

SEMINAL CONCENTRATIONS AND RELATIONSHIPS OF TRACE ELEMENTS IN ANIMAL SEMEN

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ABSTRACT

The copper concentration is significantly higher in rams (2.49 ± 0.18 mg/kg wet weight) and foxes (2.16 ± 0.53 mg/kg w.w.) in comparison with bulls (1.64 ± 0.21 mg/kg w.w.), boars (1.64 ± 0.28 mg/kg w.w.) and stallions (0.86 mg/kg w.w.). In boar semen significantly higher zinc concentration (171.74 ± 65.72 mg/kg w.w.) in comparison with stallion (86.20 ± 45.88 mg/kg w.w.), bull (83.15 ± 61.61 mg/kg w.w.), ram (60.46 ± 35.37 mg/kg w.w.) and fox semen (13.09 mg/kg w.w.) was found. Iron concentration in semen is higher in rams (40.32 ± 10.81 mg/kg w.w.), bulls (38.04 ± 22.07 mg/kg w.w.) and foxes (33.16 ± 24.36 mg/kg w.w.) and significantly lower in boar (16.14 ± 10.35 mg/kg w.w.) and stallion (12.68 mg/kg w.w.) semen. Concentration of cadmium is in all studied species relatively low ($0.05 - 0.12$ mg/kg w.w.). The highest lead concentration in ram semen (0.35 ± 0.68 mg/kg w.w.) is reported. The level of lead is in fox semen 0.08 ± 0.06 mg/kg w.w., in bull semen 0.06 ± 0.04 mg/kg w.w., in stallions 0.05 ± 0.05 mg/kg w.w. and in boar semen 0.02 ± 0.03 mg/kg w.w. The level of nickel is significantly higher in fox (0.35 ± 0.24 mg/kg w.w.), and ram (0.31 ± 0.19 mg/kg w.w.) semen in comparison with bull (0.12 ± 0.07 mg/kg w.w.) and boar semen (0.06 ± 0.08 mg/kg w.w.). The concentration of nickel in the semen of stallions is 0.20 ± 0.24 mg/kg w.w. Correlation analysis determined high positive relation between iron and zinc in bull ($r=0.723$) and stallion ($r=0.723$) semen, between cadmium and lead in ram ($r=0.976$) and boar ($r=0.973$) semen and between iron and cadmium ($r=0.783$) and iron and lead ($r=0.791$) in boar semen. High negative correlation between nickel and copper in ram ($r=-0.709$) and between copper and lead in fox semen (-0.854) was found.

INTRODUCTION

Toxic effects of various factors on testes result in a multiplicity of effects as reduced spermatozoa concentration, production of defective spermatozoa or impaired androgen production [1].

Essential trace elements, zinc and copper, are components of many important enzymes. Both elements are involved in carbohydrate or lipid metabolism and in immune functions [2]. Copper has a multilateral function in the organism – is important in iron absorption, effects the haemopoiesis and activates ferments. Copper is involved in biochemical reactions even at the cellular level, especially in the oxidation – reduction processes [3]. Zinc is an essential element for domestic animals. Zinc deficiency results in disorders of testes development and course of spermatogenesis [4,5].

Lead is a heavy metal distributed into environment, natural and antropogenic sources. It has unknown essential role in organism and its accumulation in tissues may cause several health hazards including neurotoxicity, hematotoxicity and reproductive disturbances [6]. Abnormal sperm chromatin structure is not related to blood lead concentration, but some indications of deterioration of sperm chromatin was found in men with the highest concentrations of lead within spermatozoa. Biological monitoring data did not indicate long term effects of lead on semen quantity or sperm chromatin [7]. Exposure to cadmium, via air and food, leads to renal tubular dysfunction. Cadmium has various effects on reproduction, causing follicular atresia in ovary [8], edematization of uterus [9] as well as degenerative alterations in testes [10].

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 may include that nickel reaches the testis, seminal vesicle and prostate gland [11,12,13], and there is similar report of adverse effect on spermatozoa [14].

In adult goats inductively coupled plasma spectroscopy showed the presence of copper, calcium, nickel, iron, magnesium, chromium, titanium and zinc in epididymal lumen, with fluctuating levels at different sites along the length of the epididymis. Cadmium, cobalt, lead and manganese were not found [15].

The purpose of this study was to determine copper, zinc, iron, cadmium, lead and nickel concentration in the semen of bulls, rams, boars, stallions and foxes used for artificial insemination and to find relation between these elements.

MATERIALS AND METHODS

All semen was from adult bulls (number of samples – 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 (stallions) and in natural status (boars).

The samples of semen were digested in the microwave oven MLS – 1200 MEGA (Milestone) using 5 ml HNO₃ and 1 ml HCl per 1g of sample. The program of digestion: 1st step – 250W, 5 minutes; 2nd step – 400W, 5 minutes; 3rd step – 500W, 5 minutes and 4th step – 600W, 5 minutes.

The digested samples were analysed for the presence of copper, zinc, iron, cadmium, lead and nickel by using an atomic absorption spectrophotometer (AAS), Unicam Solar 939. The flame conditions were those recommended by the instrument manufacturer for copper, zinc iron, cadmium, lead and nickel (wavelength 324.8, 213.0, 248.3, 228.8, 283.3 and 232.0 nm respectively, band pass 0.5 nm). The quantification limit copper, zinc and iron were 0.096, 0.13 and 0.12 mg/l respectively and the detection limits for copper, zinc and iron were 0.29, 0.0036 and 0.039 mg/l respectively. The quantification limits for cadmium, lead and nickel were 0.03, 0.27 and 0.22 µg/l respectively. The detection limits for cadmium, lead and nickel were 0.01, 0.08 and 0.065 mg/l respectively. The graphite furnace was optimized for maximum absorbency and linear response while aspirating known standards.

Analyzing reference materials (MBH Anal. Ltd., UK) tested the reproducibility of the method. The graphite furnaces were optimized for maximum absorbency and linear response while aspirating known standards. The standards were prepared from the individual 1000 mg/kg standard (Merck, Germany), 100 ml of five combined standards were prepared in 0.1 N HNO₃. The lamp current used was 75%. The signal type was transient for copper, zinc and iron. Measurement time was 3s. The recovery of the methods was 96 – 98% and reproducibility was better than 1.0%. All metal concentrations are expressed on a wet weight basis (original matter).

All determined values were analyzed statistically with Student's t-test, Scheffe's test and Pearson's rank using PC programs SAS and Excel.

RESULTS

The copper concentration is significantly higher ($p < 0.0001$) in ram semen (2.49 ± 0.18 mg/kg wet weight) in comparison with bull (1.64 ± 0.21 mg/kg w.w.), boar (1.64 ± 0.28 mg/kg w.w.) and stallion semen (0.86 mg/kg w.w.). In comparison with foxes the concentration is similar (2.16 ± 0.53 mg/kg w.w.). Significantly lower ($p < 0.0001$) level of copper was detected between stallions and foxes, boars as well as bulls. Significantly higher copper concentration is in fox semen in comparison with boars and bulls (Table 1).

In boar semen significantly higher zinc concentration (171.74 ± 65.72 mg/kg w.w.) in comparison with stallion (86.20 ± 45.88 mg/kg w.w.), bull (83.15 ± 61.61 mg/kg w.w.) as well as ram semen (60.46 ± 35.37 mg/kg w.w.) was detected. The lowest level of zinc was found in fox semen (13.09 mg/kg w.w.).

In iron the semen concentration is similar in rams (40.32 ± 10.81 mg/kg w.w.), bulls (38.04 ± 22.07 mg/kg w.w.) and foxes (33.16 ± 24.36 mg/kg w.w.) and significantly lower values were found in boar (16.14 ± 10.35 mg/kg w.w.) and stallion (12.68 mg/kg w.w.) semen (Table 1).

Concentration of cadmium in the semen of all studied animals is very similar ($0.05 - 0.12$ mg/kg w.w.) and any significant differences were found.

The highest concentration of lead was found in ram semen (0.35 ± 0.68 mg/kg w.w.). Lower level of this element reported in foxes (0.08 ± 0.06 mg/kg w.w.), bulls (0.06 ± 0.04 mg/kg w.w.), stallions (0.05 ± 0.05 mg/kg w.w.) and boars (0.02 ± 0.03 mg/kg w.w.).

In nickel, we found significantly ($p < 0.05-0.01$) higher level of this element in fox (0.36 ± 0.24 mg/kg w.w.) and ram (0.31 ± 0.19 mg/kg w.w.) semen in comparison with boars (0.06 ± 0.08 mg/kg w.w.). The concentration of nickel in the semen of stallions and bulls is between these values (Table 1).

Table 1. Concentration of copper, zinc and iron in the semen of bulls, rams, boars, stallions and foxes

Parameters	Copper (mg/kg) Mean ± SD	Zinc (mg/kg) Mean ± SD	Iron (mg/kg) Mean ± SD	Cadmium (mg/kg) Mean ± SD	Lead (mg/kg) Mean ± SD	Nickel (mg/kg) Mean ± SD
Bulls (n = 200)	1.64 ± 0.21*	83.15 ± 61.61	38.04 ± 2.07*	0.10 ± 0.14	0.06 ± 0.04	0.12 ± 0.07
Rams (n = 100)	2.49 ± 0.18*	60.46 ± 35.37	40.32 ± 0.81*	0.12 ± 0.12	0.35 ± 0.68	0.31 ± 0.19*
Boars (n = 20)	1.64 ± 0.28*	171.7 ± 65.7*	16.14 ± 10.35	0.05 ± 0.04	0.02 ± 0.03	0.06 ± 0.08
Stallions (n = 10)	0.86 ± 0.10	86.20 ± 45.88	12.68 ± 9.09	0.09 ± 0.11	0.05 ± 0.05	0.20 ± 0.24
Foxes (n = 10)	2.16 ± 0.53*	13.09 ± 5.22	33.16 ± 24.36	0.07 ± 0.05	0.08 ± 0.06	0.36 ± 0.24*

Copper: *p < 0.0001 (rams – bulls, boars, stallions; bulls – stallions; boars – stallions; foxes – stallions); p < 0.01 (foxes – bulls, boars)

Zinc: *p < 0.0001 (boars – foxes); p < 0.001 (boars – bulls, rams); p < 0.01 (boars – stallions)

Iron: *p < 0.01 (bulls – boars); p < 0.05 (bulls – stallions; rams – boars, stallions)

Nickel: *p < 0.05 (foxes – bulls); p < 0.01 (rams – boars; foxes – boars)

Correlation analysis determined high positive relation between iron and zinc in bull ($r=0.723$) and stallion ($r=0.723$) semen, between cadmium and lead in ram ($r=0.976$) and boar ($r=0.973$) semen and between iron and cadmium ($r=0.783$) as well as between iron and lead ($r=0.791$) in boar semen. High negative correlation has been found between nickel and copper in ram ($r=-0.709$) and between copper and lead in fox semen (-0.854).

DISCUSSION

It has been reported that the concentration of copper is the highest in ram semen (2.49 mg/kg w.w.). The copper has toxic effects on the seminiferous epithelium as well as on immune system in rams [16,17]. In the toxic phase of disease primarily the germinative epithelium is damaged. Toxic effects of copper on seminal plasma are manifested in the decrease of motile spermatozoa percentage and in decrease of malformed sperm cells [18].

Parenteral administration of iron to swine is a common practice in a pig industry to prevent and threat anaemia. Besides being a hemopoetic factor, iron plays an important role in modulating the functions of many cells in the body. Iron deficiency reduce the activity of iron-containing and iron-dependent enzymes [19]. In FeSO₄/ascorbate-incubated samples, the activities of antioxidant enzymes, superoxid dismutase, glutathione peroxidase and glutathione reductase were decreased while lipid peroxidation increased as compared to the control spermatozoa samples. Co – incubation of spermatozoa with fulleranol and FeSO₄/ascorbate increase the activities of antioxidant enzymes and prevent elevation of lipid peroxidation in a dose – dependent matter [20].

Our analyses showed that the concentration of zinc is significantly higher in boars in comparison with other males. In relation to zinc the main alteration cause the status of hypozincaemia. The zinc deficiency cause degenerative changes in spermatogenic cells after meiosis, their depletion and cumulation in the lumen of seminiferous tubules. Increased occurrence of malformed spermatids indicates impaired course of spermatogenesis. It has been stated that zinc is an indispensable element for a normal course of spermatogenesis [6]. As it is well known, that zinc can prevent toxic effect of many toxic elements. We suggest that the high zinc concentration in boar semen protect mainly their spermatozoa.

Previous studies report that low cadmium dose (40 µg/ml natrium citrate) decrease spermatozoa motility in relation to time, decrease progressive spermatozoa motility and decrease the percentage of spermatozoa with the highest motility [21]. In comparison of four different cadmium doses (0.02, 0.1, 0.2 and 2.0 mg CdCl₂/ml) it has been found that the progressive motility, path velocity and straightness are mostly affected in group

with the highest cadmium concentration [22]. In the study describing the influence of environmental cadmium on testicular proliferation in roe deer the results suggest delayed proliferation during the pre – rutting period in animals with high cadmium exposure, but other indicators of effects on the testis were not significant [23]. In relation to lead several investigators reported that heavy lead exposure causes depressed endocrine function and spermatogenesis [24,25]. Industrial lead emissions lead to dose – related oligospermia and athenospermia. In nickel, we report significantly higher levels in ram and fox semen in comparison with bulls and boars. A dose – related depression in stimulated testosterone production of mouse Leydig cells in culture following either in vivo or in vitro nickel treatment at a dose that does not induce any general toxic or significant cytotoxic action has been reported [11,13]. The data of the time – course study indicate that the effect of nickel on testosterone production is both time and concentration dependent and not due to cytotoxicity. We can conclude that in relation to nickel the most sensitive might be the spermatozoa of rams and foxes. Generally, our results suggest that there are very significant differences in the concentration of studied elements in animal semen which might directly effect the spermatozoa quality.

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