Concentration of biogenic and risk elements in wild boar testes and their interactions with sperm quality

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Abstract

The purpose of this study was to monitor concentration of selected biological and risk elements in testes and later epididymal spermatozoa motility of wild boars (Sus scrofa scrofa) as well as their association. Wild boars were hunted in region Žuhračka - Levice branch plant, Slovak Republic. Testes were surgically removed post-mortem and were transported to the laboratory. Spermatozoa were obtained by dissecting the distal portion of the cauda epididymis and were analysed by Computer assisted semen analyzer (CASA) system. Concentration of elements were measured by inductively-coupled plasma optical emission spectrometry (ICP-OES) and by cold vapor absorption spectrometer (MA-3 Solo Mercury Analyzer). Total spermatozoa motility was at the level of 44.29% and progressive motility was 18.47%. Concentration of elements in testes was in following order: K > Na > Mg > Ca > Fe > Zn > Al > Cu > Se > Mn > As > Cr > Pb > Mo > Sr > Ni > Ba > Cd > Li > Hg. Negative association was observed between Se and motility, progressive motility, further between As and velocity curved line and beat cross frequency. Mercury showed positive correlation with beat cross frequency. Our results suggest that some chemical elements accumulated from polluted environment can affect reproduction of wild animals.

Introduction

The wild boar is one of the most wide-spread wild animals in Slovakia. Based on its excellent adaptation it can inhabit countryside as well as suburbs, therefore it comes into contact with different pollutants [1]. Wild boars are exposed to pollutants mainly by soil and water, due to searching for feed in the soil by digging [2].

Expansion of industry, chemical fertilizers in agriculture and mining affects health of animals but also the human population. Reproductive system serves as a barometer, which can point out on environmental pollution [3]. Chemical elements accumulate in the organs and their amount depend on the extent of the environmental pollution in which the animal lives and on the interval of exposure [3]. Absence of some essential elements negatively affect biological functions; some elements may have a beneficial or conversely toxic effect on animal health [8].

Analysing the presence of heavy metals in wild animals and their relationship to health status is relevant to understand their toxic effects [9–11]. Detrimental effect of heavy metals can cause different health problems, diseases, furthermore, significantly affect male reproductive functions [12]. Spermatozoa, hormone production, fertility, spermatozoa viability is affected by harmful effect of heavy metals, such as cadmium, lead, mercury and arsenic [13, 14]. However, zinc, calcium and magnesium have essential function in male reproduction system such as at spermatogenesis [15], motility and concentration of spermatozoa [16], furthermore selenium [17] and copper [18] also play an important role in male fertility.

The aim of present study was to determine motility parameters of wild boar epididymal spermatozoa, to analyse selected metals in testes, and to find associations of these elements with parameters of wild boar epididymal spermatozoa.

Material And Methods

Experimental Design, Semen Collection and Processing

Sexually matured (n = 26) wild boars (*Sus scrofa scrofa*) were hunted in region Žuhračka - Levice branch plant, Slovak Republic (Fig. 1). Testes were surgically removed post-mortem and following were transported to the laboratory of Institute of Applied biology, SUA in Nitra. Spermatozoa were obtained by dissecting the distal portion of the cauda epididymis. The manual pressure was applied, and spermatozoa were collected to the tube. The acquired spermatozoa were diluted in 5 mL of saline solution (NaCl 0.9% Braun, B. Braun Melsungen AG, Germany) warmed to 37°C prior evaluation motility parameters. Testes were stored at − 20°C until analyses of trace metals.

Motility Analyses

Computer assisted semen analyzer (CASA) method with SpermVision software (Minitube, Tiefenbach, Germany) and a negative phase contrast microscope Olympus BX 51 (Olympus, Japan) with 20x magnification were used for spermatozoa analysis. Samples were placed into Makler Counting Chamber (depth 10 μm, Sefi–Medical Instruments, Germany) [19–20]. Using the boar specific set up, the following parameters were evaluated – total motile spermatozoa (MOT, %), progressive motile spermatozoa (PRO, %), velocity curved line (VCL, μm/s), amplitude of lateral head displacement (ALH, μm), beat cross frequency (BCF, Hz). Within each of the CASA system assessments, seven different fields of view of Makler Counting Chamber were evaluated [21–23].

Detection Of Trace Metals

For all operations high-purity chemicals were used. For analysis of chemical elements wild boar testes samples were kept at − 20°C until analysis. The thawed samples (~5.5 g) were mineralized (wet mineralization) in the high-performance microwave digestion system Ethos UP (Milestone Srl, Sorisole, BG, Italy) in a solution of 5 mL HNO_3 (TraceSELECT®, Honeywell Fluka, Morris Plains, USA) and 1 mL of H_2O_2 (30%, for trace analysis, Merck Suprapur®). Samples, and blank sample, were digested according to established method “animal tissue” set by manufacturer for ensuring the best result. The method consists of 15 minute heating to 200°C, maintaining this temperature for 15 min and further 15 min of active cooling. The digests cooled to 50°C were filtered through the Sartorius filter discs (grade 390) (Sartorius AG, Goettingen, Germany) into the volumetric flask and filled up with double deionized water to a volume of 50 mL [10].

The concentration of chemical elements (Al, As, Ba, Ca, Cd, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Se, Sr, Zn) in wild boar tested were analysed using inductively coupled plasma optical emission spectrophotometer (ICP-OES 720, Agilent Technologies Australia (M) Pty Ltd.). Detections limits (μg/kg) of measured elements were follows: Al 0.2, As 1.50, Ba 0.03, Ca 0.01, Cd 0.05, Cr 0.15, Cu 0.30, Fe 0.10, K

0.3, Li 0.06, Mg 0.01, Mn 0.03, Mo 0.5, Na 0.15, Ni 0.30, Pb 0.80, Se 2.00, Sr 0.01, Zn 0.20. Multielement standard solution V for ICP (Sigma-Aldrich Production GmbH, Switzerland) was used in the experiment. The validity of the whole procedure was checked by processing of duplicate samples against the certified reference material (CRM–ERM CE278K, Sigma-Aldrich Production GmbH, Switzerland) [24].

Total mercury content (Hg) was measured directly in the thawed wild board testes samples using a cold vapor absorption spectrometer MA-3 Solo Mercury Analyzer (Nippon Instruments Corporation, Bukit Batok, Singapore) in ca. 100 mg wet weight of each sample (with two repetitions). The limit of quantification for Hg was 0.02 µg/kg [25].

Statistical Analyses

For the analysis the GraphPad 9 software (GraphPad Software Inc., San Diego, CA, USA) was used. Descriptive statistical characteristics of mean, standard deviation (SD), minimum (Min) and maximum (Max) was selected for statistical evaluations. All obtained results were tested for normal Gaussian distribution using a D'Agostino–Pearson normality test and Shapiro–Wilk normality test. Furthermore, the relationship between spermatozoa quality parameters and chemical elements was evaluated using Pearson correlation. Significance was determined at $p < 0.05$ (a) and at $p < 0.01$ (A). Heatmaps with clustering were performed to visualize interactions (Pearson correlations coefficients-r) of spermatozoa quality parameters and chemical elements.

Results

Spermatozoa Quality of Wild Boars

Total spermatozoa motility after obtaining spermatozoa from epididymis was at the level of 44.29%, however the range was from 15.88–81.50%. Progressive motility of wild board spermatozoa was 18.47%. Like as in total motility, progressive motility reported wide range (2.24–53.16%). Spermatozoa velocity curved line (VCL) was at 64.07 µm/s. Amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) exhibited values 2.70 µm and 23.84 Hz respectively (Table 1).

Table 1
Wild boar spermatozoa parameters.

Parameter	Mean	SD	Min	Max
MOT (%)	44.29	18.18	15.88	81.50
PRO (%)	18.47	14.09	2.24	53.16
VCL (µm/s)	64.07	17.19	37.89	96.59
ALH (µm)	2.70	0.44	1.81	3.74
BCF (Hz)	23.84	2.84	16.55	30.06

MOT - motility, PRO - progressive motility, VCL - velocity curved line, ALH - amplitude of lateral head displacement, BCF - beat cross frequency.

Concentration Of Elements In Wild Boar Testes

The weight of the testicles obtained from wild boars ranged from 327 g to 924 g, mean weight was 641 g. The general scheme of analysed trace elements concentrations is mentioned in Table 2. Concentration of elements was in following order: K > Na > Mg > Ca > Fe > Zn > Al > Cu > Se > Mn > As > Cr > Pb > Mo > Sr > Ni > Ba > Cd > Li > Hg. The highest measured value in the testicles of wild boars was K (2.45 g/kg). On the contrary, the lowest measured value was for Hg (1.23 µg/kg).

Table 2
Selected elements detected in wild boar testes.

	Mean	SD	Min	Max
Al (mg/kg)	2.07	1.91	0.24	7.95
Ca (mg/kg)	38.06	2.49	32.75	43.28
Cr (µg/kg)	106.50	70.21	53.60	322.30
Cu (mg/kg)	1.47	0.24	0.97	1.80
Fe (mg/kg)	28.01	6.41	17.70	45.29
K (g/kg)	2.45	0.08	2.28	2.59
Li (µg/kg)	7.27	8.39	1.40	46.30
Mg (mg/kg)	128.30	3.25	120.60	133.80
Mn (mg/kg)	0.40	0.07	0.28	0.57
Se (mg/kg)	0.81	0.22	0.42	1.29
Sr (µg/kg)	25.49	9.02	11.00	61.80
Zn (mg/kg)	12.72	0.89	11.46	14.95
Na (mg/kg)	746.30	52.48	637.00	828.50
As (µg/kg)	219.20	272.70	1.50	739.30
Ba (µg/kg)	7.52	11.29	0.03	49.70
Cd (µg/kg)	7.50	12.87	0.05	50.00
Mo (µg/kg)	30.26	51.65	0.50	155.70
Ni (µg/kg)	24.31	43.26	0.30	146.10
Pb (µg/kg)	34.99	62.92	0.80	225.80
Hg (µg/kg)	1.23	0.85	0.36	3.64

Correlation Analyses

Results of correlation analysis between motility parameters and trace elements showed significant ($p < 0.01$) negative correlation between Se and MOT, furthermore significant correlation ($p < 0.05$) between Se and PRO. The negative correlation between As and VCL, respectively between As and VCL contended a significance at level $p < 0.05$. Relationship between BCF and Hg showed significant positive correlation (p

< 0.01). Results of mutual relations among motility chemical elements and motility parameters are displayed in Fig. 2.

Discussion

Several studies focused on post mortem epididymal spermatozoa quality in different animal species such as boar [26], bull [27], canine [28] and stallion [29]. Important role plays time of spermatozoa analyses post mortem. Martinez-Pastor et al. (2005) observed, that optimal time is in the range of 24 hours post mortem as it was realized in our study.

The data obtained in the present study on epididymal boar spermatozoa show slightly lower values of MOT, PRO and VCL compared which was observed by Soriano-Úbeda et al. [31] who used PBS for dilution of epididymal spermatozoa. The epididymal spermatozoa obtained from boars in study of Ydiaquez-Miranda et al. [32] showed fertilizing ability up to 72 hours during storage at 5°C. Although they recorded higher sperm motility as in our study, their analyses were performed only by light microscope.

Wild animals generally show a higher accumulation of contaminants than animals living at farms, it follows that they may be barometers of environmental pollution. The accumulation of heavy metals in wildlife animals body affect fertility, spermatogenesis and last but not least, the offspring [33].

Selenium as essential trace element, which plays an important role in the normal growth and development of humans and animals can be also toxic in several ways such as in the formation of superoxide (O_2^-) [34]. In the present study, results of MOT and PRO were affected by Se, what correlate with study of Sengupta [35], who described importance of Se in male reproduction. Deficiency of Se caused loss of spermatozoa quality, furthermore Se had toxic effect on the testes. Pilarczyk et al. [36] observed 1.14 µg/g in kidney and 0.2 µg/g of Se in liver from north-western Poland wild boars, whose results are comparable with ours in concentration of Se in testes (0.81 mg/kg). One of the sources of Se is in the body of wild boars can be soil. Previous studies observed that concentration of Se in Slovak soils is in the range 0.18–0.308 mg/kg [37–38].

Another form of As transfer to wildlife animals body is water, furthermore soil. European Union recommends arsenic limit (20 mg/kg) for agriculture soil. Parameters of wild boars' spermatozoa VCL and BCF correlated with As concentration in present study, what agrees with the results, where a correlation between As and motility of mice spermatozoa was also noted [39]. Previous study from area of Slovak Republic observed lower values of As in wild boars liver, kidney and muscles as in our study, however our results of As are under hygienic limit in comparison for kidney as well as for liver but slightly increased for hygienic limit for muscles [40].

Other of the heavy metals that affect male reproduction system and spermatozoa motility is Hg. As it was described Hg (50–300 µM) negatively affected bovine spermatozoa membrane, DNA integrity and viability [41]. Wistar rats exposed to Hg intraperitoneally in doses 5, 10 and 20 mg/kg of body weight

reported structural and functional changes on kidney and testes [42]. Compared to studies of wild boards from other countries, we observed lower values of Hg, however in kidney as well as in muscle [43].

Conclusion

In conclusion, obtained results show that concentrations of selected chemical elements from environmental pollution in testes affected spermatozoa motility parameters of wild boars. Elements, such as Se and As show negative correlation with wild boars spermatozoa parameters (MOT, PRO, respectively VCL and BCF), while Hg negatively correlated with BCF.

Declarations

Author Contribution We declare that each author contributed to the article: M. H. Jr, D. S., F. T., M. M., M. A., M. M., L. D. contributed to processing of the samples in the laboratory, analysed the data and prepared the manuscript, M. H. Jr. and D. S. contributed to the samples collection, J. Z., L. J. B., R. S. and P. M. contributed to design of the study and revision of manuscript.

Data Availability During present study, all the generated datasets and analysed data are available from the corresponding author on reason- able request.

Ethics Approval The authors followed all the valid national rules for the use and care of animals.

Consent for Publication All authors consented to the publication.

Competing Interests The authors declare no competing interests.

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Figures

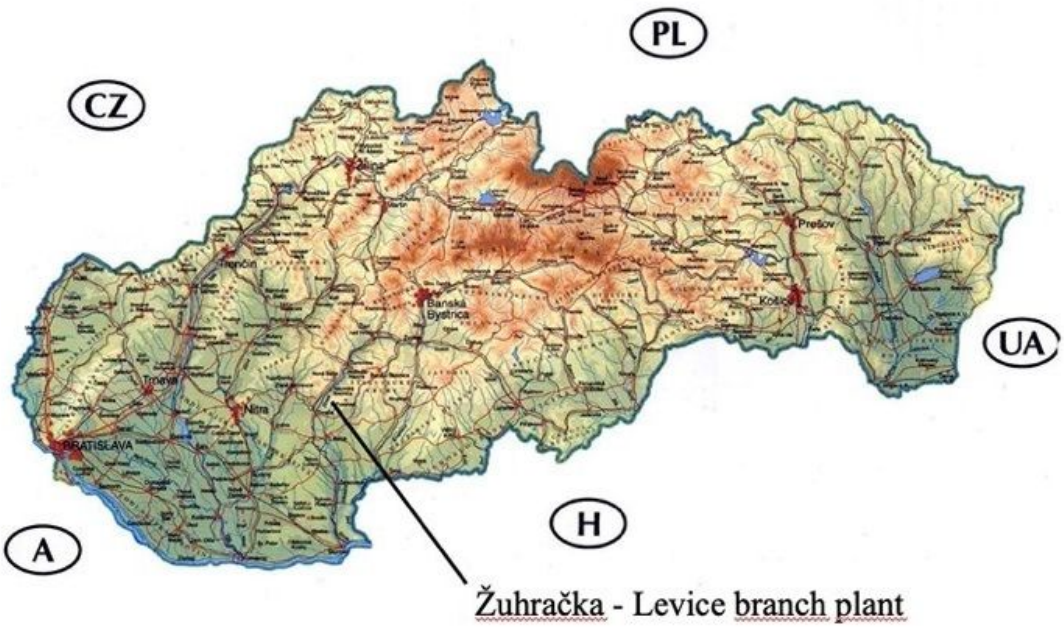

Figure 1

Region Žuhračka - Levice branch plant, Slovak Republic.

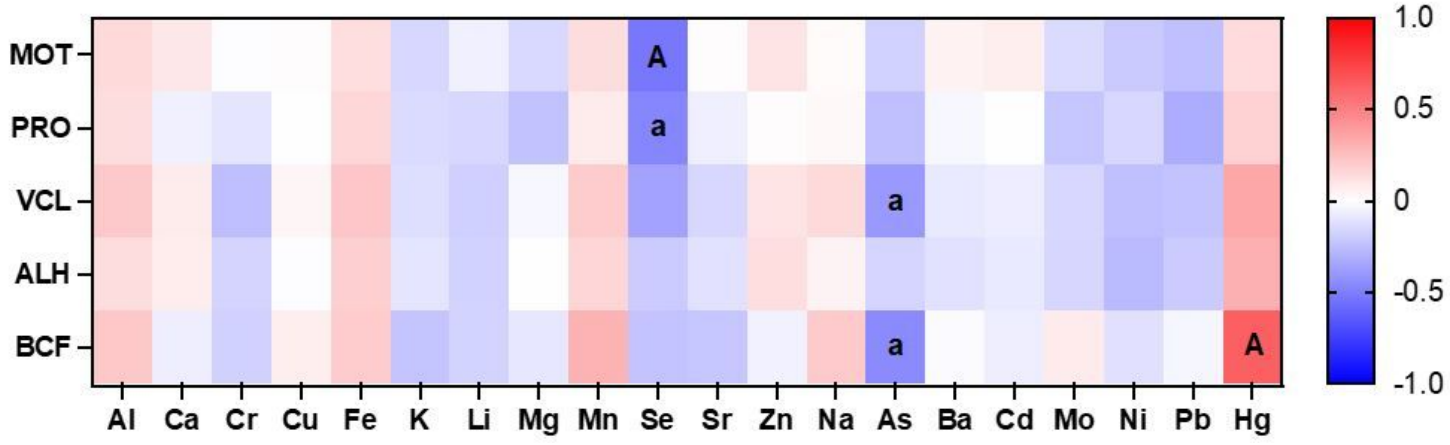

Figure 2

Correlations: spermatozoa parameters vs. chemical elements. a - significant at $p < 0.05$; A - significant at $p < 0.01$.