co). The ob- 260  
 212 tained data on hair mineral content were expressed in micro- 261  
 213 grams per gram dry weight. The obtained levels of essential 262  
 214 and toxic trace elements that were significantly different be- 263  
 215 tween the groups were compared to the existing Russian re- 264  
 216 ference values for adult women [32–34]. 265

217 Laboratory quality control was performed using the certi- 266  
 218 fied reference material (CRM) of human hair GBW09101 267  
 219 from Shanghai Institute of Nuclear Research, Shanghai 268  
 220 (China). Analysis of CRM was performed both before and 269  
 221 after analysis of the obtained hair samples. The recovery rate 270  
 222 for all trace elements analyzed was within 90–110% during all 271  
 223 measurements. 272

224 Statistical treatment of the data obtained was performed by 273  
 225 using Statistica 10.0 (Statsoft, Tulsa, OK, USA). Analysis of 274  
 226 data distribution using Shapiro-Wilk revealed non-Gaussian 275  
 227 distribution for all trace elements studied. After exclusion of 276  
 228 outliers (percentile two-sided) the group median and 25–75 277  
 229 percentile boundaries were calculated. Significance of group 278  
 230 differences was assessed using the Mann-Whitney *U* test. 279  
 231 Multiple regression analysis was used in order to assess the 280  
 232 association of anthropometric and personal characteristics 281  
 233 with hair levels of trace elements that were significantly dif- 282  
 234 ferent between the groups. The level of significance of 283  
 235  $p < 0.05$  was used for all statistical analyses applied. 284

236 **Results**

237 The obtained data demonstrate that IVF-induced pregnancy 290  
 238 was associated with significant variations in hair essential 291  
 239 trace element content (Table 2). In particular, women with 292  
 240 IVF pregnancy had 29, 46, 27, and 24% lower levels of hair 293  
 241 Cu, Fe, Si, and Zn, when compared to the controls. Moreover, 294  
 242 the incidence of low hair Fe content in the IVF-pregnant wom- 295  
 243 en (16 of 33) was significantly higher ( $p < 0.001$ ) than that of 296  
 244 the control group (16 of 99). Similarly, the prevalence of low 297  
 245 hair copper (16 of 33) detected in the IVF group significantly 298  
 246 ( $p = 0.034$ ) exceeded that of the control group (28 of 99). In 299  
 247 contrast, no significant difference in the incidence of Zn defi- 300  
 248 ciency was observed between the groups. At the same time, 25 301  
 249 of 99 women from the control group had high hair Fe content, 302  
 250 being significantly ( $p = 0.050$ ) higher than the rate in 303  
 251 IVF-pregnant women (3 of 33). No significant difference in 304  
 252 the prevalence of high Cu and Zn content in hair was detected 305  
 253 between the groups. 306

254 Significant group differences were also found for hair elec- 307  
 255 trolytes (Table 2). In particular, women with IVF pregnancy 308  
 256 had 30 and 32% lower hair Ca and Mg levels in comparison to 309  
 257 the natural pregnancy group values, respectively. At the same 310

time, hair K levels were on average higher in women with IVF 258  
 pregnancy, although not significantly. 259

260 Similar to essential trace elements and electrolytes, the hair 261  
 262 levels of toxic elements also differed between the study 263  
 264 groups (Table 3). Women with IVF pregnancy were charac- 265  
 266 terized by a significant 33% increase in hair As content in 267  
 268 comparison to the control values. At the same time, the hair 269  
 270 level of Ba in these women was 21% lower than that in wom- 271  
 272 en with natural pregnancy. Despite nearly twofold higher 273  
 274 levels of tin in hair of IVF-pregnant women, the observed 275  
 276 elevation was not significant due to a high variability of the 277  
 278 data. No significant group difference in hair Al, B, Cd, Hg, Ni, 279  
 280 Pb, and Sr was detected. In comparison to the Russian refer- 281  
 282 ence values [32], the prevalence of low (43 of 99 vs 11 of 33, 283  
 284  $p = 0.310$ ) and high (1 of 99 vs 2 of 33,  $p = 0.095$ ) hair As 285  
 286 content was nearly similar in the control and IVF-induced 287  
 288 pregnant women. 289

290 The results of multiple regression analysis demonstrated 291  
 292 that the personal anamnestic and pregnancy characteristics 293  
 294 are related to hair essential trace elements and electrolyte con- 295  
 296 tent (Table 4). In particular, the obtained data demonstrated 297  
 298 that IVF-induced pregnancy is significantly associated with 299  
 299 variations of hair Cu, Fe, and Si content. Hair copper levels 300  
 301 were also significantly associated with the number of preg- 302  
 303 nancies (first pregnancy or not), and the use of vitamin/ 304  
 305 mineral supplements. Surprisingly, neither iron supplementa- 306  
 307 tion nor its duration had a significant impact on hair Fe content 308  
 309 in women with both natural and IVF pregnancy. The results of 309  
 310 multiple regression analysis demonstrated that type of preg- 311  
 pregnancy was not significantly associated with hair Zn content. 312  
 Hair Zn levels were related to morphometric parameters 313  
 (height, weight, and BMI). Despite the presence of significant 314  
 group differences, multiple regression analysis failed to reveal 315  
 any significant effect of the studied parameters on hair calci- 316  
 um content in pregnant women (data not shown). Only 317  
 IVF-induced pregnancy was significantly associated with hair 318  
 As levels out of all the parameters. Hair magnesium levels 319  
 were significantly related to pregnancy planning. Hair Ba 320  
 levels in the pregnant women were not related to the personal 321  
 parameters (data not shown). 322

323 **Discussion**

324 The results demonstrate that women with IVF-induced preg- 325  
 326 nancy are characterized by altered hair trace element and elec- 326  
 327 trolyte content. In particular, women with IVF-induced preg- 327  
 328 nancy had significantly lower levels of essential trace ele- 328  
 329 ments (Cu, Fe, Si, and Zn) and electrolytes (Ca, Mg) in com- 329  
 330 parison to women with natural pregnancy. Surprisingly, hair 330  
 331 Ba, Au, Ga, and Li were also significantly lower in women 331  
 332 with IVF pregnancy in comparison to the control values. In 332



Hair Trace Element and Electrolyte Content in Women

t2.1 **Table 2** Medians and 25–75  
t2.2 percentile boundaries of hair  
essential element content (µg/g)  
t2.3 in women with natural and IVF-  
induced pregnancy

Element	Natural pregnancy		IVF pregnancy		P value	Reference range	References
	Median	25–75 percentile	Median	25–75 percentile			
Ca	2031	1400–3498	1429	902–2406	0.010*	494–1619	[34]
Zn	234	191–295	179	163–246	0.008*	140–315	[33]
P	173	149–199	171	153–178	0.545	135–181	[34]
Mg	155	101–228	105	57–191	0.030*	39–137	[34]
K	138	43–278	191	105–360	0.089	29–159	[34]
Na	86	55–171	102	41–187	0.749	73–331	[34]
Si	37	25–48	27	18–35	0.020*	11–37	[34]
Cu	16.8	11.5–27.3	11.9	9.8–14.9	0.002*	12.1–44.5	[33]
Fe	16.6	10.6–24.9	8.9	7.0–13.2	< 0.001*	8.9–25.6	[33]
Sr	8.2	5.0–12.7	6.4	3.2–10.9	0.141	1.6–15.2	[32]
Mn	1.1	0.7–2.2	0.8	0.5–2.4	0.191	0.3–2.1	[33]
I	0.364	0.265–0.569	0.314	0.201–0.597	0.243	–	
Se	0.356	0.280–0.456	0.381	0.332–0.451	0.552	0.094–0.504	[33]
Cr	0.078	0.05–0.158	0.070	0.047–0.126	0.373	0.060–0.400	[33]
Mo	0.021	0.016–0.026	0.022	0.019–0.027	0.446	–	
Co	0.019	0.011–0.044	0.015	0.007–0.035	0.104	0.011–0.085	[33]
Li	0.009	0.004–0.013	0.011	0.006–0.014	0.306	0.009–0.040	[32]
V	0.008	0.005–0.014	0.007	0.004–0.013	0.393	0.010–0.056	[33]

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

307 contrast, women who underwent IVF had significantly elevated hair levels of As.

308  
309 A previous study involving women following ovarian hyperstimulation demonstrated a significant decrease in iron status, as assessed by serum ferritin [35]. These findings correspond to the earlier data demonstrating the efficiency of dietary non-heme iron intake including iron supplements in reduction of the ovulatory infertility risk [9]. The role of iron supplementation in reducing the risk of adverse pregnancy outcome or infertility may be associated with increased requirements in pregnancy [36]. The results of both group comparisons and multiple regression analysis demonstrated that

IVF-induced pregnancy is significantly interrelated with hair Fe content, whereas other factors including Fe supplementation did not affect the parameter. These findings are indicative of the possible low dietary iron intake in women with IVF pregnancy. The absence of a significant influence of iron supplementation on iron status in the estimated models is at least partially in agreement with the data by Ribot et al. [37] who demonstrated that iron supplementation does not significantly influence the adverse effect of iron deficiency without anemia in early pregnancy [37]. It has been also demonstrated that consumption of vitamin/mineral supplements did not affect serum Fe levels in IVF patients [7].

t3.1 **Table 3** Medians (25–75  
t3.2 percentile) of hair toxic trace  
element levels (µg/g) in women  
t3.3 with natural and IVF-induced  
pregnancy

Element	Natural pregnancy		IVF pregnancy		P value	Reference range [32]
	Median	25–75 percentile	Median	25–75 percentile		
Al	3.9	2.4–6.2	3.7	2.3–5.9	0.670	2.8–10.5
Ba	3.8	2.3–6.2	3.0	1.0–4.4	0.007*	–
Pb	0.362	0.224–0.553	0.317	0.165–0.609	0.446	0.160–0.917
B	0.339	0.257–0.458	0.381	0.282–0.572	0.175	–
Ni	0.299	0.180–0.431	0.211	0.155–0.438	0.200	0.168–0.779
Hg	0.296	0.184–0.436	0.301	0.153–0.493	0.870	0.185–1.094
Sn	0.184	0.083–0.577	0.343	0.083–0.997	0.376	0.082–1.158
As	0.009	0.006–0.014	0.012	0.007–0.026	0.011*	0.008–0.062
Cd	0.009	0.004–0.016	0.008	0.006–0.015	0.427	0.005–0.042

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

t4.1 **Table 4** Multiple regression analysis for the association of anthropometric and personal data of pregnant women and hair trace element and electrolyte content as a dependent variable

t4.2	Element	Cu		Fe		Si		Zn		As		Mg	
t4.3	Parameter	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>
t4.4	Age, years	-0.047	0.652	0.036	0.744	0.127	0.277	-0.028	0.804	-0.119	0.271	0.080	0.470
t4.5	Age at menarche, years	0.074	0.388	0.113	0.209	-0.046	0.621	-0.010	0.914	0.018	0.838	-0.057	0.526
t4.6	Age at first sex, years	0.001	0.992	-0.123	0.208	0.045	0.660	0.050	0.610	-0.060	0.505	-0.122	0.217
t4.7	First pregnancy	0.311	0.002*	-0.008	0.939	-0.015	0.887	0.025	0.804	-0.075	0.438	-0.011	0.916
t4.8	Planned pregnancy	-0.077	0.393	0.109	0.244	0.096	0.320	-0.091	0.335	0.025	0.781	-0.214	0.024*
t4.9	Prepregnancy height, cm	0.171	0.711	-0.054	0.910	-0.169	0.736	-1.065	0.030*	0.091	0.845	0.412	0.402
t4.10	Prepregnancy weight, kg	-0.211	0.875	0.032	0.982	0.552	0.704	-3.185	0.022*	0.128	0.925	-1.281	0.378
t4.11	Prepregnancy BMI	0.354	0.776	-0.178	0.891	-0.529	0.695	-3.037	0.019*	-0.088	0.944	1.160	0.385
t4.12	Pregnancy type	-0.306	0.003*	-0.308	0.005*	-0.268	0.018*	-0.138	0.205	0.405	< 0.001 *	-0.088	0.416
t4.13	Use of V/M supplements	-0.241	0.012*	-0.076	0.446	-0.050	0.625	0.153	0.124	0.078	0.413	0.050	0.616
t4.14	Use of Fe supplements	0.009	0.927	0.004	0.968	-0.015	0.893	-0.130	0.236	-0.135	0.203	-0.022	0.814
t4.15	Days of Fe supplementation	0.109	0.297	0.041	0.711	-0.026	0.822	0.107	0.341	0.033	0.761	-0.080	0.473
t4.16	Multiple R	0.448		0.372		0.258		0.340		0.430		0.324	
t4.17	R <sup>2</sup>	0.201		0.138		0.066		0.116		0.185		0.105	
t4.18	Adjusted R <sup>2</sup>	0.119		0.050		0.029		0.025		0.102		0.013	
t4.19	p for the model	0.007		0.112		0.756		0.242		0.015		0.334	

Data presented as regression coefficient ( $\beta$ ), partial correlation coefficient (PC), and individual *p* value for every association

\*Partial correlation is significant at *p* < 0.05

331 The observed low hair Cu and Zn content in women with  
 332 IVF-induced pregnancy only partially corresponds to the ear-  
 333 lier studies. In particular, pregnant women with a history of  
 334 recurrent spontaneous abortions were found to have signifi-  
 335 cantly lower blood zinc and copper levels in comparison to  
 336 pregnant women without complicated anamnesis. Blood sele-  
 337 nium, lead, and cadmium were increased in comparison to the  
 338 control values [38]. At the same time, women with unex-  
 339 plained infertility had significantly decreased serum Zn levels,  
 340 whereas Cu levels, as well as Cu/Zn ratio were increased in  
 341 comparison to the healthy controls [39]. Another study  
 342 showed distinct patterns of blood trace elements changes in  
 343 pregnant women who underwent intrauterine insemination or  
 344 IVF. In particular, these women had a significant increase in  
 345 transferrin saturation, reduced total iron-binding capacity, and  
 346 serum Se, without any significant difference in serum copper  
 347 levels in comparison to the group of women with natural  
 348 pregnancy [40]. Despite the presence of certain indications  
 349 of the role of Se in female fertility [41], we failed to detect  
 350 any group difference in hair Se content.

351 Magnesium has been shown to play a significant role in a  
 352 variety of physiological functions, including female reproduc-  
 353 tive health [40]. Decreased hair Mg content in IVF-pregnant  
 354 women may be indicative of poor Mg status due to low Mg  
 355 intake in pregnancy [42]. In addition, women undergoing  
 356 ovarian hyperstimulation in IVF demonstrated a significant  
 357 decrease in ionized magnesium due to the influence of estro-  
 358 gens [43].

Moreover, the previous studies indicated that higher blood Zn  
 and Mg concentrations were associated with the increased prob-  
 ability of pregnancy [11]. It is notable that hair Zn content in  
 women undergoing ovarian hyperstimulation was positively as-  
 sociated with the number of oocytes collected, whereas correla-  
 tion between hair Se and the number of follicles and oocytes  
 collected after stimulation was not linear [44]. At the same time,  
 no significant difference between blood and follicular fluid zinc  
 content was revealed in infertile women undergoing IVF be-  
 tween conception and non-conception cycles [45].

Decreased hair Zn content in women with IVF pregnancy  
 may be indicative of poor zinc status due to both increased  
 requirements and low dietary intake [46]. Taking into account  
 the association between maternal zinc deficiency and poor  
 fetal outcome including neural tube defects [47], zinc status  
 in pregnant and especially IVF-pregnant women should be  
 monitored. Moreover, it has been demonstrated that Zn defi-  
 ciency may contribute to adverse health effects of certain toxic  
 substances including alcohol exposure in fetal alcohol spec-  
 trum disorders development [48].

Multiple regression model revealed the absence of a signif-  
 icant association between IVF-induced pregnancy and hair Zn  
 content; anthropometric parameters, including body weight  
 and BMI, were significant predictors. The inverse association  
 between hair Zn and body weight may be related to the bio-  
 logical function of Zn in insulin production [49] and signaling  
 [50]. Correspondingly, earlier studies have demonstrated low-  
 er indices of zinc status in obesity [51, 52].

387 The observation of lower hair levels of Ca in women with  
 388 IVF-induced pregnancy is in agreement with the findings that  
 389 women undergoing IVF treatment were characterized by low-  
 390 er dietary Ca intake [53]. Multiple studies have demonstrated  
 391 the involvement of calcium signaling in the process of in vitro  
 392 fertilization [54]. At the same time, studies aimed at assess-  
 393 ment of Ca status in women undergoing IVF are lacking.  
 394 Hypothetically, low Ca stores in the examinees may be asso-  
 395 ciated with the high prevalence of vitamin D deficiency in  
 396 women using assisted reproductive technologies [6].

397 Multiple studies have demonstrated the association be-  
 398 tween toxic trace element exposure and infertility. In par-  
 399 ticular, exposure to Hg, Pb, and Cd in women undergoing  
 400 ovarian stimulation for IVF was associated with altered  
 401 DNA methylation in whole blood [55]. However, it has  
 402 been demonstrated that Cd, Pb, and Hg in the follicle fluid  
 403 may be not only negatively associated with the outcome of  
 404 in vitro fertilization. In particular, although follicular fluid  
 405 Cd levels were associated with higher risk of embryo  
 406 cleavage and fragmentation, the metal concentration is di-  
 407 rectly related to oocyte fertilization and pregnancy [56].  
 408 Similarly, no association between hair Hg content and  
 409 IVF outcome was found [57]. We also failed to detect  
 410 any significant group difference in hair Hg, Pb, and Cd  
 411 content with respect to the type of pregnancy. Only hair  
 412 As levels were significantly higher in women with IVF  
 413 pregnancy. The observed increase in hair As content in  
 414 IVF-pregnant women is in agreement with the earlier ob-  
 415 servation of elevated urinary As levels in female partici-  
 416 pants of the US-based Study of Metals and Assisted  
 417 Reproductive Technologies [58]. It has been proposed that  
 418 the increase in urinary As in women undergoing IVF may  
 419 be associated with the frequency of sea foods consumption  
 420 [59]. A previous study demonstrated that the level of hair  
 421 As in women undergoing in vitro fertilization directly cor-  
 422 relates with follicular fluid arsenic, lead, and mercury con-  
 423 centrations [60]. Therefore, elevated hair As levels may be  
 424 indicative of the increased risk of reproductive [61, 62] and  
 425 developmental [63, 64] toxicity. Human studies demon-  
 426 strated that increased As exposure during pregnancy may  
 427 be associated with the risk of fetal loss and infant death  
 428 [65]. It is also notable that the Se/As ratio in women who  
 429 underwent IVF was significantly higher as compared to the  
 430 control group, being indicative of the antagonism between  
 431 these metalloids. In turn, it has been demonstrated that hair  
 432 Se/As ratio is characterized by a tighter association with  
 433 population health and demography as compared to hair Se  
 434 and As content separately [66].

435 Interesting data on hair Ba content were obtained, be-  
 436 ing indicative of decreased hair Ba content in women with  
 437 IVF pregnancy. The role of barium in reproductive health  
 438 is contradictory. Certain experimental studies demonstrat-  
 439 ed possible toxic effect of Ba on the reproductive system,

whereas clinical observations of Ba toxicity are inconsis- 440  
 tent [67]. 441

Taking into account antagonistic interactions between cer- 442  
 tain essential and toxic trace elements in the organism [68], the 443  
 observed decrease of essential elements in hair may predis- 444  
 pose the organism to the potentially deleterious effects of toxic 445  
 elements. In addition, the obtained data should be also taken 446  
 into account when planning infant nutrition in order to correct 447  
 deficiencies and prevent possible metal overload [69]. 448

**Conclusion** 449

The obtained data demonstrate an elevated risk of copper, 450  
 iron, zinc, calcium, and magnesium deficiency and arsenic 451  
 overload in women undergoing IVF. These findings allow to 452  
 propose that essential trace element deficiency and toxic trace 453  
 element overload may at least partially contribute to impaired 454  
 fertility in women, resulting in increased requirements for ad- 455  
 vanced reproduction technologies including IVF. Taken to- 456  
 gether, these findings underline the necessity of regular moni- 457  
 toring of micronutrient status in IVF-pregnant women in order 458  
 to prevent potential deleterious effects of altered mineral 459  
 homeostasis. 460

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 current study was approved by the Ethics Committee for Interdisciplinary 466  
 Investigations (Tomsk State University/Psychological Institute of the 467  
 Russian Academy of Education). The study was carried out in agreement 468  
 with the principles of the Declaration of Helsinki and its later amend- 469  
 ments. All women took part in the present investigation on a voluntary 470  
 basis and were informed about the experimental procedures. The in- 471  
 formed consent was signed by all participants before the investigation. 472

**Conflict of Interest** The authors declare that they have no conflict of 473  
 interest. 474

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