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7 Abstract

Introduction: Copper and iron have various effects on the male reproductive system, which can be strongly reflected in the process of spermatogenesis. The aim of our *in vitro* study was to evaluate the dose- and time-dependent effects of copper and iron on the motility, viability, structural and functional characteristics of spermatozoa.

Material and methods: Bovine semen samples were obtained from adult breeding bulls (Slovak Biological Services, Luzianky – Nitra). Spermatozoa were incubated in different culture media to which we added (3.90; 7.80; 15.60; 31.20; 62.50; 125; 250; 500; 1 000 μmol.dm^{-3}) of copper ($CuSO_4.5H_2O$; $CuCl_2$) and iron ($FeSO_4 7H_2O$; $FeCl_2$). The motility analysis was carried out using a CASA system – SpermVision™ program and Annexin-V-FLOUS was used for detection of the membrane integrity of spermatozoa. The morphological analysis we assessed the abnormal spermatozoa forms (Giemsa staining). The viability of the cells we assessed by the MTT (metabolic activity) assay.

Results: COPPER The initial spermatozoa motility in the presence of both forms of copper in physiological saline solution (PS) showed significantly (P <0.001) decreased values at concentrations ≥250 μmol.dm^{-3} and concurrently they have a cytotoxic effect on the mitochondrial complex of spermatozoa. The low concentrations (≤7.80 μmol.dm^{-3} of $CuSO_4.5H_2O/CuCl_2$) stimulated the mitochondrial activity of cells and maintained of spermatozoa motility (Time 2 h). The long-term cultivation (Time 24 h) significantly (P <0.001) reduced the average motility values in all experimental groups. The commercial medium containing triladyl, egg yolk and redistilled water increased the overall percentage of spermatozoa motility after exposure of high doses of $CuSO_4.5H_2O/CuCl_2$, but only after 1 h of cultivation. The lowest average motility values were detected after 24 h. The culture medium in composition of 20 % bovine serum albumin (BSA), triladyl, 5 % glucose and redistilled water maintained the overall percentage of spermatozoa motility during the long-term of cultivation in spite of the presence of $CuSO_4.5H_2O/CuCl_2$. The percentage of motile spermatozoa decreased after 2 h of cultivation at the highest concentrations ≥500 μmol.dm^{-3} (≥250 μmol.dm^{-3} $CuCl_2$) and after 24 h at doses ≥125 μmol.dm^{-3} $CuSO_4.5H_2O/CuCl_2$. Annexin-V fluorescence reaction was detected at the highest concentrations (500; 1 000 μmol.dm^{-3} $CuSO_4.5H_2O$ in the PS) at the beginning of incubation. The group with the highest concentration (1 000 μmol.dm^{-3} $CuSO_4.5H_2O$) and the longest time of exposure (6 h) caused significant destabilization of lipid membrane systems not only in the mitochondrial segment, but also on the head of spermatozoa (acrosomal and postacrosomal part), what subsequently led to the destruction of membranes in these parts of the spermatozoa. The total percentage of pathological abnormal spermatozoa was significantly higher in the group with the highest concentration of copper (9.63 %) in comparison to control group (4.79 %) after 24 h. Predominant morphological abnormalities were separated flagellum 2.96 %

(P <0.05), flagellum torso 2.90 % (P <0.001), knob twisted flagellum 1.10 % (P <0.001) and broken flagellum 1.06 % (P <0.001) in connection with segment (mid-piece) of spermatozoa. Detail analysis from copper chloride (1 000 μmol.dm^{-3}) determine knob twisted flagellum 2.17 % (P <0.001) associated with retention of the cytoplasmic drop 0.55 % (P <0.001).

IRON in both forms ($FeSO_4.7H_2O/FeCl_2$; in PS) maintained of overall percentage of spermatozoa motility (Time 0, 1 h). Measurement of spermatozoa motility after 2 h demonstrated increase values at the concentrations ≤125 μmol.dm^{-3} $FeSO_4.7H_2O/FeCl_2$. The experimental administration of the dose 250 μmol.dm^{-3} of $FeSO_4.7H_2O/FeCl_2$ (to Time 2 h) significantly (P <0.001) inhibited the percentage of spermatozoa motility, but did not have any negative effect on the survival of cells. Iron at concentrations ≤62.50 μmol.dm^{-3} $FeSO_4.7H_2O/FeCl_2$ after long-term periods of cultivation acts stimulating on the spermatozoa motility. The commercial medium increased average motility values in the presence of high doses of $FeSO_4.7H_2O/FeCl_2$ during short-term periods of cultivation (to Time 2 h). The spermatozoa motility parameters were maintained after 24 h at doses ≤31.20 μmol.dm^{-3} $FeSO_4.7H_2O$ (≤62.50 μmol.dm^{-3} $FeCl_2$). The culture medium BSA increased the bovine spermatozoa motility in spite of the presence of high doses of iron (to Time 2 h). A concurrently BSA maintained overall percentage of spermatozoa motility (≤250 μmol.dm^{-3} $FeSO_4.7H_2O/FeCl_2$), which was significantly different (P <0.001) at the doses ≤62.50 μmol.dm^{-3} $FeSO_4.7H_2O/FeCl_2$ during the long-term of cultivation. The highest concentrations (500; 1 000 μmol.dm^{-3} $FeSO_4.7H_2O$) caused destabilization of spermatozoa membranes. Iron (1 000 μmol.dm^{-3} $FeSO_4.7H_2O$) after 6 h of exposure even induced necrotic processes. The total percentage of pathological abnormal spermatozoa was significantly higher in the group with the highest iron concentration (9.46 %) in comparison to the control group (7.75 %) after 24 h. Predominant morphological abnormalities were knob twisted flagellum 2.71 % (P <0.001), flagellum ball 1.18 % (P <0.001), retention of the cytoplasmic drop 0.86 % (P <0.05) and broken flagellum of spermatozoa 0.67 % (P <0.05). Detail analysis from ferrous chloride (1 000 μmol.dm^{-3}) were predominantly knob twisted flagellum 2.91 % (P <0.001) with retention of the cytoplasmic drop 1.04 % (P <0.001).

Conclusion: Based on our results, we can concluded, that toxic and cytotoxic effect of copper appeared on the individual cell structure of spermatozoa depends on time. In functional aspects, all of these subtile changes could disrupt mechanism of spermatozoa motility. Iron in low doses (depending on time) has stimulating effect (*in vitro*), but on the other hand the highest concentrations of this element have adverse effects on parameters of spermatozoa motility, mitochondrial activity, morphology and membrane integrity of spermatozoa.

Key words: copper, iron, spermatozoa, culture media, motility, viability, morphology and cell membrane integrity