Effect of Nickel on Male Reproduction

Lukac N.1*, Massanyi P.1, Krockova J.1, Toman R.1, Danko J.2, Stawarz R.3, Formicki G.3

1Department of Animal Physiology, Slovak University of Agriculture in Nitra, Slovakia
2Institute of Anatomy, University of Veterinary Medicine and Pharmacy in Kosice, Slovakia
3Institute of Biology, Pedagogical University of Krakow, Poland

*Corresponding Author: norbert.lukac@uniag.sk

Abstract In this study distribution of nickel as a risk factor of environment in testis and its effects on the testicular structure in experimental animals and effect on spermatozoa was analyzed. In this study the effect of Ni on the testicular structure after an experimental intraperitoneal (i.p.) administration, concentration of nickel in semen of different animal species and the effect of in vitro spermatozoa incubation with nickel on the spermatozoa motility and membrane changes is reported. Our findings clearly suggest a negative effect of nickel on the structure as well as on the function of seminiferous epithelium. In experimental groups with nickel a significant (p<0.001) decrease of germinal epithelium in comparison with control group was observed. The analysis of nickel showed that the concentration of this element in stallion semen was 0.20 mg/kg, in bull 0.12 mg/kg, ram 0.31 mg/kg, boar 0.06 mg/kg and in fox semen 0.36 mg/kg. Concentrations from 125 μM Ni/ml in various time periods of culture stimulate spermatozoa motility after 30 minutes (p<0.001), but later inhibit spermatozoa motility. After a culture of spermatozoa with addition of 125 µM Ni/ml and 240 minutes a typical Annexin-V fluorescence reaction was detected. Fluorescence was detected in mitochondrial segment of bovine spermatozoa. In spermatozoa exposed to higher nickel concentration the Annexin–V positive reaction was detected also on the spermatozoa head membrane. Nickel in very low concentrations (7.8 μM Ni/ml) stimulates the spermatozoa motility but in higher concentrations (>250 μM Ni/ml) cause decrease of spermatozoa motility in vitro.

Keywords Nickel, Spermatozoa, Testis, Semen

1. Introduction

High quantity of nickel is known to be injurious for animal and human health. Its effects on various aspects of reproduction have been previously 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 on adverse effect on spermatozoa [1, 2]. Nickel is an essential trace element. Its industrial application has a broad spectrum and primarily it is used in alloys. Other uses are in electroplating, welding, flame cutting, flame spraying and mold making. Nickel is also used in the manufacture of artificial jewelry, coinage, cutlery, cooking utensils and dental or surgical prostheses [3]. Since 1970s more nickel containing enzymes have been discovered in prokaryotes bacteria (e.g. urease, hydrogenase, methyl-S-coenzyme M reductase, carbon monoxide dehydrogenase, nickel superoxide dismutase, etc.). Nickel is also an essential trace metal that is vital for growth enhancement in very low doses (ppb) for birds and mammals [4]. Animal studies have indicated that nickel may reach the testis, seminal vesicle and prostate gland [5], and there are reports of its adverse effect on spermatozoa [6, 7]. Its action on spermatozoa motility, morphology and count, is an important parameter for the evaluation of male fertility.

The purpose of this study was to determine the effect of nickel on the testicular structure and possible spermatozoa alterations in vivo and in vitro under condition.

2. Material and Method

Firstly nickel was intraperitoneally (i.p.) administered in a single dose to adult (3 month old) mice (ICR, Mus musculus albicans). The animals (n=15) were divided into 3 groups (A, B, C). Five animals (group A) were injected i.p. with a single dose of 20 mg NiCl2 per kg of body weight. Other five animals (group B) received a single dose of 40 mg NiCl2 per kg of body weight. Animals were killed in extremis 48 hours after application of nickel (NiCl2, Sigma, St. Louis, MO). The last group (C) was the control without nickel injection. After killing visceral organs were weighted and testis of nickel–treated and untreated mice were fixed in 10% formalin. Animals were killed in extremis 48 hours after application of nickel (NiCl2, Sigma, St. Louis, MO). The last group (C) was the control without nickel injection. After killing visceral organs were weighted and testis of nickel–treated and untreated mice were fixed in 10% formalin, dehydrated in a graded series of ethanol and embedded in paraffin wax. Whole testes were sectioned on a microtome. At least five sections from each animal were measured using light microscope (Carl Zeiss NU–2) and software for analysis Image ProPlus (Media Cybernetic). The relative
volume of germinal epithelium, interstitium and lumen; the diameter of seminiferous tubule; the height of germinal epithelium; the diameter of lumen of seminiferous tubule and the perimeter of seminiferous tubule (μm) were measured with respect to each testis based on micromorphological criteria. For apoptosis assay MEBSTAIN Apoptosis kit Direct (Immunotech, France) to detect TUNEL reactions was used. After fixation in 4% paraformaldehyde, 3 – 5 μm thick sections were prepared for staining. The samples were deparaffinized using xylene and ethanol (100 – 80%) and treated with proteinase K (PK+). DNA nick end was labeled using TdT reaction, stained with DAPI and mounted. After the procedure samples were analyzed on Leica fluorescent microscope.

Secondly the analysis of nickel in animal semen (stallion, bull, ram, boar, fox) and its relation to spermatozoa quality was analyzed. 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 samples were digested in a microwave oven (MLS-1200 MEGA, Milestone, USA). 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 recovery of the methods was 69%~98% and reproducibility was higher than 1.0%.

Effect of nickel on in vitro culture of bovine spermatozoa with nickel (NiCl₂) on the spermatozoa motility and membrane changes was analyzed as third experiment. Bovine semen was collected from Holstein –Friesian breed bulls (n=10). Semen samples were diluted in commercial diluent (Triladyl; Minitüb, Tiefenbach, Germany). In vitro culture with nickel (NiCl₂, Sigma Aldrich, USA) was treated with various concentrations (7.8, 15.6, 31.25, 62.5, 125, 250, 500 and 1000 mM) and in four time periods (30, 60, 120 and 240 min) at 3 °C. All equipment was sterilized and decontaminated before the experiment. Each sample from control and experimental groups was analyzed for motility on the CASA system (Sperm Vision, Minibube, Germany). Fluorescence analysis of bovine spermatozoa incubated in different nickel concentration was analyzed by fluorescence technique with Annexin V FLUOS, DAPI and Propidium Iodide stain mark with the Leica fluorescent microscope (Leica Micosystem, Germany) using a 488 nm wavelength filter. Spermatozoa with disordered membrane exhibited green fluorescence, whilst live spermatozoa remained unstained [8]. From final data, basic statistical characteristics (mean, standard deviation, variation, median, minimum, maximum values) were calculated using PC program Graph Pad Prism ver. 6.0 and the differences between groups were analyzed by F – test in the level of significance of P<0.05.

3. Results and Discussion

This study describes the effect of nickel on structural changes in the testes, the concentration of nickel in semen of mature animals and in vitro effects of nickel on spermatozoa motility and membrane integrity.

Our results clearly show negative effect of nickel on spermatogenesis. The decrease of the relative volume of germinal epithelium indicates alterations of the spermatozoa production.

We have determined that the concentration of seminal nickel is significantly higher in fox and ram (0.31±0.19 mg/kg) in comparison with bull (0.12±0.07 mg/kg) and boar (0.06±0.08 mg/kg). The concentration of nickel in stallion semen was 0.20±0.24 mg/kg. Correlation analysis in bulls as well as in rams showed high positive relation between nickel and separated flagellum. On the other hand [9] it was reported that nickel has influence on cyclic nucleotide gated channels (CNG) which are important in spermatozoa physiology. Nickel deprivation significantly decreased spermatozoa motility and density in the epididymides, epididymal transit time of spermatozoa, and testes spermatozoa production rate. Nickel deficiency also significantly decreased the weights of the seminal vesicles and prostate glands. Although, a short-term dose of nickel induced only a minimal lipid peroxidation response (LPO), multiple doses elicited a moderate increase in LPO in whole homogenates and higher dose-related increases in both mitochondrial and microsomal fractions [10]. This was associated with a significant increase in DNA damage in the testis as evidenced by increased single-strand breaks. These findings suggest that testicular toxicity of nickel compounds may be related to enhanced production of reactive oxygen species, probably mediated through oxidative damage to macromolecules, including damage of DNA. Lindemann et al. [11] investigated a selective effect of Ni²⁺ on wave initiation in bull spermatozoa flagella. Although spontaneous coordination of flagellum waves is lost after exposure to Ni²⁺, other function of the flagellum remain intact. Wave motility can be restored to Ni²⁺-inhibited spermatozoa by including a permanent bend onto the flagellum by micromanipulation. Although Ni²⁺-treated cells have a functional sliding tubule mechanism, and consequently the axoneme can propagate bends and it appears that these retained functions are not sufficient to cause spontaneous bend initiation. The findings show that bend initiation are inhibited by Ni²⁺, and therefore is an independent process separate from the sliding tubule mechanism responsible for wave propagation.

Experiment analysing in vitro effect of nickel on bovine spermatozoa detected that nickel in low concentrations (7.8 mM Ni²⁺) stimulates the bovine spermatozoa motility but in higher concentrations (>250 mM Ni²⁺) causes a decrease of spermatozoa motility in vitro. After culture of spermatozoa with addition of 125 mM Ni²⁺ for 240 min a typical Annexin V fluorescence reaction was detected. Fluorescence was detected in the mitochondrial segment of
bovine spermatozoa. In spermatozoa exposed to higher nickel concentrations the Annexin V-positive reaction was also detected on the spermatozoa head membrane. At a concentration of 500 mM Ni$^{2+}$, necrotic processes (PI fluorescence) were even detected. In the group with the highest concentration and the longest time of exposure (1000 mM Ni$^{2+}$; 240 min), apoptotic Annexin-positive regions were detected not only in the mitochondrial part, but also in the spermatozoa head (acroosomal and postacrosomal part), showing significant alteration of spermatozoa membrane integrity. The effects of nickel sulfate on the frequency of dominant lethal mutations and two-strand DNA breaks (TSBs) were also studied [12]. The results indicated that the spermatogenesis stages most sensitive to nickel sulfate are spermatozoids, late spermatocytes and stem spermatogonia. Early studies on nickel essentiality with rats and goats indicated that nickel deprivation impaired reproductive performance. Nickel has also been found to influence cyclic nucleotide gated channels – which are important in spermatozoa physiology. Nickel deprivation significantly decreased spermatozoa motility and density in the epididymides, epididymal transit time of spermatozoa and testes spermatozoa production rate. Nickel deficiency also significantly decreased the weight of the seminal vesicles and prostate glands [13].

4. Conclusions

Results of this study clearly confirm toxic effect of nickel on male reproductive abilities and the necessity of monitoring nickel concentrations in animal semen. All detected results suggest that the nickel has a negative effect on testicular structure affecting mainly the spermatozoa development as well as the steroidogenesis. Previously determined alterations in spermatozoa structure in vivo were completed by detection of the dose dependent effect of nickel on spermatozoa motility and particularly by the detection of fine spermatozoa structural changes associated with nickel toxicity.

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