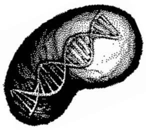

Journal of Microbiology, Biotechnology and Food Sciences

Kňažická et al. 2012 : 2 (2) 414-425

REGULAR ARTICLE

IN VITRO ASSESSMENT OF IRON EFFECT ON THE SPERMATOZOA MOTILITY PARAMETERS

Zuzana Kňažická[1], Jana Lukáčová[1], Eva Tvrdá[1], Agnieszka Greń[2], Zofia Goc[2], Peter Massányi[1], Norbert Lukáč[1]*

Address: [1]Slovak University of Agriculture, Department of Animal Physiology, Tr. Andreja Hlinku 2, 949 76, Nitra, Slovak republic

[2]Pedagogical University, Department of Animal Physiology and Toxicology, Podbrzezie 3, 31 054 Cracow, Poland

*Corresponding author: zuzanaknazicka25@gmail.com

ABSTRACT

Iron (Fe) is an essential element but on the other hand it could induce changes in reproductive system. The general objective of this *in vitro* study was at first to examine dose- and time-dependent effects of iron (ferrous sulphate heptahydrate - $FeSO_4.7H_2O$) on the spermatozoa motility parameters, secondly expand the knowledge concerning direct action of this metal on the fertilization potential of the spermatozoa. The motility analysis was determined after exposure to concentrations of 3.9; 7.8; 15.6; 31.2; 62.5; 125; 250; 500; 1000 $\mu mol.dm^{-3}$ of $FeSO_4.7H_2O$ using the Sperm Vision^{TM} CASA (Computer Assisted Semen Analyzer) system during different time periods (Time 0 h, 2 h and 24 h). The highest percentage of motile spermatozoa was detected in the control group (95.41±1.32%) (Time 0 h). After 2 h of cultivation with ferrous sulphate heptahydrate the motility spermatozoa significantly ($P<0.001$) increased at the concentrations ≤ 125 $\mu mol.dm^{-3}$. The experimental administration at the doses ≥ 125 $\mu mol.dm^{-3}$ $FeSO_4.7H_2O$ inhibited the overall percentage of spermatozoa motility during Time 24 h. The identical spermatozoa motility was detected also for the percentage of progressive motile spermatozoa during all time periods. Detailed evaluation of spermatozoa distance average (DAP) and velocity average (VAP) path as well as amplitude of lateral head displacement (ALH) revealed decrease in groups with

concentrations ≥ 125 μmol.dm^{-3} $FeSO_4.7H_2O$ in comparison with the control group during the long-term cultivation. Based on these results, we can conclude that the iron at the low concentrations maintains the spermatozoa motility parameters. This essential element has probably direct action on the fertilization potential of the spermatozoa, what could be used in assisted reproductive technologies.

Keywords: iron, bovine spermatozoa, motility parameters

INTRODUCTION

Natural environmental factors and differentiated anthropogenic pollutants, as well as many other sources strongly influence the reproductive material located in the semen, both in animals and humans. Chemical elements constitute an important group of ecophysiological influence among these sources **(Fergusson, 1990).**

Iron (Fe) is an important for the organism, because it plays an active part in oxidative/reduction reactions and electron transport associated with cellular respiration. This essential element has crucial role in human body as part of metalo-proteins like haemoglobin or myoglobin, enzymes, neurotransmitters, they are also involved in energetic reactions **(Dorea, 2000).**

Iron and iron compounds are not essentially toxic for animals and human organisms **(Marzec-Wróblewska *et al.,* 2012).** Nevertheless, disturbances in the regulative absorption mechanism can appear due to pathological conditions or prolonged intake of high Fe doses. In these cases Fe is bound in the form of ferric phosphate (haemosiderin) or into proteins, and is distributed in the liver **(Semczuk and Kurpisz, 2006; Kabata-Pendias and Mukherjee, 2007).** Its toxicity may be connected with catalysing many deleterious reactions in cells and tissues **(Reilly, 2004).** High doses of Fe could affect a wide range of mechanisms **(Defrere *et al.,* 2008),** tissue damage **(Reilly, 2004)** or lesion proliferation **(Defrere *et al.,* 2008).** On the other hand, Fe deficiency reduces the activity of iron-containing and iron-dependent enzymes **(Mudron *et al.,* 1996).**

This element plays also a vital role also in fertility and is essential for normal growth and development of the foetus **(Dorea, 2000).** However, at high doses has a harmful consequence on the reproductive system, which can be strongly reflected in the final stage of spermatogenesis associated with pathological disorders **(Carriquiriborde *et al.,* 2004;**

Defrere *et al.*, 2008). High doses of Fe can lead to increased sperm DNA damage **(Perera *et al.*, 2002).**

Currently, there is little information available on impacts of iron on the fertilization potential of the spermatozoa; therefore this study was performed to gain more information in this field. Specifically, we evaluated the dose-dependent effects of this essential metal on the spermatozoa motility parameters during different time periods.

MATERIAL AND METHODS

Semen samples and *in vitro* culture

Bull semen samples were obtained from adult breeding bulls (n=4) (Slovak Biological Services, Nitra, Slovak Republic). The samples had to accomplish the basic criteria given for the corresponding breed. After collecting the samples, they were stored in the laboratory at room temperature (22-25 °C). Each sample was diluted in physiological saline solution (PS) (sodium chloride 0.9% w/v; Bieffe Medital, Grosotto, Italia; pH - 5.5; osmolarily - 301 $mOsmol.kg^{-1}$), using a dilution ratio of 1:40, depending on the original spermatozoa concentration. We used physiological saline solution as culture medium where various concentrations of iron (group I – 3.9; H – 7.8; G – 15.6; F – 31.2; E - 62.5; D - 125; C - 250; B - 500; A - 1000 $\mu mol.dm^{-3}$) were added, in the form of ferrous sulphate heptahydrate ($FeSO_4.7H_2O$; Sigma-Aldrich, St. Louis, USA). The spermatozoa with iron were cultivated in the laboratory at room temperature (22-25 °C). We compared the control group (Ctrl) (medium without $FeSO_4.7H_2O$) with the experimental groups (exposed to different concentrations of $FeSO_4.7H_2O$).

Spermatozoa motility

The motility analysis was carried out using a CASA (Computer Assisted Semen Analyzer) system – SpermVision™ program (MiniTűb, Tiefenbach, Germany) with the Olympus BX 51 microscope (Olympus, Tokyo, Japan) at cultivation Times 0 h, 2 h and 24 h. Each sample was placed into the Makler Counting Chamber (deph 10 µm, Sefi-Medical Instruments, Haifa, Israel) and the following parameters were evaluated: percentage of motile spermatozoa (MOT - %; motility > 5 $\mu m.s^{-1}$); percentage of progressive motile spermatozoa (PROG - %; motility > 20 $\mu m.s^{-1}$); distance average path (DAP; µm); velocity average path

(VAP; μm.s^{-1}) and amplitude of lateral head displacement (ALH; μm). Results of analysis were collected of four repeated experiments at each concentration (n = 32).

Statistical analysis

Obtained data were statistically analyzed with the help of PC program GraphPad Prism 3.02 (GraphPad Software Incorporated, San Diego, California, USA). Descriptive statistical characteristics (mean, minimum, maximum, standard deviation and coefficient of variation) were evaluated. One-way analysis of variance (ANOVA) and the Dunnett´s multiple comparison test were used for statistical evaluations. The level of significance was set at ^{A} (P<0.001); ^{B} (P<0.01); ^{C} (P<0.05).

RESULTS AND DISCUSSION

The effects of different metals depend on the chemical form, length of exposure, dose, duration, route of administration and animal species **(Mathur *et al.,* 2010; Kňažická *et al.,* 2012).** Our experiment shows dose- and time-dependent effects of iron (in the form $FeSO_4.7H_2O$) on the spermatozoa motility parameters (Table 1-5).

Table 1 Spermatozoa motility (MOT; %) exposed to iron ($FeSO_4.7H_2O$) during different time periods

Groups	**Control Ctrl**	**1000 A**	**500 B**	**250 C**	**125 D**	**62.50 E**	**31.20 F**	**15.60 G**	**7.80 H**	**3.90 I**
	$FeSO_4.7H_2O$ (μmol.dm^{-3})									
0 h										
x	95.41	94.25	94.23	94.01^{C}	94.98^{C}	93.61^{A}	93.26^{A}	94.09^{A}	94.54^{A}	94.38^{A}
minimum	93.14	90.29	90.12	90.24	91.01	87.77	88.80	88.31	87.38	90.35
maximum	97.53	97.26	98.01	99.03	96.55	98.42	98.07	97.84	98.51	97.80
S.D.	1.32	1.68	2.26	2.32	1.30	2.45	2.72	2.30	2.37	2.23
CV (%)	1.39	1.79	2.40	2.47	1.36	2.62	2.92	2.44	2.51	2.36
2 h										
x	83.56	75.76	83.35	83.87	87.98^{A}	88.02^{A}	93.97^{A}	90.15^{A}	90.34^{A}	91.96^{A}
minimum	75.75	72.18	71.42	70.76	78.12	79.56	89.01	82.40	88.09	89.04
maximum	93.33	82.22	94.25	94.54	97.50	95.23	97.08	97.75	93.50	94.36
S.D.	6.83	3.55	7.79	8.45	3.83	5.48	2.63	4.39	1.93	1.92
CV (%)	8.17	4.68	9.34	10.08	4.36	6.23	2.80	4.87	2.13	2.08
24 h										
x	35.45	0.00	1.34	32.68	32.98	48.03^{A}	61.65^{A}	70.23^{A}	72.94^{A}	76.82^{A}
minimum	20.00	0.00	0.51	26.82	28.00	36.47	41.86	48.07	57.14	68.00
maximum	48.57	0.00	2.22	37.93	44.89	61.70	76.74	77.50	90.19	83.33
S.D.	5.44	0.00	0.60	4.51	5.17	8.40	12.00	7,52	11.93	4.04
CV (%)	15.36	0.00	44.57	13.80	15.69	17.50	19.47	10.70	16.36	5.25

Legend: x – mean, S.D. – standard deviation, CV (%) – coefficient of variation
$^{A}P<0.001$; $^{B}P<0.01$; $^{C}P<0.05$

Initially (Time 0 h), similar values of percentage of motile spermatozoa were detected in all groups (Table 1). After 2 h of cultivation we proved that the average motility values significantly ($P<0.001$) increased at the concentrations ≤ 125 μmol.dm^{-3} of $FeSO_4.7H_2O$ in comparison with the control group. Other data were not significant in comparison with the control group. The experimental administration at the doses ≥ 125 μmol.dm^{-3} of $FeSO_4.7H_2O$ inhibited the overall percentage of spermatozoa motility during the long-term cultivation (Time 24 h). However, a significant ($P<0.001$) increase of spermatozoa motility at the concentrations ≤ 62.50 μmol.dm^{-3} of $FeSO_4.7H_2O$ was recorded. Identical spermatozoa motility was detected also for the percentage of progressive motile spermatozoa (> 20 μm.s^{-1}) during all time periods (Table 2).

Table 2 Progressive spermatozoa motility (PROG; %) exposed to iron ($FeSO_4.7H_2O$) during different time periods

Groups	Control Ctrl	1000 A	500 B	250 C	125 D	62.50 E	31.20 F	15.60 G	7.80 H	3.90 I
	$FeSO_4.7H_2O$ (µmol.dm^{-3})									
0 h										
x	92.91	91.90	92.13	91.76	92.80	91.80	91.10	92.41	92.84	92.81
minimum	89.44	88.34	87.20	87.61	90.69	86.66	86.31	88.31	89.51	87.50
maximum	96.27	96.26	97.02	96.73	95.34	96.00	96.70	97.84	95.55	96.70
S.D.	1.54	1.95	2.36	2.68	1.53	2.61	3.28	2.44	1.71	2.33
CV (%)	1.66	2.13	2.56	2.92	1.65	2.84	3.60	2.64	1.84	2.51
2 h										
x	80.83	67.96^{A}	80.14	81.57	85.18	85.45	89.41^{C}	87.79^{C}	88.08^{C}	89.97^{C}
minimum	74.35	64.91	62.90	66.15	76.56	75.00	86.81	78.70	83.15	87.50
maximum	89.65	75.55	94.25	92.72	90.90	93.65	91.66	96.62	92.15	91.95
S.D.	5.94	3.80	9.81	9.66	3.43	5.68	1.55	4.89	2.52	1.73
CV (%)	7.35	5.59	12.24	11.85	4.02	6.65	1.73	5.57	2.86	1.93
24 h										
x	27.53	0.00^{A}	1.42^{A}	25.04	25.33	39.11^{A}	59.27^{A}	61.65^{A}	61.97^{A}	67.85^{A}
minimum	21.42	0.00	0.20	19.17	21.73	28.00	40.47	55.26	42.85	49.23
maximum	35.18	0.00	2.12	29.41	29.56	53.70	75.12	68.29	83.01	72.54
S.D.	4.42	0.00	0.69	3.44	2.61	6.94	13.68	4.59	14.73	7.26
CV (%)	16.04	0.00	55.33	13.73	10.32	17.76	23.08	7.45	23.77	10.70

Legend: x – mean, S.D. – standard deviation, CV (%) – coefficient of variation

$^{A}P<0.001$; $^{B}P<0.01$; $^{C}P<0.05$

The distance average path (DAP) analysis revealed no significant differences ($P>0.05$) among experimental groups and the control group at Time 0 h (Table 3). Concentration 500 µmol.dm^{-3} of $FeSO_4.7H_2O$ in short-term periods of cultivation act stimulating on the spermatozoa motility, but later (Time 24 h) significantly ($P<0.001$) inhibiting of selected parameter. Evaluation of velocity average path (VAP) showed increase in all $FeSO_4.7H_2O$ addition groups ($P<0.001$) in comparison with the control group in the Time 0 h. Parameter of VAP detected that spermatozoa exposed to low iron concentrations (≤ 31.20 µmol.dm^{-3}) after 24 h of cultivation ($P<0.001$) are more active as those in control group, but in relation to higher iron concentrations (≥ 250 µmol.dm^{-3} of $FeSO_4.7H_2O$) significant ($P<0.05$) decrease was observed (Table 4). Measurement of the amplitude of lateral displacement (ALH) at Time 2 h was lower in the experimental groups A, B (≥ 500 µmol.dm^{-3} of $FeSO_4.7H_2O$) compared

to the control group, but the differences were not significant (*P*>0.05). The experimental administration at the doses ≤ 62.5 μmol.dm^{-3} of $FeSO_4.7H_2O$ significantly (*P*<0.001) stimulated ALH during Time 24 h (Table 5).

Table 3 Distance average path (DAP; μm) exposed to iron ($FeSO_4.7H_2O$) during different time periods

Groups	**Control** **Ctrl**	**1000** **A**	**500** **B**	**250** **C**	**125** **D**	**62.50** **E**	**31.20** **F**	**15.60** **G**	**7.80** **H**	**3.90** **I**
	$FeSO_4.7H_2O$ (μmol.dm^{-3})									
0 h										
x	35.57	36.95	36.78	36.46	36.96	35.37	35.29	36.31	36.69	36.49
minimum	35.01	30.04	33.46	32.03	33.28	32.49	30.04	33.25	33.42	30.59
maximum	36.86	42.88	41.70	43.39	41.14	39.50	38.55	38.40	41.27	38.59
S.D.	0.63	3.80	2.18	4.12	2.50	2.39	3.16	1.52	1,75	2.03
CV (%)	1.76	10.26	5.92	11.29	6.76	6.75	8.94	4.18	4.77	5.56
2 h										
x	23.82	19.49^{C}	24.02	24.81	27.05	27.43	29.73^{A}	29.30^{A}	29.39^{A}	29.54^{A}
minimum	19.47	15.27	19.03	16.80	25.65	19.68	24.73	20.11	24.01	22.95
maximum	31.88	22.77	30.36	32.74	29.25	32.68	34.69	36.34	35.88	37.83
S.D.	5.47	2.13	4.00	6.87	1.16	4.60	3.24	4.23	4.43	4.65
CV (%)	22.95	10.94	16.65	27.68	4.28	16.77	10.89	14.41	15.08	15.72
24 h										
x	17.36	0.00^{A}	0.32^{A}	12.91^{C}	14.92	20.36	24.18^{A}	26.63^{A}	26.68^{A}	27.13^{A}
minimum	14.41	0.00	0.14	8.65	12.17	13.84	15.92	16.52	19.55	21.07
maximum	22.59	0.00	0.54	15.52	19.25	35.97	30.96	34.91	34.70	37.49
S.D.	1.84	0.00	0.17	1.83	2.21	7.78	5.98	6.26	6.74	4.89
CV (%)	10.60	0.00	53.76	14.15	14.81	38.20	24.74	23.49	25.25	18.02

Legend: x – mean, S.D. – standard deviation, CV (%) – coefficient of variation

$^{A}P<0.001$; $^{B}P<0.01$; $^{C}P<0.05$

Results of this study extended our previous observation on the motility, as well as on viability spermatozoa after metal additions. Previous study showed that the highest iron dose (200 μmol.dm^{-3}) decreased the percentage of spermatozoa motility and progressively decreased progressive of motile spermatozoa in relation to time. However, we found that iron in all tested doses (1 - 200 μmol.dm^{-3}) has not cytotoxic effect on mitochondrial complex, but its potential toxicity could be reflected in the others pathways of cells **(Kňažická *et al.,* 2011).**

In this present study we found that the progressive motility, path distance and velocity as well as amplitude are mostly affected in groups with the highest iron concentrations.

Table 4 Velocity average path (VAP; µm.s^{-1}) exposed to iron ($FeSO_4.7H_2O$) during different time periods.

Groups	**Control Ctrl**	**1000 A**	**500 B**	**250 C**	**125 D**	**62.50 E**	**31.20 F**	**15.60 G**	**7.80 H**	**3.90 I**
					$FeSO_4.7H_2O$ (µmol.dm^{-3})					
0 h										
x	81.89	90.49^{A}	91.53^{A}	92.38^{A}	91.54^{A}	93.98^{A}	99.30^{A}	93.22^{A}	90.56^{A}	91.27^{A}
minimum	78.34	81.39	80.57	80.16	86.51	80.62	89.32	85.28	88.06	83.55
maximum	87.37	102.70	109.30	101.60	97.43	102.30	110.90	105.60	94.65	98.12
S.D.	2.62	7.06	9.56	8.50	3.41	5.69	5.49	5.32	2.19	4.43
CV (%)	3.20	7.81	10.45	9.20	3.73	6.06	5.53	5.71	2.41	4.86
2 h										
x	74.68	44.87^{A}	67.18^{A}	74.24^{A}	76.45^{A}	76.06^{A}	78.06^{A}	85.87^{A}	88.64^{A}	90.12^{A}
minimum	67.15	40.33	51.65	65.15	60.25	66.13	55.92	70.21	80.82	82.42
maximum	85.09	50.90	77.65	81.02	86.48	91.22	96.31	99.19	101.10	95.54
S.D.	4.31	3.53	6.51	4.29	9.05	9.80	13.45	9.46	6.66	3.81
CV (%)	5.78	7.87	9.69	5.78	11.84	12.88	17.23	11.02	7.52	4.23
24 h										
x	36.93	0.00^{C}	0.00^{C}	27.27^{C}	30.93	34.41	49.35^{A}	65.67^{A}	70.75^{A}	75.76^{A}
minimum	30.73	0.00	0.00	18.54	25.52	29.77	32.95	62.00	67.34	70.11
maximum	48.54	0.00	0.00	32.11	37.75	40.62	66.46	69.63	74.85	81.66
S.D.	3.76	0.00	0.00	3.86	3.49	2.20	13.64	2.63	2.72	4.29
CV (%)	10.18	0.00	0.00	14.17	11.28	6.40	27.65	4.00	3.85	5.67

Legend: x – mean, S.D. – standard deviation, CV (%) – coefficient of variation

$^{A}P<0.001$; $^{B}P<0.01$; $^{C}P<0.05$

Excessive doses Fe cause destructive effect on the testicular function and spermatogenesis **(Merker *et al.,* 1996)**, but its physiological level is required for normal spermatozoa production. In general, the semen contains a certain amount of Fe. According to **Gamčík *et al.* (1992)**, bull spermatozoa have approximately 1.26 µmol.dm^{-3} of Fe. **Eghbali *et al.* (2010)** recorded, that the total Fe content of the buffalo seminal plasma was 40.68±0.75 mg.L^{-1}. They came to conclusion, that the Fe content of seminal plasma is important for the preservation of sperm motility and viability after ejaculation, and its presence in the seminal plasma will help spermatozoa to maintain their functions.

Table 5 Amplitude of lateral head displacement (ALH; μm.s^{-1}) exposed to iron ($FeSO_4.7H_2O$) during different time periods.

Groups	**Control Ctrl**	**1000 A**	**500 B**	**250 C**	**125 D**	**62.50 E**	**31.20 F**	**15.60 G**	**7.80 H**	**3.90 I**
	$FeSO_4.7H_2O$ (μmol.dm^{-3})									
0 h										
x	4.46	4.38	3.78^{A}	3.62^{A}	4.04^{A}	4.44	4.48	4.54	4.68	4.65
minimum	4.13	3.85	3.14	3.33	3.60	3.98	3.91	3.98	3.94	4.44
maximum	4.87	5.13	4.25	4.19	4.35	4.81	5.11	5.11	5.37	5.08
S.D.	0.19	0.40	0.36	0.21	0.21	0.28	0.34	0.39	0.36	0.20
CV (%)	4.34	9.07	9.44	5.80	5.27	6.25	7.66	8.52	7.62	4.20
2 h										
x	4.31	3.82	4.16	4.38	4.40	4.70	5.00^{A}	5.03^{A}	5.11^{A}	5.03^{A}
minimum	3.47	3.35	3.38	3.92	3.63	3.34	4.15	3.93	4.58	3.80
maximum	5.47	4.48	5.32	4.68	4.81	6.57	5.86	6.27	6.03	5.72
S.D.	0.78	0.38	0.71	0.21	0.39	1.24	0.61	0.57	0.36	0.57
CV (%)	18.12	10.07	16.99	4.85	8.98	26.44	12.26	11.32	6.94	11.40
24 h										
x	3.19	0.00	1.47	2.81	2.86	3.52^{A}	4.16^{A}	4.14^{A}	4.26^{A}	4.41^{A}
minimum	2.64	0.00	1.24	2.04	2.24	2.82	3.61	3.60	3.32	3.55
maximum	3.66	0.00	1.85	3.34	3.21	4.33	4.94	4.82	5.38	5.14
S.D.	0.27	0.00	0.33	0.41	0.26	0.44	0.45	0.43	0.60	0.46
CV (%)	8.48	0.00	22.55	14.46	8.96	12.43	10.77	10.26	14.12	10.35

Legend: x – mean, S.D. – standard deviation, CV (%) – coefficient of variation
$^{A}P<0.001$; $^{B}P<0.01$; $^{C}P<0.05$

Disproportionate levels of divalent ferrous iron (Fe^{2+}) reduce size of testes **(Lucesoli *et al.,* 1999).** Smaller testes and reduced sperm production may be related to the elevated Fe^{2+} concentrations **(Merker *et al.,* 1996).** Iron overload increases oxidative stress in testes and epididymal sperm causing infertility **(Huang *et al.,* 2001).**

The administration of Fe to rats results in testicular atrophy, morphological changes in the testes, impaired spermatogenesis, epididymal lesions and impaired reproductive performance **(Crawford, 1995; Whittaker *et al.,* 1997).** The mechanism(s) involved in the production of these testicular changes by Fe is not fully understood. Iron accumulation is associated with either acute or chronic Fe overload led to a subtle Fe increase in the testes that was associated with oxidative damage to lipids, proteins and DNA **(Lucesoli and Fraga, 1995; 1999).**

CONCLUSION

The obtained data from this *in vitro* study proved that iron (≤ 250 µmol.dm^{-3} $FeSO_4.7H_2O$) sustains of spermatozoa motility and thus of the energy metabolism, which is a key factor supporting spermatozoa function. Additionally, we found that iron at low concentrations (≤ 62.50 µmol.dm^{-3}) increase the overall of motile spermatozoa, but at high doses acts as a toxic element during the long-term cultivation. Our results point out that iron in acceptable doses has probably direct action on the fertilization potential of the spermatozoa, what could be used in assisted reproductive technologies (IVF, ICSI).

Acknowledgments: This work has been supported by the SPP Foundation (The Scholarship Programme Smart Heads, Slovak republic).

REFERENCES

CARRIQUIRIBORDE, P. - HANDY, R. D. - DAVIES, S. J. 2004. Physiological modulation of iron metabolism in rainbow trout (*Oncorbynchus mykiss*) fed low and high iron diets. In *Journal of Experimental Biology*, vol. 207, 2004, p. 75-86.

CRAWFORD, R. D. 1995. Proposed role for a combination of citric acid and ascorbic acid in the production of dietary iron overload: a fundamental cause of disease. In *Biochemical and Molecular Medicine*, vol. 54, 1995, p. 1–11.

DEFRERE, S. - LOUSSE, J. C. - GONZÁLEZ–RAMOS, R. - COLETTE, S. - DONNEZ, J. - VAN LANGENDONCKT, A. 2008. Potential involvement of iron in the pathogenesis of peritoneal endometriosis. In *Molecular Human Reproduction*, vol. 14, 2008, p. 377–385.

DOREA, J. G. 2000. Iron and copper in human milk. In *Nutrition*, vol. 16, 2000, p. 209-220.

EGHBALI, M. - ALAVI-SHOUSHTARI, S. M. - ASRI-REZAEI, S. – ANSARI, M. H. K. 2010. Effects of the seminal plasma iron and lead content on semen quality of water buffalo *(Bubalus bubalis)* bulls. In *Veterinary Research Forum*, vol. 3, 2010, p. 142-148.

FERGUSSON, J. E. 1990. The Heavy Elements: Chemistry, Environmental Impact and Health Effects. Pergamon Press, England: Oxford, 1990.

GAMČÍK, P. - KOZUMOLÍK, J. - MESÁROŠ, P. - SCHVARC, F. - VLČEK, Z. - ZIBRÍN, M. 1992. *Andrology and artifieial insemination of farm animals (in Slovak).* Bratislava: Príroda, 1992. 299 p.

HUANG, Y. L. - TSENG, W. C. - LIN, T. H. 2001. *In vitro* effects of metal ions (Fe^{2+}, Mn^{2+}, Pb^{2+}) on sperm motility and lipid peroxidation in human semen. In *Journal of Toxicology and Environmental Health*, *Part A,* vol. 62, 2001, p. 259-267.

KABATA-PENDIAS, A. - MUKHERJEE, A. B. 2007. Trace Elements from Soil to Human. Germany: Springer-Verlag, Heidelberg, 2007. 550 p.

KŇAŽICKÁ, Z. – LUKÁČ, N. - GREN, A. – FORMICKI, G. - MASSÁNYI, P. 2012. In vitro effects of copper on the motility and viability of spermatozoa. In *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 1, 2012, no. 6, p. 1529-1539.

KŇAŽICKÁ, Z. – TVRDÁ, E. – MASSANYI, P. - GOC, Z. – KILIAN, K. – SCHNEIDGENOVÁ, M. – FORMICKI, G. – STAWARZ, R. – LUKÁČ, N. 2011. The effects of iron on the motility and viability of spermatozoa *in vitro*. In *Risk Factors of Food Chain 2011* (Proceedings of 11th International Conference) Poland: Iwonicz – Zdroj, 2011, p. 144-156.

LUCESOLI, F. - CALIGIURI, M. - ROBERTI, M. F. - PERAZZO, J. C. - FRAGA, C. C. 1999. Dose dependent increase of oxidative damage in the testes of rats subjected in acute iron overload. In *Archives of Biochemistry and Biophysics,* vol. 372, 1999, p. 37-43.

LUCESOLI, F. - FRAGA, C. G. Oxidative stress in testes of rats subjected to chronic iron intoxication and alpha–tocopherol supplementation. In *Toxicology*, vol. 132, 1999, p. 179–186.

LUCESOLI, F. - FRAGA, C. G. Oxidative damage to lipids and DNA concurrent with decrease of antioxidants in rat testes after acute iron intoxication. In *Archives of Biochemistry and Biophysics*, vol. 316, 1995, p. 567–571.

MARZEC-WRÓBLEWSKA, U. – KAMIŃSKI, P. – ŁAKOTA, P. 2012. Influence of chemical elements on mammalian spermatozoa. In *Folia Biologica*, vol. 58, 2012, p. 7-15.

MATHUR, N. - PANDEY, G. - JAIN, G. C. 2010. Male reproductive toxicity of some selected metals: A review. In *Journal of Biological Sciences,* vol. 10, 2010, p. 396-404.

MERKER, H. J. - VORMANN, J. - GUNTHER, T. 1996. Iron-induced injury of rat testis. In *Andrologia,* vol. 28, 1996, p. 267-273.

MUDRON, P. - BAUMGARTNER, W. - KOVAC, G. - BARTKO, P. - ROSIVAL, I. - ZEZULA, I. 1996. Effects of iron and vitamin E administration on some immunological parameters in pigs. In *Dtsch Tierarztl Wochenschr*, vol. 103, 1996, p. 131-133.

PERERA, D. - PIZZEY, A. - CAMPBELL, A. - KATZ, M. - POTER, J. - PETROU, M. - IRVINE, D. S. - CHATTERJEE, R. 2002. Sperm DNA damage in potentially fertile

homozygous ß-thalassaemia patients with iron overload. In *Human Reproduction,* vol. 17, 2002, p. 1820-1825.

REILLY, C. 2004. The Nutritional Trace Metals. London: Blackwell Publishing, Ltd, Oxford, 2004. 238 p.

SEMCZUK, M. - KURPISZ, M. 2006. The Andrology. Wyd. Lek. PZWL, Warszawa. (in Polish). 2006.

WHITTAKER, P. - DUNKEL, V. C. - BUCCI, T. J. - KUSEWITT, D. F. - THURMAN, J. D. - WARBRITTON, A. - WOLFF, G. L. 1997. Genome–linked toxic responses to dietary iron overload. In *Toxicologic Pathology,* vol. 25, 1997, p. 556–564.