Slovak J. Anim. Sci., 42, 2009 SUPPLEMENT 1: 46-50

ISSN 1337-9984

Scientific paper formerly presented

RELATIONSHIP BETWEEN TRACE ELEMENT CONCENTRATIONS AND SPERMATOZOA QUALITY IN RABBIT SEMEN

N. LUKÁČ[1*], P. MASSÁNYI[1], J. KROČKOVA[1], P. NAĎ[2], J. SLAMEČKA[3], Ľ. ONDRUŠKA[3], G. FORMICKI[4], J. TRANDŽÍK[5]

[1]Slovak Agricultural University, Department of Animal Physiology, Nitra, Slovak Republic,
[2]University of Veterinary Medicine, Department of Nutrition and Dietetics, Kosice, Slovak Republic,
[3]Animal Production Research Centre, Nitra, Slovak Republic,
[4]Institute of Biology, Krakow Pedagogical University, Krakow, Poland,
[5]Constantine the Philosopher University, Department of Zoology and Anthropology, Nitra, Slovak Republic

ABSTRACT

Concentrations of cooper, zinc, iron, manganese, cadmium, lead and nickel in the semen of rabbits (n=27) in relation to spermatozoa quality were evaluated. Samples were analyzed on an atomic absorption spectrophotometer (Unicam Solar 939).
For analysis of pathological spermatozoa semen samples were fixed in Hancock´s solution and stained with Giemsa dye. For each rabbit at least 500 spermatozoa were evaluated. Concentrations of trace elements determined in rabbit semen were following: cooper - 20.10±4.09 mg/kg, zinc - 81.20±59.43 mg.kg^{-1}, iron - 19.65±7.26 mg.kg^{-1}, manganese - 30.87±1.81 mg.kg^{-1}, cadmium - 0.095±0.10 mg.kg^{-1}, lead - 0.092±0.04 mg.kg^{-1}, and nickel - 0.297±0.11 mg.kg^{-1}. Total percentage of pathological spermatozoa was 16.21±7.99% with predominance of knob-twisted flagellum, separated flagellum and flagellum ball. Correlations between the copper concentration and acrosomal changes (r=-0.75), total pathological spermatozoa and flagellum ball form (r=0.674) and between total pathological spermatozoa and knob- twisted flagellum (r=0,762) were detected.
This study demonstrates that rabbit semen is specific for high concentration of cooper. Also a significant effect of zinc on the occurrence of pathological spermatozoa was revealed.

Key words: semen; cooper; zinc; iron; manganese; cadmium; lead; nickel; rabbit

INTRODUCTION

Many recent studies have indicated an increasing prevalence of various abnormalities of the reproductive system in males. Possible factors explaining this phenomenon include stress, lifestyle, and a variety of endocrine–altering chemicals in the environment, that can be linked to decreased male reproductive capacity, as indicated mainly by the results of experimental animal studies (Massanyi et al., 2003; 2004; 2005).

Rabbit males have relatively low fertility rate as compared to other mammals. Thus, the rabbit male may be at greater risk from reproductive toxicants. Rabbit males have markedly smaller relative testis size and the lowest rate of daily spermatozoa production per gram of testes (by a factor of more than 3) as compared to the mouse, rat, or monkey; the percentages of progressively motile spermatozoa and morphologically normal spermatozoa in rabbit semen are also lower than in any of the animal models (Russell et al., 1990).

Trace elements are essential for the function of various enzymes and other proteins. The effects of trace element biochemistry and physiology on parameters of fertility are presented for zinc, selenium, iodine, copper and manganese (Leonhard–Marek, 2000).

Lead and cadmium are highly toxic metals for

***Correspondence:** E-mail: norolukac@gmail.com
doc. Ing. Norbert Lukáč, PhD., Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak Agricultural University, Tr. A. Hlinku 2, SK–949 76 Nitra, Slovak Republic;
Phone: +42137414284

rabbits and other mammals. Both are pervasive in the animal environment and accumulate in the body over a lifetime, including prenatal life (especially lead). Many experimental animal studies show that lead and cadmium can adversely affect the mammalian male reproductive system. On the other hand, relatively few data are available regarding possible reproductive effects of Pb and/or Cd in men, and generally conflicting results have been reported in reviews on this subject. Nevertheless, the results of several studies suggest that relatively high occupational exposure to Pb, as indicated by blood Pb (BPb) levels, can reduce human semen quality (decreased number, motility, and altered morphology of spermatozoa), whereas reproductive endocrine function is either not affected or is only marginally affected (Telisman et al., 2000).

High concentration of Mn^{2+} or Pb^{2+} significantly inhibits spermatozoa motility without an accompanying change in seminal MDA (malondialdehyd) levels. Incubation with Fe^{2+} significantly inhibits spermatozoa motility (Huang et al., 2001).

The purpose of this study was to determine cooper, zinc, iron, manganese, cadmium, lead and nickel concentration in rabbit semen, to determine the occurrence of pathological spermatozoa and to find possible relationships between the trace elements and the spermatozoa quality.

MATERIALS AND METHODS

All semen was collected from adult rabbits (n=27). Semen was processed at the animal breeding farm (Animal Production Research Centre, Nitra, Slovak Republic).

Atomic absorption spectroscopy (AAS – Unicam Solar 939) is routinely used to detect and quantify trace element contents in rabbit seminal plasma (Jurasovic and Telisman, 1993). Consequently, AAS was selected as the analytical technique to determine contents of Cu, Zn, Fe, Mn, Cd, Pb and Ni in semen of each animal (Massanyi et al., 2004; 2005).

Basic characteristics of rabbit semen samples were analyzed by the routine laboratory technique (Massanyi et al., 2003, Chrenek et al., 2007). Spermatozoa motility was estimated subjectively using optical microscopy.

For analysis of pathological spermatozoa, semen samples were fixed in Hancock`s solution and stained with Giemsa. The frequency of abnormal spermatozoa was quantified microscopically at 500x magnification, and following abnormal morphological changes were evaluated: knob-twisted flagellum, separated flagellum, flagellum torso, broken flagellum, retention of cytoplasmatic drop, acrosomal changes, large head, small head, flagellum ball, and other pathological spermatozoa (Gamcik et al., 1992).

The mean ± S.D. values for each sample were calculated. Data from analyzed samples were statistically evaluated using t–test. To determine significance between total number of pathological spermatozoa and trace element level a two-way analysis of variance (ANOVA) and correlation analysis were performed using GraphPad version 3.01 software.

RESULTS AND DISCUSSION

Analysis of basic rabbit semen parameters (Table 1) showed that all observed characteristics were at physiological rates. All males were exploited in reproductive process.

Table 1: Basic parameters of analyzed rabbit semen samples

	Mean	S.D.
Semen volume (ml)	0.60	0.30
Motility (%)	80	10.25
Spermatozoa concentration (x 10^9.ml^{-1})	0.32	0.11
pH	7.5	0.22
Osmolarity (mOsm)	368	14.25

S.D. – standard deviation

The concentration of copper in rabbit semen was 20.10±4.09 $mg.kg^{-1}$ wet weight ($w.w^{-1}$). The zinc concentration was 81.20±59.43 $mg.kg^{-1}$ $w.w^{-1}$, concentration of iron -19.65±7.26 $mg.kg^{-1}$ $w.w^{-1}$ and manganese concentration was 30.87±1.81 $mg.kg^{-1}$ $w.w^{-1}$. Concentration of potentially toxic trace elements as cadmium, lead and nickel in rabbit semen was relatively low. Average concentration of cadmium was 0.095±0.10 $mg.kg^{-1}$ $w.w^{-1}$, concentration of lead in rabbit semen was 0.0915±0.044 $mg.kg^{-1}$ $w.w^{-1}$ and concentration of nickel was 0.297±0.11 $mg.kg^{-1}$ $w.w^{-1}$ (Table 2).

Table 2: Concentration of trace elements in rabbit semen

Parameter n=27	Cu	Zn	Fe	Mn	Cd	Pb	Ni
mean	20.10	81.20	19.65	34.44	0.09559	0.09148	0.2975
S.D.	4.090	59.43	7.266	1.816	0.1060	0.04435	0.1087
S.E.	0.7871	11.44	1.398	0.3496	0.02040	0.008535	0.02092
C.V. (%)	20.35	73.19	36.98	5.27	110.88	48.48	36.55
G.M.	19.67	56.15	17.64	34.39	0.05022	0.07706	0.2050
Maximum	26.01	260.40	36.39	37.84	0.3900	0.1700	0.4600

S.D. – standard deviation; S.E. – standard error; C.V. – coefficient of variation; G.M. – geometric mean

Total percentage of pathological spermatozoa in rabbit semen was 16.21±7.99% (Table 3). From this total number, 3.92% had knob-twisted flagellum, 4.78% - separated flagellum, 0.79% - flagellum torso, 1.11% - broken flagellum, 0.99% - retention of the cytoplasmic drop, 0.54% - acrosomal changes, 0.04% - large heads, 0.22% - small heads, 3.08% - flagellum ball and 0.74% represented other forms of pathological spermatozoa.

Table 3: Occurrence of pathological spermatozoa in rabbit semen (%)

Pathological change (%)	**Mean**	**S.D.**
Total number	16.21	7.99
Separated flagellum	4.78	4.12
Knob-twisted flagellum	3.92	3.34
Flagellum ball	3.08	3.00
Broken flagellum	1.11	0.69
Retention of cytoplasmic drop	0.99	0.90
Flagellum torso	0.79	0.58
Acrosomal changes	0.54	0.42
Small head	0.22	0.21
Large head	0.04	0.02
Other pathological spermatozoa	0.74	0.53

S.D. – standard deviation

Analysis of variance showed significant ($p<0.001$) difference between the concentration of zinc and total number of pathological spermatozoa in rabbit semen (Figure 1).

Correlation analysis showed significant positive correlation between total pathological spermatozoa and flagellum ball (r=0.674), and between total pathological spermatozoa and knob-twisted flagellum (r=0.762). Negative correlation was found between copper concentration and acrosomal changes (r=-0.75).

The role which heavy metals play in the etiology of reproductive pathology has been discussed for several decades. Exposures to lead and cadmium have been reported to reduce male fertility in both humans and rodents and there are several hypotheses that suggest how reduced male fertility might result from incorporation of heavy metals into spermatozoa chromatin (Bench et al., 1999; Bennetts and Aitken 2005; Casswel et al., 1987).

Heavy metals transported into the egg by spermatozoa may also pose a significant risk to the developing embryo via their toxicity. However, some of the information implicating the involvement of heavy metals in the etiology of reduced male fertility has been anecdotal and there are few studies that demonstrate the capacity of semen and spermatozoa to accumulate these metals (Bench et al., 1999). Negative correlation was found between copper concentration and acrosomal changes (r=-0.75) in rabbit semen. Our results also suggest an effect of copper and zinc on the occurrence of pathological spermatozoa in semen. In this study we have detected a significant correlation between total pathological spermatozoa and flagellum ball (r=0.674), and between total pathological spermatozoa and knob-twisted flagellum (r=0,762).

Statistically significant ($p<0.05$) positive coefficients of correlation were found between the copper concentration in blood plasma and the total number of spermatozoa in ejaculate of bulls (r= 0.33), between

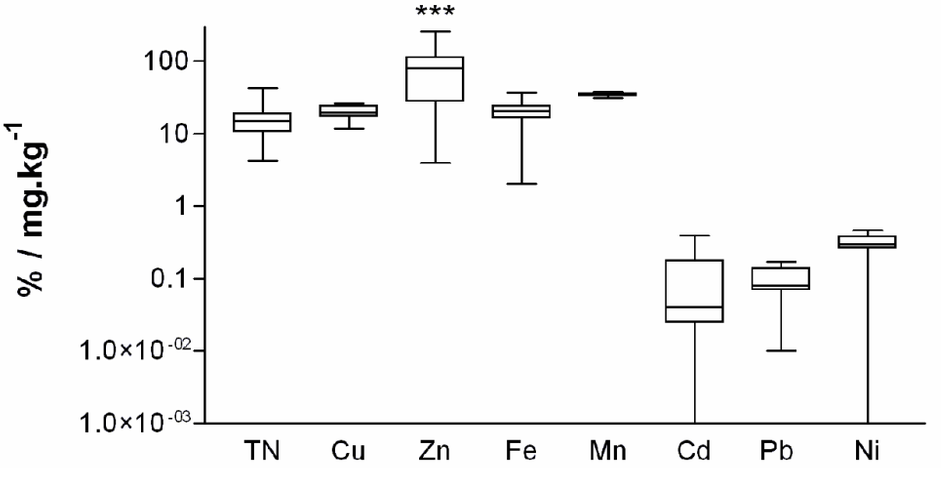

Fig. 1: Relationship between total number (TN) of pathological spermatozoa and concentration of selected trace elements in rabbit semen

the copper concentration in blood plasma and the total number of spermatozoa with progressive motility (r=0.35). Positive coefficients of correlation were found between the copper concentration in seminal plasma and the mean monthly volume of bull's semen (r=0.36, p<0.01), between the copper concentration in seminal plasma and spermatozoa motility (r=0.33, p<0.05), and copper concentration in seminal plasma and total number of spermatozoa with progressive motility in bovine semen (r=0.28, p<0.05) (Machal et al., 2002).

In a human study, the concentrations of calcium, magnesium, zinc and copper in the blood and seminal plasma were not different between the subfertile and fertile group. Weak correlations were demonstrated between blood plasma zinc concentrations and spermatozoa count, spermatozoa motility, and abnormal spermatozoa morphology. Zinc and magnesium concentrations in seminal plasma correlate with spermatozoa count. Strong correlations were found between calcium, magnesium, and zinc in seminal plasma (Wonga et al., 2001).

Trace elements are essential for the function of various enzymes and other proteins. Zinc ions are involved in processes of cell division, development and differentiation, and in the control of gene expression (Danek, 2002).

Mean levels of minerals in the blood and seminal plasma also vary between different bull's breeds (Dhami et al., 2001). The differences between bull breeds are significant only for iron and copper in the blood and for copper in seminal plasma. Zinc levels are higher in seminal plasma than in the blood plasma: iron and copper levels were identical, while copper and manganese levels were higher in the blood compared to the seminal plasma in all the breeds.

Treatment of rats with cadmium causes a significant decrease in spermatozoa concentration, motility, weight of testes and epididymis, and increases the number of dead and abnormal spermatozoa (Fatma et al., 2004).

Early studies on nickel, essentially in 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 spermatozoa physiology. Nickel deprivation significantly decreases spermatozoa motility and density in the epididymis, epididymal transit time of spermatozoa, and testis spermatozoa production rate (Yokoi et al., 2003).

The oral exposure to nickel may affect the histology of testes, epididymides, seminal vesicles and spermatozoa morphology in mice (Pandey et al., 1999; Zemanova et al., 2007).

In comparison to other animals, lower copper concentrations are reported in stallion, bull, boar, and ram semen. Rabbit semen is characterized by very high copper concentration. In bull, ram, and fox semen a higher level of iron in comparison to rabbit semen was found. The level of cadmium and lead in all studied animals does not show any significant differences, but on the other hand, there is a high nickel concentration in rabbit semen in comparison to stallion, bull, and boar semen (Massanyi et al., 2003; 2004).

ACKNOWLEDGMENT

This study was supported by the VEGA grant No. 1/4347/07 of Slovak Ministry of Education and the APVV grant No. 0299–06.

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