

Original Research Paper

# Selenium and Cadmium Tissue Concentrations and The CASA Sperm Motility Analysis After Administration to Rats

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**Abstract:** The accumulation of Selenium (Se) and Cadmium (Cd) and their effects on sperm motility parameters in rats were investigated. Male rats were dosed with Cd (group A: 2 mg kg<sup>-1</sup> b.w., intraperitoneally, group B: 30 mg L<sup>-1</sup>, *per os* in drinking water) and Se (group D: 2 mg kg<sup>-1</sup> b.w., intraperitoneally, group E: 5 mg L<sup>-1</sup>, *per os* in drinking water). 36 h after an intraperitoneal (i.p.) and after 90 days of per oral (p.o.) administration of Cd and Se, the samples of liver, kidney, adipose tissue and muscle tissue (*m. quadriceps femoris*) were collected and content of Cd was analyzed using an Electrothermal Atomic Absorption Spectrometry (ETAAS) and Se using a Hydride Generation Atomic Absorption Spectrometry (HG-AAS) methods. Sperm motility parameters were performed using a Computer-Assisted Sperm Analysis (CASA) method. A significant increase in Cd ( $p < 0.0001$ ) and Se ( $p < 0.01$ ) in liver, Cd ( $p < 0.05$ ) in kidney and adipose tissue and Cd ( $p < 0.0001$ ) and Se ( $p < 0.01$ ) in muscle tissue were found after an i.p. administration of Cd. After the per oral Cd administration, increases in Cd ( $p < 0.0001$ ) in liver, Cd and Se ( $p < 0.0001$ ) in kidney, Se in adipose tissue ( $p < 0.05$ ) and in muscle tissue ( $p < 0.0001$ ) contents were observed. In group D, increase in Se in liver and kidney ( $p < 0.0001$ ), in muscle ( $p < 0.05$ ) and the decrease in kidney Cd contents ( $p < 0.0001$ ) were found. Significant increase in Se in liver, kidney, adipose tissue ( $p < 0.0001$ ) and in muscle ( $p < 0.01$ ) contents were recorded in group E. All evaluated sperm motility parameters significantly decreased in groups B, D and E. Intraperitoneal administration of Cd caused the significant decrease in the motility ( $p < 0.01$ ), progressive motility ( $p < 0.001$ ), Straightness (STR) and Beat-Cross Frequency (BCF) ( $p < 0.05$ ) in exposed males. The concentration of selenium in tissues changes in relation to cadmium intake and vice-versa. Increased intake of Se negatively affects the sperm motility. Cd intake affected the sperm motility more significantly after a per oral administration than that exposed intraperitoneally. Both elements can increase their levels in the organism and it can result to symptoms of reduced male fertility or infertility.

**Keywords:** Selenium, Cadmium, Accumulation, Sperm Motility, CASA

## Introduction

Cadmium (Cd) is an extremely toxic metal commonly found in industrial workplaces. Exposure to cadmium occurs as a result of atmospheric emission during Cd production and processing, from combustion of fossil energy sources, waste and sludge, phosphate fertilizers and deposition of waste and slag at disposal sites. Meat, fish and fruits generally contain up to 50 µg Cd/kg fresh weight, whereas vegetables, potatoes and grain products may contain up to 150 µg Cd/kg

fresh weight. Higher concentrations are found in the kidneys of animals slaughtered for food, in wild mushrooms and in seafood such as mussels and oysters (Fried and Rozman, 2008).

Cadmium has a strong preferential affinity for the liver and the kidney over a wide range of exposure levels. In general, about 50% of the total body burden is found in these two organs. The kidney and liver are considered to be the major organs which accumulate cadmium and are probably the most susceptible organs to cadmium effects (Kim *et al.*, 2013; Yuan *et al.*, 2014;

Roggeman *et al.*, 2014). Cadmium causes tissue damage in humans and animals and many toxicological studies have found the functional and structural changes in the kidneys, liver, lungs, bones, ovaries and fetal effects (Massanyi *et al.*, 2007; Martiniakova *et al.*, 2011; Stolakis *et al.*, 2013; Oh *et al.*, 2014; Dkhil *et al.*, 2014; Wallin *et al.*, 2014). Several studies have illustrated that the testis is exceedingly sensitive to cadmium toxicity, likely due to disruption of the blood-testis barrier via specific signal transduction pathways and signaling molecules (Siu *et al.*, 2009). Exposure to cadmium has been reported to induce testicular and epididymal damage (De Souza Predes *et al.*, 2010; Toman *et al.*, 2011) and may contribute to male infertility by reducing sperm quality in both humans and rodents (Benoff *et al.*, 2009; Roychoudhury *et al.*, 2010; Asadi *et al.*, 2014). Cadmium has been suggested to interact with essential metals, such as zinc, selenium and others and it then disturbs their metabolism. On the other hand, zinc and selenium are believed to be the antagonists of cadmium toxic effects (Galazyn-Sidorczuk *et al.*, 2012; Afridi *et al.*, 2014; García-Sevillano *et al.*, 2014).

Selenium (Se) is known due to its antioxidant role in living systems and therefore it is considered to be an essential element for humans and animals. However, routine selenium supplementation is not recommended if the Se intake is equal to 80 µg per day or greater (Burk, 2002). The best way of adequate selenium intake is via food and increase of its content in plants by soil or foliar application is investigated during last decade (Ducsay and Ložek, 2006; Zhao and McGrath, 2009). The treatment with Se during Cd exposure has been demonstrated to have beneficial effects on Cd-induced toxicity (El-Sharaky *et al.*, 2007; Dzobo and Naik, 2013; El-Boshy *et al.*, 2014). However, the co-effect of the trace element on the toxicity caused by Cd is not yet well studied. Alterations in selenium consumption and metabolism are reflected by changes in the activity of a selenoenzyme, glutathione peroxidase. Moreover, selenium together with genetic variations in selenoprotein genes may influence susceptibility to cancer risk (Gupta *et al.*, 2013). Selenium significantly impacts sensitive metabolic regulations of male reproductive system. In the form of selenoenzymes and selenoproteins (PHGPx/GPx4, selenoprotein P and others) is essential for testicular development and sperm functions (Shalini and Bansal, 2005). Selenium treatment significantly increased serum testosterone level that was reduced by Cd. Se treatment ameliorated Cd-induced reduction in testicular steroidogenic acute regulatory and 17β-hydroxysteroid dehydrogenase activities. The protective potential of Se against Cd-induced reproductive toxicity seems to be due to up-regulation of steroidogenic activity leading to increase in testosterone synthesis for an optimal sperm quality and spermatogenesis (Ren *et al.*, 2012). Many authors

suggest the use of selenium alone or in combination with other antioxidants for fertility promotion and treat some forms of male sub- or infertility (Safarinejad and Safarinejad, 2009; Moslemi and Tavanbakhsh, 2011). Shi *et al.* (2014) noted that dietary sodium selenite can influence the population of spermatogonial stem cells of roosters during spermatogenesis and also oxidative stress can modulate behavior of the stem cells through regulating some key factors during spermatogenesis. On the other hand, less known are effects of excess of selenium on the male reproductive system, complex mechanism of actions and relation between the results of different types of experiments. Kaushal and Bansal (2009) reported prooxidant effects of selenium on germ cells after administration of an excess of sodium selenite to the mouse in their feed.

Computer Assisted Semen Analysis (CASA) is automatic or semi-automatic sperm motility analysis system based on image analysis, providing the detailed information of more than 10 motility parameters. The main advantage of CASA consists in a precise establishment of sperm motility, eliminating also the subjectivity of the usual microscope sperm motility assessment (Rusu *et al.*, 2011).

The aim of the work was to determine the effect of cadmium and selenium administered separately on the status of both elements in selected rat tissues and their effects on the sperm motility.

## Materials and Methods

### Experimental Design

Fifty males Wistar rats were divided to five groups, two cadmium-treated groups (A and B), two selenium-treated groups (D and E) and control, untreated group (C), each containing 10 males. The males were housed in plastic cages (Tecniplast, Italy) in an environment maintained at 20-24°C, 55±10% humidity, with access to water and food (feed mixture M3, Machal, Czech Republic) *ad libitum*. Sexually mature male rats were used in groups A and D. Young, 4 weeks old males of groups B and E were used to differ the organism response to cadmium and selenium exposure in sexually mature males and those reaching the sexual maturity at the end of the experiments.

Adult males of group A were administered a single intraperitoneal (i.p.) dose (2 mg kg<sup>-1</sup> b.w.) of cadmium (CdCl<sub>2</sub>, Reachem, Slovak Republic) and young, 4 weeks old rats of group B were dosed with a daily Cd dose of 30 mg L<sup>-1</sup> in drinking water for 90 days. Adult male rats of group D were administered a single intraperitoneal dose (2 mg kg<sup>-1</sup> b.w.) of selenium (Na<sub>2</sub>SeO<sub>3</sub>, Sigma, USA) and young, 4 weeks old males of group E were dosed with a daily Se dose of 5 mg L<sup>-1</sup> in drinking water for 90 days.

### Tissue Se and Cd Content Analysis

The liver, kidney, adipose tissue and muscle tissue (*m. quadriceps femoris*) were sampled 36 h after Cd and Se intraperitoneal administration (groups A and D) and 90 days after the daily Cd and Se per oral (p.o.) intake (groups B and E). The samples were weighed and stored at -20°C and then analyzed. Cadmium was analyzed using the Electrothermal Atomic Absorption Spectrometry (ETAAS, Varian SpectrAA 220, The Netherlands) and selenium was determined using the hydride generation atomic absorption spectrometry (HG-AAS, Varian SpectrAA 220 with VGA-76 hydride generator, The Netherlands) (EL, s.r.o. Spišská Nová Ves, Slovak Republic).

### Sperm Motility Analysis

Spermatozoa obtained immediately after sacrifice of rats from the cauda epididymis were subsequently diluted with tempered (37°C) phosphate buffered saline solution (20 µL). The sample was located into the Makler counting chamber (Sefi-Medical Instruments, Germany). Analysis was realized using a CASA system SpermVision™ (Minitüb, Tiefenbach, Germany) with Olympus BX 51 (Olympus, Japan) microscope. In the samples, the following sperm motility parameters were determined: % of motile spermatozoa, % of progressive motility, 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, Hz).

### Statistical Analysis

The values of control and experimental animal analyses were expressed as mean ± SD. The results were analyzed by one-way Analysis of Variance (ANOVA) followed by Scheffe's test for *post hoc* comparisons using

statistical software Stata 9 (StataCorp LP, TX, USA). Differences were considered significant at  $p < 0.05$ .

## Results

### Cd and Se Tissue Content

Rats exposed to cadmium showed significant accumulation of cadmium in liver, kidney and muscle tissue when dosed intraperitoneally and in liver and kidney after per oral administration (Table 1). Selenium content significantly increased in liver and muscle tissue after the i.p. exposure to 2 mg Cd/kg body weight and also significantly increased in kidney, adipose and muscle tissues after the daily per oral intake of 30 mg Cd/L in drinking water during 90 days. In our study, selenium administration significantly ( $p < 0.0001$ ) decreases the cadmium concentration in the kidney in the intraperitoneally exposed group. The selenium content significantly increased in liver, kidney and muscle tissue after the i.p. Se administration and in all observed tissues in the per oral selenium exposed group (Table 1).

### Sperm Motility

Results of sperm motility parameters are shown in Table 2. For quantitative analysis of motility changes, CASA was performed after cadmium or selenium exposure to rats. When males were injected with single intraperitoneal dose of cadmium, total motility and progressive motility decreased significantly ( $p < 0.01$  and  $p < 0.001$ , respectively), accompanied by significant decline in STR and BCF parameters ( $p < 0.05$ ). Rats exposed to cadmium in drinking water for 90 days showed more significant changes in all measured sperm motility parameters than those in group A. All motility parameters decreased significantly when compared to untreated control.

In contrast, a single intraperitoneal dose of selenium had no significant effect on the sperm motility except of BCF which decreased significantly ( $p < 0.05$ ). In case of per oral intake of selenium in drinking water for 90 days, there were similar results recorded like after cadmium per oral administration.

Table 1. Cd and Se concentration in selected rat tissues after the cadmium and selenium administration (mg/kg)

Group		Liver	Kidney	Adipose tissue	Muscle tissue
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control group (C)	Cd	0.006±0.001	0.012±0.002	0.005±0.001	0.006±0.001
	Se	1.085±0.262	1.527±0.239	0.137±0.048	0.250±0.001
Group A	Cd	20.27±9.30****	11.30±12.9*	1.17±1.47	0.088±0.051****
	Se	2.775±1.378**	1.781±1.207	0.179±0.165	0.32±0.077*
Group B	Cd	0.065±0.025****	0.284±0.153****	0.011±0.016	0.013±0.025
	Se	1.292±0.22	2.46±0.538****	0.398±0.331*	0.607±0.113****
Group D	Cd	0.068±0.003	0.006±0.0019****	0.005±0.001	0.005±0.001
	Se	3.105±1.09****	7.23±4.00****	0.122±0.062	0.304±0.061*
Group E	Cd	0.010±0.009	0.012±0.007	0.005±0.001	0.006±0.002
	Se	3.266±1.271****	4.256±0.977****	0.424±0.112****	0.423±0.149**

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\*\* $p < 0.0001$ ; SD-Standard Deviation

Table 2. Sperm motility parameters in rats after the cadmium and selenium administration

Parameter	Group C <i>Mean±SD</i>	Group A <i>Mean±SD</i>	Group B <i>Mean±SD</i>	Group D <i>Mean±SD</i>	Group E <i>Mean±SD</i>
Motility (%)	50.31±9.15	24.47±18.36 **	16.72±20.38***	39.23±22.82	12.16±6.23***
Progressive motility (%)	27.69±7.34	10.16±9.73***	6.97±15.23**	19.45±14.92	3.87±3.69***
DAP (µm)	21.95±3.29	23.03±6.53	10.39±9.61**	21.70±5.00	8.63±7.24***
DCL (µm)	33.91±4.98	37.77±11.42	16.14±14.57**	33.71±8.12	13.54±10.14***
DSL (µm)	17.65±2.15	16.39±4.16	7.65±6.36***	16.87±3.58	6.99±5.75
VAP (µm/s)	52.73±9.25	56.75±16.76	25.43±24.69**	54.46±12.30	21.16±17.52***
VCL (µm/s)	81.10±13.88	92.76±30.83	39.57±37.33**	84.34±19.85	33.05±24.34***
VSL (µm/s)	42.37±5.92	40.57±10.58	18.76±16.28**	42.31±8.67	17.29±14.10***
STR (%)	0.81±0.04	0.74±0.08*	0.35±0.24***	0.78±0.05	0.40±0.28***
LIN (%)	0.53±0.04	0.49±0.12	0.24±0.18***	0.53±0.05	0.28±0.20**
WOB (%)	0.65±0.03	0.64±0.11	0.31±0.23***	0.66±0.03	0.33±0.25***
ALH (µm)	4.52±1.26	5.57±1.06	2.52±1.93*	5.54±0.95	2.49±2.00*
BCF (Hz)	22.37±2.71	19.81±1.46*	7.99±5.79***	17.92±3.08*	7.66±5.91***

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; SD-Standard Deviation

All parameters of sperm motility significantly decreased in comparison with the control group.

## Discussion

The kidney and liver are recognized as the main cadmium storage tissues (Toman and Massányi, 1996; Jihen *et al.*, 2008; Kolesarova *et al.*, 2008; Roggeman *et al.*, 2014). Excessive exposure to both elements causes increase in their contents in the internal organs. In our experiments, cadmium accumulated in liver and kidney after i.p. and p.o. administration of Cd and also in muscle tissue after i.p. injection of this metal. Selenium increased in similar way after Se exposure but it was also found in adipose tissue of per oral selenium group in concentration significantly higher than that of the control. Selenium appears to antagonize cadmium, especially in acute exposures. In a mouse study, after acute cadmium exposure, a significant decrease in cadmium levels was observed in the kidneys and liver following an eight-week daily selenium supplementation (Jamba *et al.*, 1997). Meyer *et al.* (1982) reported that increased cadmium intake increases cadmium and zinc levels and decreases iron concentration linearly. Also hepatic copper concentration tended to decrease with cadmium supplementation. In our experiment, selenium content significantly increased in liver and muscle tissue after the i.p. exposure to 2 mg Cd/kg body weight and also significantly increased in kidney, adipose and muscle tissues after the daily per oral intake of 30 mg Cd/L in drinking water during 90 days. It suggests that increasing intake of cadmium mobilizes the selenium storage in the tissues which can be manifested by its increase in the main storage organs, but also in the muscle tissue. Similar result was reported by (Ognjanovic *et al.*, 2008) who have found that increased Cd concentration in the liver and kidneys caused increases in Se concentration, although it was not administered additionally. Selenium is known to induce the synthesis of metal-binding protein,

Metallothionein (MT), in tissues. Liu *et al.* (2014) suggested that the selenium is more potent in induction of MT and was a stronger inducer than ZnSO<sub>4</sub> in its effective dose range. Therefore selenium may be developed into a novel and safe MT inducer. This action can be a reason of the selenium increase in tissue content after the cadmium enters the body.

Selenium was found to have a protective effect by decreasing Cd content in the liver and kidneys (Chen *et al.*, 1975). In fact, intraperitoneal administration of selenium caused the significant decrease in Cd content in kidney in our experiment. However, it has also been observed that simultaneous administration of cadmium and selenium (200 ppm and 0.1 ppm, respectively) in drinking water for five weeks did not decrease Cd concentration in the liver and kidney and only affected the toxic effects of Cd in these organs (Jihen *et al.*, 2008). Dietary selenium did not affect the concentration of cadmium in tissues in our experiment. Similarly, no decrease in cadmium, zinc, iron or copper in rat liver was found after selenium intake in food (Meyer *et al.*, 1982). Deficiency of the element in animals makes them susceptible to injury by certain types of oxidative stress (Burk, 2002). This protection includes the capability of Se to alter the distribution of Cd in tissues and induces binding of the Cd-Se complexes to proteins, which are similar to metallothioneins (Jamba *et al.*, 1997; Combs and Gray, 1998; Ognjanovic *et al.*, 2008). The lipid peroxidation, one of the main manifestations of the oxidative damage, plays an important role in the toxicity of many xenobiotics. Intoxication with cadmium causes a significant increase of lipid peroxidation in liver and kidneys of rats (Ognjanovic *et al.*, 2008) which are also the main organs cumulating the cadmium. Therefore, increase in selenium content in these organs can be connected with the selenium protective role in oxidative stress induced by cadmium.

Cadmium and selenium are known to affect the male fertility. Cd is well known for its negative effects on male reproductive system (De Souza Predes *et al.*,

2010), semen quality (Telisman *et al.*, 2000; Xu *et al.*, 2003; El-Demerdash *et al.*, 2004; Bennetts and Aitken, 2005; Thompson and Bannigan, 2008; Benoff *et al.*, 2009; Tvrdá *et al.*, 2013) and testes structural changes and disruption of spermatogenesis (Siu *et al.*, 2009; Jahan *et al.*, 2014).

On the other hand, low doses of elements such as Se may have protective effects on male reproductive functions (Benoff *et al.*, 1997; Olson *et al.*, 2005) and may eliminate the harmful effects of Cd or other metals (Telisman *et al.*, 2000; Xu *et al.*, 2003). Increase in concentrations of these elements in organism may interact with testis functions and spermatogenesis as well as with sperm morphology and motility. During the past decades, the quality and fertility potential of sperm have decreased dramatically. Sperm motility has a high correlation with fertility and is an early and sensitive endpoint for evaluating chemical effects on male fertility (Lifeng *et al.*, 2006). The efficacy of CASA has been demonstrated for use with a variety of species in assessing male reproductive quality as well as the impact of various treatments on sperm motility; CASA allows an objective assessment of different cell characteristics: Motion, velocity and morphology (Verstegen *et al.*, 2002).

Our results obtained by motion analysis in group A (i.p. cadmium injection) revealed a significant decline in the percentage of motile spermatozoa ( $p < 0.01$ ), percentage of spermatozoa with progressive motility ( $p < 0.001$ ) and significant decrease in STR and BCF ( $p < 0.05$ ). These findings confirm the positive relationship between cadmium levels and sperm motility decrease, supporting the hypothesis that environmental cadmium exposures may contribute significantly to reduced sperm motility (Jeng *et al.*, 2014). Chronic exposure to environmentally relevant cadmium may result in dose- and time-dependent decreases in sperm count and sperm motility (Benoff *et al.*, 2009). Decline in sperm motility negatively affects the fertilization success (Gist *et al.*, 2000). We confirm, that in group B (per oral Cd exposure), the sperm motility was affected more seriously than that in the intraperitoneal Cd group (A). All motility parameters significantly declined in comparison to control group (Table 2). Spermatids are transformed into sperm during spermatogenesis by different formation processes. Disruption at this stage of development can cause impairment of sperm condensation, motility and morphology (De Jager *et al.*, 2006; Roychoudhury *et al.*, 2010). The decrease in sperm motility may be explained by cadmium effects on microtubules and sperm mitochondrial function (Oliveira *et al.*, 2009). The impairment of the structures of the sperm tail may result to the sperm motility decrease.

Evaluation of semen quality parameters in group D (intraperitoneal Se administration) has shown that selenium was not able to affect the sperm motility significantly. The only significant change in this group

was the BCF decrease. Selenium in excess causes oxidative stress in testicular germ cells, damage of RNA and DNA, reduction of the expression of genes required for synthesis of proteins essential for spermatogenesis (Kaushal and Bansal, 2009; Ranawat and Bansal, 2009). Optimal production of spermatozoa in rats occurs first in the 45th postnatal days and optimal production is achieved first in the 75th days (Russell, 1992). Consequently, subchronic administration of selenium in our design of experiment might potentially influence also testicular development. All evaluated parameters of semen quality in group E (per oral Se exposure) significantly decreased, similarly like in group with per oral administration of cadmium.

Reduction in motility parameters like STR and BCF has damaging effects on sperm motility. Important Velocity Parameters (VSL, VCL and VAP) directly express sperm motion and decline in sperm velocity, percentage of motile sperm, BCF and ALH parameters can also adversely affect fertility (Kato *et al.*, 2001; Ban *et al.*, 1999). ALH is calculated from the amplitudes of the lateral deviations of sperm head about the axis of progression (Mukhopadhyay *et al.*, 2010). It is a valuable measurement, as this is one of the parameters affecting the outcome of *in vitro* fertilization and sperm penetration ability (Verstegen *et al.*, 2002). Moreover, reduction in other motility parameters like STR and BCF has damaging effects on sperm motility and are indicators of sperm vigor (Duty *et al.*, 2004). Negative correlations were detected between Se concentrations in seminal plasma and total sperm head defects and between Se concentrations in serum and VAP, VSL, STR and LIN in cats (Villaverde *et al.*, 2014). Authors suggested that selenium along with zinc were the potential candidates for male fertility markers. The protective effects of selenium in sperm include Reactive Oxygen Species (ROS) neutralization. One of the antioxidant system enzymes in the semen is Glutathione Peroxidase (GPx) containing selenium in the form of selenocysteine. In sperm it is located mainly in the mitochondrial matrix (Pecker *et al.*, 1997). A specific selenoenzyme, sperm nuclei Glutathione Peroxidase (snGPx) protects of sperm DNA against oxidative damage and might be more efficient in ROS degradation (Pfeifer *et al.*, 2001). However, Lovercamp *et al.* (2013) noted that supplementing a basal diet with organic or inorganic selenium did not affect semen quantity or sperm quality in fresh ejaculates in boars nor did it appear to have any beneficial latent effects in extended semen stored post collection.

All motility parameters are closely related to sperm mitochondrial section, site of energy production. Kaur and Parshad (1994) reported morphological damage of mitochondrial section of spermatozoa after per oral feeding of 4 ppm sodium selenite to the rats. These results

support our findings of reduced sperm motility parameters. Data suggest that the mitochondrial section might be directly or indirectly the target site of spermatozoon damage caused by selenium.

## Conclusion

Increased intake of cadmium caused redistribution of selenium in the main storage organs, liver and kidney and in adipose and muscle tissues. On the other hand, selenium intake decreased Cd content in the kidney. Increased cadmium and selenium intake and accumulation in the organism resulted in significant decline in sperm motility variables, including the most important velocity parameters. The results indicate that excessive per oral cadmium and selenium intake decreases the sperm motility in rats more significantly than after a single intraperitoneal dose.

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## Conflict of Interest

The researchers have no any potential conflict of interest.

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## Author's Contributions

**Robert Toman:** Designed and supervised the research, participated in all experiments, coordinated the data analysis and contributed to the writing of the manuscript.

**Svatoslav Hluchy:** Participated in all experiments and contributed to the writing of the manuscript.

**Peter Massanyi and Norbert Lukac:** Realized the CASA analysis and contributed to the writing of the manuscript.

**Maria Adamkovicova and Michal Cabaj:** Participated in all experiments, executed the statistical tests and contributed to the writing of the manuscript.

**Zuzana Hajkova:** Collected the tissue samples and executed the statistical tests.

## Ethics

All experiments and procedures were carried out under the approval by the Ethical Committee and State Veterinary and Food Administration of the Slovak Republic, No. Ro 811/07-221.

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