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REGULAR ARTICLE

RELATIONSHIP BETWEEN LEVEL OF COPPER IN BOVINE SEMINAL PLASMA AND SPERMATOZOA MOTILITY

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ABSTRACT

The aim of this study was to evaluate relationship between copper (Cu) concentration of bovine seminal plasma and spermatozoa motility. Semen samples were collected from 13 breeding bulls. The motility analysis was carried out using the Computer Assisted Sperm Analysis (CASA) system. The mean value for the percentage of motile spermatozoa (MOT) was 92.46±3.99% and the progressive motility of the spermatozoa (PROG) as 90.23±4.02%. The seminal plasma Cu concentrations were analyzed by UV/VIS spectrophotometry. The total Cu concentration of the seminal plasma was 4.28±1.47 µM/L. The correlation analysis revealed a strong negative correlation between MOT and seminal plasma Cu concentration (r_p=-0.781; P<0.01) as well as between PROG and Cu content in the seminal plasma (r_p=-0.726; P<0.01). The data obtained from this study clearly indicated that concentration of copper in seminal plasma negatively affects the spermatozoa motility parameters and subsequently might cause reproductive alteration in male sexual functions.

Keywords: copper, bovine spermatozoa, semen analysis, seminal plasma, motility parameters

INTRODUCTION

The natural environmental factors and differentiated anthropogenic pollutants, as well as many other sources strongly influence the reproductive material located in the semen, both in animals and humans **(Fergusson, 1990; Marzec-Wroblewska *et al.,* 2012).** Mammalian semen is known to contain a big variety of chemical elements **(Marzec-Wroblewska *et al.,* 2012)**, whose influence on spermatozoa viability has been extensively studied in animals as well as in humans **(Kanwal *et al.,* 2000; Massányi *et al.,* 2003a,b; Eghbali *et al.,* 2008; Atig *et al.,* 2012).** Some of them are crucial for a proper sperm cell function (e. g. sodium, magnesium, calcium, potassium), others are required in relatively narrow limits (zinc, manganese, copper, iron, selenium) **(Massányi *et al.,* 2003a,b; 2004; Tvrdá *et al.,* 2012).**

The possible influence of metals ions, and especially copper (Cu), on the male infertility is a matter of great interest. Copper is an important trace and essential element for the all organisms **(Craig *et al.,* 2009)**, because has a great positive role in physiological and regulatory processes **(Dobrzanski *et al.,* 1996)**. Moreover, it is a component of a number of metalloenzymes and metalloproteins **(Agarwal *et al.,* 1990)**, which are involved in energy and antioxidant metabolism **(Haliwell and Gutteridge, 2000; Aydemir *et al.,* 2006).**

Copper is a normal constituent of semen bound to the tail midpiece of spermatozoa **(Manu, 1974)** and present in seminal plasma, ampullar and seminal vesicular fluids and also released by other structures of the reproductive system (i.e. epididymis) **(Valsa *et al.,* 1994)**. Copper deficiency affects the development of sperm cells **(Van Niekerk and Van Niekerk, 1989; Leonhard-Marek, 2000)**. On the other hand, the high doses of copper ions (Cu^{2+}) have a toxic effect on the epididymis **(Xu *et al.,* 1985)**, testes, scrotum of mammals **(Skandhan, 1992; Eidi *et al.,* 2010)**, which may ultimately lead to a reduced fertility **(Pesch *et al.,* 2006)**. Increased levels of metal ions in semen **(Umeyama *et al.,* 1986)** or seminal plasma **(Stanwell-Smith *et al.,* 1983)** appear to be significantly and positively correlated with male infertility. **Meeker *et al.* (2008)** found evidence of an inverse association between high Cu levels and semen quality, which is consistent with a number of animal and human studies **(Skandhan, 1992; Huang *et al.,* 2000; Massanyi *et al.,* 2004; Yuyan *et al.,* 2007)**. Our previous *in vitro* studies evaluated the negative effects of a wide range of concentrations of Cu as a risk factor of environment on the motility of spermatozoa and subsequent pointed out a cytotoxic effect of Cu on the mitochondrial complex **(Knazicka *et al.,* 2012a,b).**

Since, that Cu plays an essential role in spermatogenesis and fertility; this study was carried out to determine relationship of seminal plasma Cu concentration and spermatozoa motility.

MATERIAL AND METHODS

Biological material

Bovine semen samples were obtained from 13 adult breeding bulls (Slovak Biological Services, Nitra, Slovak Republic). The samples had to accomplish the basic quality criteria given for the corresponding breed. The semen was obtained on a regular collection schedule using an artificial vagina. After collecting the samples, they were stored in the laboratory at room temperature (22-25 °C) and basic measurements were performed – volume (mL), pH, concentration (x10^9/mL) and osmolarity (mOsmol/kg) (Table 1). Each sample was diluted in physiological saline solution (PS) (sodium chloride 0.9% w/v, Bieffe Medital, Grosotto, Italia; pH – 5,5; osmolarity – 301 mOsmol/kg), using a dilution ratio of 1:40, depending on the original spermatozoa concentration.

Spermatozoa motility analysis

The spermatozoa motility was carried out using the Computer Assisted Semen Analysis (CASA) system – SpermVision^{TM} program (MiniTűb, Tiefenbach, Germany) with the Olympus BX 51 phase contrast microscope (Olympus, Tokyo, Japan) equipped with heating plate (37°C). Each sample was placed into the Makler Counting Chamber (depth 10 µm, Sefi-Medical Instruments, Haifa, Israel) and the following parameters were evaluated: MOT - percentage of motile spermatozoa (%; motility > 5 µm/s); PROG - percentage of progressive motile spermatozoa (%; motility > 20 µm/s); DAP - distance average path (µm); DCL - distance curved line (µm); DSL - distance straight line (µm); VAP - velocity average path (µm/s); VCL - velocity curved line (µm/s); VSL - velocity straight line (µm/s); STR – straightness (VSL:VAP); LIN – linearity (VSL:VCL); WOB – wobble (VAP:VCL); ALH - amplitude of lateral head displacement (µm) and BCF – beat cross-frequency (H_z).

Analysis of seminal plasma Cu concentration

Subsequently, after measurements the samples were centrifuged (10 min, 9500 rpm, 4 °C) to obtain the cell sediment and seminal plasma fraction (supernatant). The analysis of Cu in the seminal plasma was performed BioLaTest (PLIVA-Lachema, Brno, Czech Republic) commercial kit. The measurement was based on a colorimetric reaction between Cu(I) ions and bathocuproine (BCP) forming a stable orange coloured complex, which was easy to detect photometrically at 480 nm (Genesys 10 spectrophotometer, ThermoFisher Scientific Inc., Madison, USA). Concentrations were expressed as μM/L.

Statistical analysis

Statistical analysis of the results was carried out using the statistical program GraphPad Prism 3.02 (GraphPad Software Incorporated, San Diego, California, USA). Descriptive statistical characteristics (arithmetic mean, minimum, maximum, standard deviation and coefficient of variation) were evaluated. Pearson´s correlation coefficient (two tailed) test was used to examine correlations between all the analyzed parameters of the semen. The level of significance was set at ^{A} ($P<0.001$); ^{B} ($P<0.01$); ^{C} ($P<0.05$).

RESULTS AND DISCUSSION

Semen volume, pH, concentration, viability and motility of spermatozoa as well as composition of the seminal plasma are common parameters to assess spermatozoa quality **(Alavi and Cosson, 2006).** These factors are directly related to the fertilization success **(Bozkut *et al.*, 2009).**

The results of basic semen parameters showed that all observed characteristics were at physiological rates (Table 1). The semen has a very high buffering capacity, much higher than that of most other fluids in the body **(Meacham, 2002)**. The bovine semen maintains a slightly acidic pH **(Gamcik, 1992),** which was in accordance with our results (pH of 6.57). The semen is notable also for its high osmolarity, which is substantially higher than that of blood plasma. The osmolarity of semen depends greatly on the concentration of sugars and other organics concentrations as well as ionic salt concentrations **(Mandal and Bhattacharyya, 1987; Owen and Katz, 2005)**. In our experiment semen has a target osmolarity of 297.20 mOsmol/kg. Some researchers have noted that osmolarity increases measurably with semen aging **(Velazquez *et al.,* 1977).**

Table 1 The basic parameters of analyzed bull semen samples (n=13).

PARAMETERS	x±S.D.
pH	6.57±0.18
Spermatozoa concentration (x10^9/mL)	3.42±0.96
Semen volume (mL)	6.25±2.45
Osmolarity (mOsmol/kg)	297.20±3.44
Motility (MOT; %)	92.46±3.99
Progressive motility (PROG; %)	90.23±4.02
Seminal plasma copper concentration (μM/L)	4.28±1.47

Legend: x – arithmetic mean; S.D. – standard deviation

The seminal plasma is a reliable biological marker for evaluating vitality, sperm metabolism, motility and others relevant semen parameters **(Maxwell *et al.,* 1996; Asadpour, 2012).** Results of present study showed that the seminal plasma Cu concentration was in the range 2.14-6.89 μM/L with an average value of 4.28±1.47 μM/L. **Eidi *et al.* (2010)** examined seminal plasma levels of Cu and its relationship with human semen parameters. Their study demonstrated significant negative correlation between seminal plasma Cu concentration and pH (r_p=-0.173; P<0.05) as well as sperm concentration (r_p=-0.114; P<0.05). Subsequently, they confirmed that high concentration of Cu is related to lowering pH of seminal plasma, acidic pH, with changing condition of seminal plasma due to decrease motile or alive percent of spermatozoa. The excess Cu in seminal plasma is detrimental for male reproductive capacity by reducing spermatozoa count, motility, vitality and morphology.

The differences in opinion concerning the Cu content in seminal plasma of different species of animals were detected. The mean total Cu value of the buffalo seminal plasma in the study of **Eghbali *et al.* (2008)** was recorded as 2.51±0.04 mg/kg wet weight. Comparing with other authors we found out that according to **Massanyi *et al.,* (2003a,b)** the seminal plasma Cu concentration was significantly higher (P<0.01) in the rams (2.49±0.18 mg/kg), fox (2.16±0.53 mg/kg) than that in the bulls (1.64±0.21 mg/kg), boars (1.64±0.28 mg/kg) and stallions (0.86±0.10 mg/kg). The concentration of Cu in rabbit semen was assessed **Lukac *et al.* (2009)** on the level 20.10±4.09 mg/kg wet weight, while rabbit semen is characterized by very high Cu concentration. **Machal *et al.* (2002)** state that the mean Cu level in seminal plasma of bulls was 38.17 μM/L, which in comparison with our results is too high a concentration.

Table 2 The average values of spermatozoa motility parameters of analyzed bovine semen samples.

PARAMETERS MOTILITY	x	minimum	maximum	S.D.	CV (%)
MOT (%)	92.46	82.22	99.39	3.99	4.32
PROG (%)	90.23	80.55	96.66	4.02	4.46
DAP (μm)	37.73	29.26	54.07	5.08	13.48
DCL (μm)	60.78	47.83	88.77	8.34	13.73
DSL (μm)	31.43	23.21	47.82	4.92	15.65
VAP (μm/s)	89.42	68.47	137.70	12.76	14.27
VCL (μm/s)	143.70	110.90	208.10	20.20	14.06
VSL (μm/s)	74.83	55.93	123.40	12.21	16.32
STR	0.83	0.74	0.91	0.04	4.47
LIN	0.52	0.40	0.66	0.06	11.07
WOB	0.62	0.49	0.74	0.05	8.67
ALH (μm)	4.82	2.99	6.14	0.69	14.30
BCF (Hz)	34.65	27.41	44.60	3.36	9.70

Legend: MOT – percentage of motile spermatozoa (%); PROG - percentage of progressive motile spermatozoa (%); DAP- distance average path (μm); DCL – distance curved line (μm); DSL – distance straight line (μm); VAP- velocity average path (μm/s); VCL – velocity curved line (μm/s); VSL – velocity straight line (μm/s); STR – straightness (VSL:VAP); LIN – linearity (VSL:VCL); WOB – wobble (VAP:VCL); ALH - amplitude of lateral head displacement (μm) and BCF – beat cross-frequency (H_z).
x – arithmetic mean, S.D. – standard deviation, CV (%) – coefficient of variation

The mean value for the percentage of motile spermatozoa (quantity of movement) was 92.46±3.99%. The CASA analysis showed 90.23±4.02% of progressive motile spermatozoa (quality of movement). The other motility parameters with statistical differences are shown in the Table 2. **Eghbali *et al.* (2008)** recorded, that spermatozoa motility was 92.24±0.51% in excellent group, 81.66±0.62% in good group and moderate group 71.66±1.05%, which were significantly different.

The high concentration of Cu in seminal plasma is correlated with reduced spermatozoa motility **(Rebrelo *et al.,* 1996; Eidi *et al.,* 2010).** In our case, the correlation analysis revealed a strong negative correlation between percentage of motile spermatozoa and seminal plasma Cu concentration (r_P=-0.781; P<0.01) as well as between progressive of motile spermatozoa and Cu content in the seminal plasma (r_P=-0.726; P<0.01), which is in agreement with the report of **Eidi *et al.* (2010)** and **Akinloye *et al.* (2011).** The findings of other authors are however controversial in the comparison with our results. **Eghbali *et al.* (2008)** demonstrated a positive correlation between seminal plasma Cu concentration and spermatozoa motility with viability. **Machal *et al.* (2002)** reported a statistically significant (P<0.05) positive coefficients of correlation between the Cu concentration in seminal plasma and spermatozoa motility (r_P=0.330) and the total number of sperm cells with progressive motility (r_p=0.280). These their results correspond with the studies of **Dhami *et al.* (1994)** and **Leonhard-Marek (2000).** In a similar study, **Jockenhövel *et al.* (1990)** showed significant correlation between seminal plasma Cu concentrations and spermatozoa count, motility and normal morphology. The toxic effects of Cu on the seminal plasma are manifested in the decrease of motile spermatozoa percentage and in the increase of malformed spermatozoa **(Gamcik *et al.*, 1990; Vrzgulova *et al.*, 1993; Massanyi *et al.*, 2005).**

Table 3 The Pearson´s coefficient of correlations (r_p-values) for relationship between seminal plasma Cu concentration and selected spermatozoa motility parameters.

	Cu	MOT	PROG
Cu	1		
MOT	-0.781^{B}	1	
PROG	-0.726^{B}	0.962^{A}	1

Legend: The correlation analysis was based on the value of the correlation coefficient: ±0.111 to ±0.333: *low correlation*; ±0.334 to ±0.666: *moderate correlation*; ±0.667 to ±0.999: *high correlation*.
MOT – percentage of motile spermatozoa (%); PROG - percentage of progressive motile spermatozoa (%); $^{A}P<0.001$; $^{B}P<0.01$; $^{C}P<0.05$

CONCLUSION

The data obtained from this study clearly indicated that concentration of copper in seminal plasma negatively affects the spermatozoa motility parameters and subsequently might cause reproductive alteration in male sexual functions. Therefore, it is important to systematically evaluate its presence in the seminal plasma.

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