Seminal concentrations of nickel in various animals and correlation to spermatozoa quality

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Abstract

In this study the analysis of nickel in animal semen (stallion, bull, ram, boar, fox) and its relation to spermatozoa quality was analyzed. The analysis of nickel showed that the concentration of this element in stallion semen 0.20 $mg.kg^{-1}$, in bull 0.12 $mg.kg^{-1}$, in ram 0.31 $mg.kg^{-1}$, in boar 0.06 $mg.kg^{-1}$ and in fox 0.36 $mg.kg^{-1}$. Seminal nickel concentration was significantly higher ($P<0.05$) in foxes than that in bulls and significantly higher ($P<0.01$) in rams and foxes in comparison with in boars.
In stallion semen we determined 17.09±3.66% of total pathological spermatozoa, with the dominancy of knob twisted flagellum, separated flagellum and flagellum torso. In bulls we found 11.80±4.88% of total pathological spermatozoa, with the dominancy of separated flagellum, flagellum torso and knob twisted flagellum. In ram semen was occurrence of pathological spermatozoa 17.17±3.76%, and separated flagellum, flagellum torso, knob twisted flagellum were the most frequent forms of pathological spermatozoa. The total percentage of pathological spermatozoa was 9.82±1.47% in boar. From all observed pathological spermatozoa evaluated in boars the highest numbers were with separated flagellum, flagellum torso and other pathological spermatozoa. The total percentage of pathological spermatozoa was 7.75±1.33% in fox. From this total number the most frequent changes were knob twisted flagellum, separated flagellum and broken flagellum.
Correlation analysis in bulls indicated a high positive correlation between seminal nickel and separated flagellum ($r=0.76$) and medium positive correlation between nickel and flagellum torso ($r=0.62$), in rams high positive correlation between nickel and separated flagellum ($r=0.77$). In relation to studied nickel medium positive correlation was found between nickel and separated flagellum ($r=0.43$), nickel and other pathological spermatozoa ($r=0.45$) in boars.

Introduction

Development in industry and agriculture produce an infiltration of elements in the food chain. This also promotes the uneven distribution of essential elements in the animal body and changes their interactions. The amount of an element which accumulates in the organs depends on the interval of exposure, the quantity ingested, the production and reproduction phases of the animals, as well as their age and breed. Element toxicity upon the biological systems of animals is affected by the route and form of ingestion as well as by the interaction between essential and toxic elements (Bires et al., 1997).

Some metals are essential for life, others have unknown biological functions, either favorable or toxic, and some others have the potential to produce disease. Those causing toxicity are the ones, which accumulate in the body through the food chain, water and air.
Many recent studies have indicated an increasing prevalence of various abnormalities of the reproductive system in animals as well as human males. There is growing concern about the considerable decrease in sperm density over the last 50 years in general populations worldwide, especially in the United States and in Europe. The data reporting the metal concentration are mainly related to human semen and less in animal (stallion, bull, coypu) semen (Sorensen et al., 1999). Generally, studies reporting effect of various metals on spermatogenesis in nontoxic environment that may influence individual susceptibility to adverse effects are lacking. Toxic insults to the testes can result in a multiplicity of effects, namely, reduced spermatozoa concentrations and the production of defective spermatozoa, as well as impaired andogen production and its consequence. The most obvious manifestation of testicular toxicity is reduced fertility (Cigankova et al., 1994, 1998).
High quantity of nickel is known to be injurious for animal and human health. Its effects on various aspects of reproduction have been described. Animal studies refer that nickel has negative effects on the structure and function of testis, seminal vesicle, and prostate gland, and there is similar report of adverse effect on spermatozoa (Pandey and Srivastava, 2000; Forgacs et al., 2001).
The purpose of this study was to determine nickel concentration in the semen of bull, ram, boar, stallion and fox used for artificial insemination and to analyze the occurrence of pathological spermatozoa and to find relations between nickel and the spermatozoa quality.

Material and methods

Animals and semen samples
Semen samples were obtained from adult bulls (n=200), rams (n=100), boars (n=20), stallions (n=10), and foxes (n=10). Semen was processed at the animal breeding station (State Breeding Institute, Nitra, Slovak Republic) to frozen-thawed pellets (bulls, rams, foxes), frozen-thawed insemination tubes (stallion) and in natural status (boars).

Semen analysis
Semen samples were digested in a microwave oven (MLS-1200 MEGA, Milestone, USA) using 5 ml HNO_3 and 1 ml HCl.g^{-1} sample. The digested samples were analyzed for nickel by means of an atomic absorption spectrophotometer (Unicam Solar 939, USA). The flame conditions were those recommended by the instrument manufacturer for nickel (wavelength 232.0 nm, band pass 0.5 nm). The quantification limits for nickel were 0.22 µg.l^{-1} and the detection limits 0.065 mg.l^{-1}. Analyzing reference materials (MBH Anal. Ltd., UK) were employed to test the reproducibility of the method. The graphite furnaces were optimized for maximum absorbancy and linear response while aspirating known standards. The standard was prepared from the individual 1.000 mg.kg^{-1} standard (Merck, Germany) and 100 ml of five combined standards were prepared in 0.1 mol.l^{-1} HNO_3. The lamp current used was 75%. The measurement time was 3 sec. The recovery of the methods was 69%~98% and reproducibility was higher than 1.0%. The concentration of nickel was expressed in mg.kg^{-1} wet weight of the tissue.

Analysis of pathological spermatozoa
For analysis of pathological spermatozoa semen samples fixed with Hancock's solution and stained with Giemsa were prepared. All slides were analyzed at the magnification 500 x. For each animal at least 1000 spermatozoa were evaluated and the percentage of pathological spermatozoa was recorded. These pathological changes were classified: knob twisted flagellum, separated flagellum, flagellum torso, broken flagellum, retention of cytoplasmic drop, acrosomal changes, large heads, small heads, flagellum ball, and other pathological spermatozoa (teratoid spermatozoa; a spiral twisted flagellum; deformation of the mitochondrial part; acrosomal changes; others).

Statistical analysis
Data were expressed in mean±SD and analyzed statistically with Student`s t-test, Scheffe`s test and Pearson`s rank using PC program SAS and Excel.

Results and discussion

The analysis of nickel showed that the concentration of this element in stallion was 0.20 $mg.kg^{-1}$, in bull was 0.12 $mg.kg^{-1}$, in ram was 0.31 $mg.kg^{-1}$, in boar semen was 0.06 $mg.kg^{-1}$, and in fox was 0.36 $mg.kg^{-1}$. All the values obtained are listed in Table 1.
Seminal nickel concentration was significantly higher ($P<0.05$) in foxes (0.36±0.24 $mg.kg^{-1}$) than that in bulls (0.12±0.07 $mg.kg^{-1}$), and significantly higher ($P<0.01$) in rams and foxes than that in boars.

Table 1.
Concentration of nickel in animal semen

Concentrations of nickel in semen ($mg.kg^{-1}$)				
	mean	sd	minimum	maximum
stallion	0.20	0.24	0.03	0.61
bull	0.12	0.07	0.03	0.28
ram	0.31	0.19	0.04	0.65
boar	**0.06**	0.08	0.00	0.28
fox	0.36	0.24	0.05	0.83

Significant difference: ram vs boar; fox vs boar.

In stallion semen we determined 17.09 ± 3.66% of total pathological spermatozoa, with the dominancy of knob twisted flagellum (4.93%), separated flagellum (4.71%), and flagellum torso (1.60%). In bull semen we found 11.80 ± 4.88% of total pathological spermatozoa, with the dominancy of separated flagellum (3.85%), flagellum torso (2.83%), and knob twisted flagellum (2.04%). In ram semen was occurrence of pathological spermatozoa 17.17 ± 3.76%, and separated flagellum (9.28%), flagellum torso (3.27%), and knob twisted flagellum (1.89%) were the most frequent forms of pathological spermatozoa. The total percentage of pathological spermatozoa was 9.82 ± 1.47% in boar. From this total number 3.18% had separated flagellum, 2.26% knob twisted flagellum, 0.88% flagellum torso, 0.85%

flagellum ball, and 1.83% other forms of pathological changes. From all observed pathological spermatozoa evaluated in boars the highest numbers were with separated flagellum (32.38%), flagellum torso (23.01%), and other pathological spermatozoa 18.64 ±10.54%. The total percentage of pathological spermatozoa was 7.75±1.33% in fox. From this total number the most frequent changes were 3.22% had knob twisted flagellum, 1.99% separated flagellum, 0.33% broken flagellum, 0.30% flagellum torso, and 1.02% other forms of pathological changes (teratoid, spermatozoa, a spiral twisted flagellum, deformation of the mitochondrial part, acrosomal changes, others). Correlation analysis in bulls indicated a high positive correlation between seminal nickel and separated flagellum (r=0.76) and medium positive correlation between nickel and flagellum torso (r=0.62), in rams high positive correlation between nickel and separated flagellum (r=0.77).In relation to studied nickel medium positive correlation was found between nickel and separated flagellum (r=0.43), nickel and other pathological spermatozoa (r=0.45) in boars. Other correlation were low in the range of 0.1-0.33.

Table 2.
Occurrence of pathological spermatozoa (%) in relation to all studied spermatozoa in stallions

Pathological change	**mean**	SD	Min	Max
Total number	**17,09**	1,47	6,70	11,80
Knob-twisted flagellum	**4,93**	1,66	2,77	7,80
Separated flagellum	**4,71**	3,24	1,21	10,58
Flagellum torso	**1,60**	0,55	0,65	2,25
Broken flagellum	**0,87**	0,55	0,28	1,62
Retention of cytoplasmic drop	**0,78**	0,34	0,37	1,36
Acrosomal changes	**0,74**	0,59	0,18	1,72
Large head	**0,67**	0,44	0,19	1,38
Small head	**0,51**	0,48	0,00	1,45
Flagellum ball	**0,34**	0,21	0,10	0,73
Other pathological speratozoa	**1,94**	0,96	0,71	3,23

Table 3.
Occurrence of pathological spermatozoa (%) in relation to all studied spermatozoa in bulls

pathological change	**mean**	SD	Min	Max
Total number	**11,80**	4,88	4,60	21,70
Knob-twisted flagellum	**2,04**	1,26	0,50	4,60
Separated flagellum	**3,85**	2,25	0,80	9,60
Flagellum torso	**2,83**	1,57	0,40	5,80
Broken flagellum	**0,59**	0,45	0,20	1,90
Retention of cytoplasmic drop	**0,19**	0,24	0,00	0,60
Flagellum ball	**0,47**	0,43	0,00	1,40
Other pathological speratozoa	**1,83**	1,02	0,30	3,70

Table 4.
Occurrence of pathological spermatozoa (%) in relation to all studied spermatozoa in rams

pathological change	**mean**	SD	Min	Max
Total number	**17,17**	3,76	12,30	21,30
Knob-twisted flagellum	**1,89**	1,33	0,40	5,10
Separated flagellum	**9,28**	3,33	5,60	14,10
Flagellum torso	**3,27**	1,36	0,80	4,80
Broken flagellum	**0,60**	0,24	0,30	1,10
Retention of cytoplasmic drop	**0,24**	0,21	0,00	0,70
Flagellum ball	**0,46**	0,26	0,20	1,10
Other pathological spermatozoa	**1,43**	0,73	0,50	2,80

Table 5.
Occurrence of pathological spermatozoa (%) in relation to all studied spermatozoa in boars

pathological change	**mean**	SD	Min	Max
Total number	**9,82**	1,47	6,70	11,80
Knob-twisted flagellum	**2,26**	0,52	1,40	3,40
Separated flagellum	**3,18**	0,68	2,00	4,60
Flagellum torso	**0,88**	0,32	0,40	1,50
Broken flagellum	**0,42**	0,35	0,00	1,70
Retention of cytoplasmic drop	**0,23**	0,34	0,00	1,40
Large head	**0,03**	0,07	0,00	0,20
Small head	**0,14**	0,29	0,00	1,30
Flagellum ball	**0,85**	0,36	0,30	1,80
Other pathological speratozoa	**1,83**	1,02	0,30	3,70

Table 6.
Occurrence of pathological spermatozoa (%) in relation to all studied spermatozoa in foxes

pathological change	**mean**	SD	Min	Max
Total number	**7,75**	1,33	5,47	9,42
Knob-twisted flagellum	**3,22**	1,01	2,08	4,57
Separated flagellum	**1,99**	0,82	0,86	3,12
Flagellum torso	**0,30**	0,18	0,08	0,66
Broken flagellum	**0,33**	0,18	0,08	0,66
Retention of cytoplasmic drop	**0,28**	0,17	0,10	0,57
Acrosomal changes	**0,21**	0,25	0,00	0,66
Large head	**0,09**	0,11	0,00	0,28
Small head	**0,01**	0,03	0,00	0,09
Flagellum ball	**0,30**	0,22	0,10	0,67
Other pathological speratozoa	**1,02**	0,48	0,59	1,70

Early studies on nickel essentiality with rats and goats indicated that nickel deprivation impaired reproductive performance. Nickel also has been found to influence cyclic nucleotide gated channels (CNG) – which are important in sperm physiology. Nickel deprivation significantly decrease spermatozoa motility and density in the epididymides, epididymal transit time of spermatozoa, and testes sperm production rate. Nickel deficiency also significantly decreased the weight of the seminal vesicles and prostate glands (Xu et al., 2003). Treatment of rats with cadmium cause a significant decrease in sperm concentration, motility, weight of testes and epididymis, and increase the number of dead and abnormal sperm (Hunt et al., 1992; Mertin et al., 1992).
In comparison with other animals lower copper concentration are reported in stallion, bull, boar semen and higher in rams semen. In bull and ram semen higher level of iron in comparison with fox semen was found. Fox semen is also characteristic with very low zinc concentration. The level of cadmium and lead in all studied animals show any significant differences, but on the other hand there is a high nickel concentration in fox semen in comparison with stallion, bull and boar semen (Massányi et al., 2000, 2003).

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