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Trace elements content in semen and their interactions with sperm quality and RedOx status in freshwater fish *Cyprinus carpio*: A correlation study

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ABSTRACT

Objective of the present study was to investigate interactions between trace elements content and RedOx status, as well as sperm quality parameters (motility features, DNA fragmentation) in fish spermatozoa in natural conditions. Reproductively mature male freshwater fish (n = 16) of *Cyprinus carpio* breed were used in the study. Trace elements content was determined in fish milt samples by inductively-coupled plasma optical emission spectrometry (ICP-OES) and by cold-vapor atomic absorption spectroscopy (CV-AAS). Sperm quality evaluation was realized by computer-assisted sperm analysis (CASA) quantifying several parameters: concentration, total motility, progressive motility, distance average path, distance curved line, distance straight line, velocity average path, velocity curved line, velocity straight line, straightness, linearity, amplitude of lateral head displacement and beat cross frequency. The general scheme of descending concentrations of trace metals in semen samples was following: Zn > Fe > Cu > As > Sr > Ni > Mn > Se > Pb > Cr > Cd > Hg. Total motility of spermatozoa was relatively high (91.45%), however progressive motility was not even half of this value (39.47%). Sperm DNA fragmentation values were relatively low (4.00–6.29%). The percentage of immotile spermatozoa showed a significant correlation with all RedOx status parameters and also with DNA fragmentation. Positive statistically significant correlations were observed between trace elements (Mn, Se, Sr, and Zn) and some qualitative spermatozoa parameters (velocity and distance parameters). Cu and Hg content shows similar negative associations with progressive motility. Hg also interacted with production of malondialdehyde. Overall, the present study suggests application of multi-component mixtures of environmentally related trace elements concentrations when assessing the potential reproductive risk.

1. Introduction

In general, it is well known that reproductive system may be considered as barometer of environmental contamination [1]. The main contaminants of environment targeting the male reproductive system are heavy metals [2,51], pesticides [3–6] and endocrine disruptors [7–10].

A number of studies have demonstrated a possible involvement of trace elements in the dys/function of male reproductive system in terms

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of endocrine activity, gametes production and gamete quality, specifically in men [11–14], boar [15], fox [16], buffalo [3], as well as in fish species, namely African catfish [17], Sea bass - *Dicentrarchus labrax* [18], Rainbow trout - *Oncorhynchus mykiss* [19], Banded knifefish - *Gymnotus carapo* [20], and Common carp [21,22]. Other studies demonstrated associations of trace metals with oxidative status parameters, associated with sperm motility parameters in bull *in vivo* [23] and in fish (*Acipenser ruthenus*) in *in vitro* conditions [24]. Several trace metals are considered essential for reproduction [25], however combined higher levels of these elements in seminal plasma may have adverse effect on sperm motility, oxidative stress production and thus may interfere with physiological processes responsible for successful fertilization [26–28]. In terms of bioaccumulation and hard degradability or non-degradability, environmental pollutants such as heavy metals are a serious risk factor for the aquatic organisms. Fish exposed to metals may be affected through a direct effect on the testes resulting in decreased fertility and embryonic development [29].

Bergamo et al. [52] in pilot study described human semen as sensitive biomarker of highly polluted living environment, with the combined measurement of trace elements in association with the overall assessment of semen quality, RedOx parameters and sperm DNA damage. Similar studies in animals, not to mention in fish, are unknown as majority of studies is focused on effect of one metal in high concentrations. This kind of research in fish is insufficient mainly from the perspective of their natural environment contaminated with the multicomponent mixture of toxicants to which they are constantly exposed [30]. Thus, the first objective of the present study was to investigate interactions between trace elements (As, arsenic; Cd, cadmium; Cr, chromium; Cu, cuprum; Fe, iron; Mn, manganese; Ni, nickel; Pb, lead; Se, selenium; Sr, strontium; and Zn, zinc) content and oxidative stress (OS) indices (ROS, reactive oxygen species; TAC, total antioxidant capacity; MDA, malondialdehyde; CP, carbonyl protein), as well as sperm quality parameters (motility and velocity, DNA fragmentation) in fish spermatozoa in natural conditions. The second aim of the study was to evaluate the link between sperm quality (motility and DNA fragmentation) and seminal antioxidant system.

2. Material and methods

2.1. Fish

Reproductively mature male freshwater fish (n = 16) of *Cyprinus carpio* breed were harvested from the experimental pond located at the affiliation of Slovak University of Agriculture in Nitra, University Farm Kolíňany (48°21′14.6″N 18°13′03.2″E) [31]. Fish stocking was realized in March 2015. Catching of the fish was realized from May to June 2015. The freshwater fish common carp (*Cyprinus carpio*) were caught by seine net. In total, 16 male fish were collected. The fish were transferred in polyethylene bags to the laboratory within 20 min for semen collection after catching. Fish were manipulated by a competent person in accordance to the provisions of the national law. After standard ichthyology evaluation (age: 6–9 years; body weight: 1826 ± 295 g; total length: 452 ± 22 mm; standard length: 383 ± 19 mm) semen collection was performed.

2.2. Semen sample collection

Fish were transferred to the laboratory of Department of Poultry Science and Small Animal Husbandry where were humanely sacrificed. Testes were surgically removed *post mortem* and the milt was collected from the sperm duct in order to avoid the contamination or early activation by urine [32].

2.3. Semen quality evaluation - CASA analysis

The CASA analysis was conducted at Department of Animal Physiology using the Computer Assisted Sperm Analyzer method with SpermVision software (Minitub, Tiefenbach, Germany) and the microscope Olympus BX 51 (Olympus, Japan). Semen samples were placed into Makler counting chamber (10 µm, Sefi-Medical Instruments, Germany) [23]. Every single output of the CASA system is the result of 4 diverse sub-measurements of 4 different fields of Makler Counting Chamber. CASA assessments determined the values of total motility (MOT), progressive motility (PRO), distance average path (DAP), distance curved line (DCL), distance straight line (DSL), velocity average path (VAP), velocity curved line (VCL), velocity straight line (VSL), straightness (STR), linearity (LIN), amplitude of lateral head displacement (ALH), beat cross frequency (BCF) of spermatozoa [33–35]. Immotile cells (IMC) were calculated according the following formula: IMC = 100% − MOT.

Table 1
Concentration of selected trace metals in semen samples.

Parameter (unit)	Mean	Median	S.D.	CV%	SEM	Min-max
As (mg/kg)	0.76	0.71	0.49	65.52	0.13	0–1.77
Cd (µg/kg)	22.96	10.14	27.49	119.70	6.87	0–87.73
Cr (mg/kg)	0.18	0.17	0.05	27.34	0.01	0.12–0.32
Cu (mg/kg)	2.16	2.18	0.39	18.52	0.09	1.51–2.89
Fe (mg/kg)	15.51	14.85	5.43	34.99	1.36	8.71–27.92
Hg (µg/kg)	3.54	3.42	1.72	48.65	0.43	1.61–7.18
Mn (mg/kg)	0.44	0.39	0.19	44.89	0.05	0.18–0.82
Ni (mg/kg)	0.48	0.33	0.39	79.97	0.09	0.11–1.36
Pb (mg/kg)	0.25	0.27	0.17	66.81	0.04	0–0.55
Se (mg/kg)	0.33	0.00	0.56	166.15	0.14	0–1.67
Sr (mg/kg)	0.60	0.63	0.38	63.72	0.10	0.19–1.60
Zn (mg/kg)	50.08	51.28	46.03	91.92	11.51	6.59–177.57

S. D. - standard deviation, CV% - coefficient of variation, SEM - standard error, Min -minimum and max – maximum.

2.4. Measurements of RedOx status in semen (ROS, TAC, PC and MDA)

ROS production in samples was quantified by the chemiluminescence assay based on the luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione; Sigma-Aldrich) probe [36]. Luminol (2.5 µL, 5 mmol/L) was added to 100 µL of semen prior to sample analysis. Negative controls were prepared by replacing the semen with 100 µL of PBS (Dulbecco's Phosphate Buffer Saline without calcium chloride and magnesium chloride; Sigma-Aldrich). Positive controls consisted of 100 µL PBS, 2.5 µL luminol and 50 µL hydrogen peroxide (H_2O_2, 30%; 8.8 M; Sigma-Aldrich). Chemiluminescence was measured in 96-well plates in 15 replicates of 1 min long cycles of Glomax Multi + Combined Spectro-Fluoro Luminometer (Promega Corporation, Madison, WI, USA) set up [37,38]. The results are expressed as relative light units (RLU)/s/g protein.

Total antioxidant capacity (TAC) was determined applying the improved chemiluminescence antioxidant assay which utilizes the horseradish peroxidase conjugate and luminol [39]. 5–100 µmol/L Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich) was used as the standard and a signal reagent, consisting of 0.1 mol/L Tris-HCl (Sigma-Aldrich), 12 mol/L H_2O_2 (Sigma-Aldrich), 41.8 mmol/L 4-iodophenol (Sigma-Aldrich) and 282.2 mmol/L luminol (Sigma-Aldrich), was employed to induce the chemiluminiscent reaction. Chemiluminescence was quantified on 96-well plates in ten consecutive one minute long cycles using the Glomax Multi + Combined Spectro-Fluoro Luminometer (Promega Corporation). The results are expressed as µmol Trolox Eq./g protein.

Carbonyl group quantification was carried out through the conventional 2,4-dinitrophenylhydrazine (DNPH) method. Briefly, 1 mL of sample was added to 1 mL of DNPH (10 mM in 2 NHCl; Sigma-Aldrich), mixed, and incubated in the dark at room temperature for an hour. Following the addition of 1 mL of trichloroacetic acid (20% w/v; Sigma-Aldrich), the mixture underwent the 10 min of incubation at 4 °C and