

## The influence of smoking on semen quality, seminal microelements and $\text{Ca}^{2+}$ -ATPase activity among infertile and fertile men

T.A. Kumosani<sup>a</sup>, M.F. Elshal<sup>a,\*</sup>, A.A. Al-Jonaid<sup>a</sup>, H.S. Abduljabar<sup>b</sup>

<sup>a</sup> Department of Biochemistry, Faculty of Science, King Abdulaziz University, P.O. Box: 80203 Jeddah, 21589, Saudi Arabia

<sup>b</sup> Department of Obstetric and Gynecology, King Abdulaziz University Hospital, Jeddah, Saudi Arabia

Received 15 December 2007; received in revised form 17 June 2008; accepted 13 July 2008

Available online 5 August 2008

### Abstract

**Objective:** Tobacco smoking is now increasing rapidly throughout the developing world and is one of the biggest threats to current and future world health. Several studies have addressed the role of cigarette smoking on semen quality, but the exact mechanisms remain inconclusive. In order to evaluate the detrimental effects of smoking on semen quality among Saudi subjects, the levels of different seminal parameters in smokers were compared to non-smokers.

**Patients and methods:** A total of 159 semen samples (61 smokers and 98 non-smokers) from men attending an infertility clinic for routine infertility workup were sub-grouped into fertile or infertile and were compared based on standard semen analysis (according to WHO guidelines), content of metals (magnesium, zinc and cadmium) and plasma membrane  $\text{Ca}^{2+}$ -ATPase activity of sperms.

**Results:** Cadmium concentration was found significantly higher in smokers than in non-smokers either in fertile or infertile group ( $2.9 \pm 0.4$  vs  $1.4 \pm 0.7$ ;  $2.9 \pm 0.5$  vs  $1.3 \pm 0.7 \mu\text{g L}^{-1}$ ; respectively). Together with this increase in seminal Cd a significant decrease in  $\text{Ca}^{2+}$ -ATPase activity ( $21.5 \pm 2.8$  vs  $33.71 \pm 1.2$ ;  $20.7 \pm 1.5$  vs  $35.07 \pm 2.9 \text{ mmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ,  $p < 0.05$ ), decrease in seminal zinc ( $109.8 \pm 8.1$  vs  $189.7 \pm 9.9 \text{ mg L}^{-1}$ ,  $p < 0.01$ ) and decrease in sperm motility ( $41.9\% \pm 2.9$  vs  $46.01\% \pm 2.5$ ;  $9.8\% \pm 2.4$  vs  $15.3\% \pm 2.7$ ,  $p < 0.05$ ) were found.

**Conclusion:** Our data demonstrate that cigarette smoking affects both  $\text{Ca}^{2+}$ -ATPase activity and motility of the spermatozoa. These effects may be attributed to increased seminal cadmium and reduced zinc concentrations.

© 2008 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.

**Keywords:** Semen quality; Sperm; Smoking; Motility; Morphology; Magnesium; Zinc; Cadmium;  $\text{Ca}^{2+}$ -ATPase activity

### Introduction

Major changes in the seminal fluid components appear to be related to abnormal spermatozoal function and fertilizing capacity [1]. Several studies over the past two decades have suggested that the quality of semen is declining in industrialized countries throughout the world, arousing concern about male fertility in the future. That decline in semen quality including a decrease in sperm concentration and semen volume in normal men over a relatively short period of time suggests that the cause (s) are more likely to be environmental rather than genetic [2].

Studies have revealed the presence of upwards of 4000 chemicals in cigarettes; many are toxic and around 40 cause cancer [3]. Many of these chemicals may be referred to as toxic

heavy metals [4]. Among them, cadmium is of major interest since it is tightly associated with respiratory stress and injures of the respiratory tract, lung cancers, nephropathy and proteinuria, osteomalacia and osteoporosis and male infertility [5–10].

Several experimental studies in rats and mice have provided evidence that Cd disrupts spermatogenesis, damages supporting testicular tissue, and reduces male fertility [11–13]; but the mechanisms that lead to these effects remain unknown. Cadmium toxicity in animal cells has been endorsed by interference with essential metals including calcium, magnesium and zinc which are primary determinants of sperm cell function [14–17].

$\text{Ca}^{2+}$  is considered a prime regulator of sperm motility, capacitation and in the initiation of the acrosome reaction processes [18–21]. In addition, Feng and coworkers found that calcium plays a role in cell growth and differentiation during spermatogenesis [22]. Whereas Zn is thought to be one of the factors that affect spermatozoa motility in seminal plasma, it

\* Corresponding author.

E-mail address: [melshal2002@yahoo.com](mailto:melshal2002@yahoo.com) (M.F. Elshal).

exerts this effect by controlling  $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$ -ATPase enzyme activity [15].

The objective of the present study is to study the effect of smoking on the quality of semen and to investigate the possible relationship between semen quality and the concentration of microelements specially cadmium, magnesium and zinc. We also aimed at investigating the effect of smoking on plasma membrane  $\text{Ca}^{2+}$ -ATPase of sperms of fertile and infertile subjects.

## Materials and methods

This study were undertaken in Jeddah, Kingdom of Saudi Arabia and lasted for 18 months. A total number of 159 randomly recruited men attending an infertility clinic for routine infertility workup were included in the study. They were divided according to their cigarette smoking habit into 61 smokers and 98 non-smokers. Seventy subjects were considered infertile since they had no child after a period of unprotected intercourse for more than one year and had an abnormal semen analysis (Table 1). The rest 89 subjects were considered fertile since their female partner had conceived within the last two years. A detailed background history and physical examination were done on subjects.

Semen specimens were collected through masturbation after 3 days abstinence. Semen samples were incubated for 30 min at 37 °C for liquefaction. A routine semen analysis was performed upon liquefaction according to WHO to measure volume, pH, sperm concentration, motility and morphology [23]. The remaining semen sample was centrifuged at 1000 ×g for 10 min; the seminal plasma was separated for three equal parts and stored at -80 °C until further analyses.

Determination of magnesium (Mg), calcium (Ca), zinc (Zn) and cadmium (Cd) was carried out according to a method of Beaty using Perkin Elmer Atomic absorption spectrophot-

ometer model 3100 with the HGA-600 Graphite Furnace, and the Model 3100 Enhanced Data System [24]. Plasma membrane  $\text{Ca}^{2+}$ -ATPase activity of sperm was determined by measuring of inorganic phosphate (Pi), released into seminal plasma from ATP according to the method of Lin and Morales [25].

## Statistical analysis

Statistical analysis of the data was performed using software SPSS v. 13.0 for Windows (SPSS Inc., Chicago, IL). Student's *t*-test was used to check significant differences between groups with variables normally distributed; when not, a non-parametric Mann-Whitney *U*-test was performed. Correlation tests were performed by the Pearson or Spearman correlation coefficient following the distribution of the variables. Values of  $p < 0.05$  were considered significant. All results were expressed as mean ± standard error mean (SEM).

## Results

The age of fertile subjects ranged from 26 to 42 years with mean of  $33.9 \pm 2.7$  years; whereas the age of the infertile subject ranged from 29–45 years with mean of  $37.1 \pm 2.0$  years. There was no correlation between age, weight, diet, place of birth, smoking type, seminal fluid pH with other parameters studied.

The number of coitus of fertile subjects ranged from 2 to 6 times per week with mean of  $4 \pm 2$  times; whereas the number of coitus of the infertile subjects ranged from 1 to 5 times per week with mean of  $3 \pm 2$  times. The difference was significant between the two groups ( $p < 0.001$ ).

The mean value of seminal fluid volume did not show any significant difference between smokers and non-smokers in both fertile and infertile groups. For fertile group, spermatozoa count did not differ significantly between smokers and non-smoker. In other hand, the number of sperms in the infertile smokers group was significantly lower than that of the infertile non-smokers group (Table 1).

The motility of spermatozoa was significantly lower in fertile smokers and infertile smokers than that of fertile non-smokers and infertile non-smoker group (Table 1). The mean value of sperm abnormal morphology and seminal fluid pH did not show any significant difference between smokers and non-smokers in both fertile and infertile groups (Table 1).

Smoking was found associated with significantly reduced level of zinc and significantly increased cadmium levels in fertile and infertile groups (Table 1). Meanwhile, the seminal content of magnesium and calcium did not reach statistical significant difference between smokers and non-smokers in neither the fertile nor the infertile groups (Table 1). There was a significantly lower plasma membrane  $\text{Ca}^{2+}$ -ATPase activity in smokers than non-smokers either in fertile or infertile subjects (Fig. 1).

## Discussion

Cigarette smoking is an important cause of increased mortality and morbidity in developed countries and the

Table 1  
Effects of smoking on seminal parameters and microelements in fertile and infertile subjects

Parameters	Fertile (N=89)		Infertile (N=70)	
	SM (n=23)	Non-SM (n=66)	SM (n=38)	Non-SM (n=32)
Age (years)	34.9±1.8	33.5±0.9	37.1±1.4	37.1±1.2
Body weight (kg)	72.3±14.5	69.7±17.7	75.8±17.2	78.1±19.4
Volume (mL)	3.05±0.3	3.3±0.2	2.8±1.9	2.4±1.3
Seminal Ph	7.9±0.2	7.8±0.2	7.7±0.3	7.8±0.2
Count (million)	114.0±7.9	130.1±8.1	3.0±0.7*	5.3±1.13
Motility (%)	41.9±2.9*	46.0±2.5	9.8±2.4*	15.3±2.7
Abnormal morphology (%)	47.7±3.6	42.3±2.4	66.1±5.6	61.8±5.9
Magnesium (µg L <sup>-1</sup> )	123.2±6.3	130.2±1.3	132.3±2.5	132.9±2.4
Calcium (µg L <sup>-1</sup> )	21.7±3.1	18.6±5.2	19.5±2.7	18.1±3.1
Cadmium (µg L <sup>-1</sup> )	2.9±0.4**	1.4±0.7	2.9±0.5**	1.3±0.7
Zinc (mg L <sup>-1</sup> )	109.8±1.1**	189.7±3.8	107.3±1.7**	170.9±0.9

SM = smokers.

Non-SM = non-smokers.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

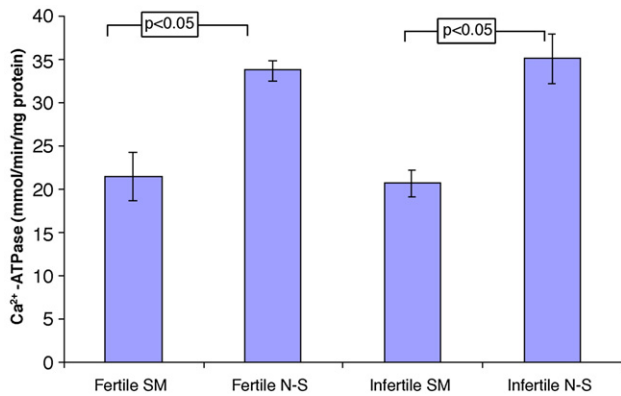


Fig. 1. Spermatozoal plasma membrane Ca<sup>2+</sup>-ATPase activity in fertile smokers, non-smokers; and infertile smokers, non-smokers.

prevalence is increasing in the developing world as well. Fertility problems are more likely in couples who smoke and maternal smoking in pregnancy is associated with intrauterine growth retardation [26].

In Saudi Arabia, a recent survey has showed that 22.3% (34% males, 11.1% females) of 1505 adolescents were found current cigarette smokers, 5.8% of them (11.1% males, 0.7% females) were daily smokers [27]. Numerous studies were carried out in Saudi Arabia to demonstrated the effect of smoking on health [28–36], however, no study investigated the effect of tobacco smoking on male infertility.

Several investigations have been conducted on the relationship between cigarette smoking and male infertility, but few studies have indicated the direct effect of heavy metals on semen quality. Cigarette smoke contains up to 6.67 µg cadmium/cigarette [37]. For a 70-kg human, this data indicates that up to 13 µg/kg cadmium per week could be inhaled from smoking 20 cigarettes per day [38]. Recently, a study on human subjects has found that cigarette smoking enhances the levels of Cd and Pb in seminal plasma and blood and the extent of oxidative damage associated with a decrease in components of the anti-oxidant defenses in the sperm of infertile males [39]. Moreover, Chia and his team found that cigarette smoking appears to affect sperm density, especially in heavy smokers and they concluded that cadmium present in cigarettes could be a possible causative agent for the low sperm density among smokers [40].

In the present study, a significant increase in cadmium concentration was found in smokers compared to non-smoker either in fertile or infertile subjects; that increase was accompanied by a decrease in sperm quality (count, and motility), seminal zinc, seminal magnesium ion concentrations and plasma membrane Ca<sup>2+</sup>-ATPase activity of sperms. The data presented here is consistent with that of Omu and coworkers. They suggested that the high levels of cadmium in smokers with asthenozoospermia were evidence of the possible toxic effect of this trace element and this may be one of the causes of asthenozoospermia [1]. In addition, our results are consistent with other research groups found the mean cadmium concentrations in seminal plasma of normospermic patients were higher in smokers compared with non-smokers [41–43].

Calcium plays a predominant role in regulating many functional processes of spermatogenesis and fertilization including acrosome reaction and motility [44,45]. Ca<sup>2+</sup> gradients across the plasma membrane, required for Ca<sup>2+</sup> homeostasis and signaling, are maintained in part by plasma membrane Ca<sup>2+</sup>-ATPase activity [22,46]. In somatic cells, Ca<sup>2+</sup>-ATPase activity was found to be affected by heavy metals specially cadmium. Cadmium induces extensive membrane damage including a significant decrease in the activities of membrane bound ATPases (Na<sup>+</sup>/K<sup>+</sup>-ATPase, Ca<sup>2+</sup>-ATPase, Mg<sup>2+</sup>-ATPase), the indicators of membrane function in testis, lymphocytes, muscle cells, liver, and kidney [47–49]. Moreover, cadmium ion has been found to be a non-competitive inhibitor of red cell Ca<sup>2+</sup>-ATPase activity [50].

Our finding that Ca<sup>2+</sup>-ATPase activity is significantly reduced in smokers which was associated with increased cadmium concentration and decreased motility suggests an association of smoking and sperm motility. Based on these data, our results may support previous data [45] on the role of plasma membrane Ca<sup>2+</sup>-ATPase activity in the regulation of spermatozoa motility.

Cadmium is a natural antagonist to zinc [42,43]. Zinc is essential for living organisms as more than 300 enzymes require Zn for their activity including that of the oxidant defense system [51]. It also plays an important role in the DNA replication, transcription, and protein synthesis, influencing cell division and differentiation [52,53]. Zinc deficiency or depletion is regarded as a grave side effect of cigarette smoking [54]. In addition, it is suggested that the reduction in seminal zinc secretion may jeopardize the content of chromatin zinc, and thereby the stability of the sperm chromatin. This may then contribute to reproductive failure or have consequences for fetal development [14,54]. On the other hand, Zinc therapy was found to improve sperm quality by modulating the putative effect of cadmium through its enhancement of T-helper 2 cytokine expression (seminal IL-4) and down-regulation of T-helper 1 cytokines (TNF-alpha and IFN-gamma) [55].

Our data showed a significant decrease in zinc ion concentration among smokers group compared with non-smoker group irrespective of their fertility status which challenge with the result obtained by Eggert-Kruse et al. that showed no statistically significant relationship of zinc in seminal plasma or serum with semen quality parameters [56].

Moreover, Zn has been reported to play an important role in the regulation of Ca<sup>2+</sup>-pumps through its ability to control Ca<sup>2+</sup>-ATPase activity that regulate intracellular Ca<sup>2+</sup> in animal cells [15–17]. In the present study, the lower seminal Zn levels in the smokers were associated with lower plasma membrane Ca<sup>2+</sup>-ATPase activity of sperms. These results are in agreement with that of Eberle and coworkers who found that zinc deficiency caused a significant decline in the activity of the Ca–Mg-ATPase in erythrocytes membranes [57]. Taken together, these data may support previous data on the effect of zinc on sperm motility, sperm chromatin integrity and capacitation.

Similar to zinc, magnesium is a crucial element in cell physiology which is present in a high concentration in semen. Magnesium is important for nearly all enzymatic systems, as it modifies specific enzyme substrates and plays a fundamental

role as a cofactor in more than 300 enzymatic reactions involving energy metabolism (ATP synthesis and utilization) and nucleic acid synthesis [58]. In addition, magnesium may play a role in spermatogenesis, in particular in sperm motility [59]. In agreement with previous results by Wong and coworkers, we found insignificant decrease in Mg concentrations in fertile smokers than fertile non-smokers that was consistent with reduced spermatozoa motility [59].

In summary, we suggest that the deleterious effects of smoking on semen quality especially spermatozoa motility may be due to changes occurred in plasma membrane  $\text{Ca}^{2+}$ -ATPase activity of sperms which is involved in maintaining motility of the spermatozoa. These effects may be attributed to increased seminal content of cadmium and reduced seminal zinc concentration.

## References

- [1] Omu AE, Dashti H, Mohamed AT, Mattappallil AB. Significance of trace elements in seminal plasma of infertile men. *Nutrition* 1995;11:502–5.
- [2] Giwerman A, Carlsen E, Keiding N, Skakkebaek NE. Evidence for increasing incidence of abnormalities of the human testis: a review. *Environ Health Perspect* 1993;101:S65–71.
- [3] Bernhard D, Rossmann A, Wick G. Metals in cigarette smoke. *IUBMB Life* 2005;57:805–9.
- [4] Järup L. Hazards of heavy metal contamination. *Br Med Bull* 2003; 68:167–82.
- [5] Waalkes MP. Cadmium carcinogenesis. *Mutat Res* 2003;533:107–20.
- [6] Benoff S, Hurley IR, Barcia M, Mandel FS, Cooper GW, Hershlag A. A potential role for cadmium in the etiology of varicocele-associated infertility. *Fertil Steril* 1997;67:336–47.
- [7] Yang JM, Arnush M, Chenc QY, Wu XD, Pang B, Jiang XZ. Cadmium-induced damage to primary cultures of rat Leydig cells. *Reprod Toxicol* 2003;17:553–60.
- [8] Chia SE, Ong CN, Lee ST, Tsakok FH. Blood concentrations of lead, cadmium, mercury, zinc, and copper and human semen parameters. *Arch Androl* 1992;29:177–83.
- [9] Lin FJ, Fitzpatrick JW, Iannotti CA, Martin DS, Mariani BD, Tuan RS. Effects of cadmium on trophoblast calcium transport. *Placenta* 1997; 18:341–56.
- [10] He J, Reynolds K, Chen J, et al. Cigarette smoking and erectile dysfunction among Chinese men without clinical vascular disease. *Am J Epidemiol* 2007;166:803–9.
- [11] Laskey JW, Rehnberg GL, Laws SC, Hein JF. Reproductive effects of low acute doses of cadmium chloride in adult male rats. *Toxicol Appl Pharmacol* 1984;73:250–5.
- [12] Clark JT, Jimenez B, Evans SL, Barrow R, Winfree M, Mrotek JJ. Cadmium-induced sexual dysfunction does not involve increased hepatic metabolism of testosterone nor increased circulating levels of corticosterone. *Physiol Behav* 1994;56:975–81.
- [13] Waalkes MP, Rehm S, Devor DE. The effects of continuous testosterone exposure on spontaneous and cadmium-induced tumors in the male Fischer (F344/NCr) rat: loss of testicular response. *Toxicol Appl Pharmacol* 1997;142:40–6.
- [14] Bertelsmann H, Sieme H, Behne D, Kyriakopoulos A. Is the distribution of selenium and zinc in the sublocations of spermatozoa regulated? *Ann N Y Acad Sci* 2007;1095:204–8.
- [15] Karacagil M, Sade M, Türkyilmaz RK. The effect of zinc, arginine, fructose and seminal supernatant of normal semen on the triple adenosine triphosphatase activities of the spermatozoa from males with oligoasthenozoospermia. *Andrologia* 1985;17:383–8.
- [16] Martelli A, Rousselet E, Dycke C, Bouron A, Moulis JM. Cadmium toxicity in animal cells by interference with essential metals. *Biochimie* 2006; 88:1807–14.
- [17] Serres C, Feneux D, Berthon B. Decrease of internal free calcium and human sperm movement. *Cell Motil Cytoskeleton* 1991;18:228–40.
- [18] Ren D, Navarro B, Perez G, et al. A sperm ion channel required for sperm motility and male fertility. *Nature* 2001;413:603–9.
- [19] Publicover S, Harper CV, Barratt C.  $[\text{Ca}^{2+}]_i$  signalling in sperm — making the most of what you've got. *Nat Cell Biol* 2007;9:235–42.
- [20] Williams KM, Ford WC. Effects of Ca-ATPase inhibitors on the intracellular calcium activity and motility of human spermatozoa. *Int J of Androl* 2003;26:366–75.
- [21] Schuh K, Cartwright EJ, Jankevics E, et al. Plasma membrane  $\text{Ca}^{2+}$ -ATPase 4 is required for sperm motility and male fertility. *J Biol Chem* 2004;279:28220–6.
- [22] Feng HL, Hershlag A, Han YB, Zheng LJ. Localizations of intracellular calcium and  $\text{Ca}^{2+}$ -ATPase in hamster spermatogenic cells and spermatozoa. *Microsc Res Tech* 2006;69:618–23.
- [23] WHO. Laboratory Manual for the Examination of Human Semen Cervical Mucus Interaction. WHO. Cambridge: The Press Syndicate of the University of Cambridge; 1987.
- [24] Beatty DR. Concepts, instrumentation and techniques in atomic absorption spectrophotometry. USA: Perkin-Elmer Corporation; 1988.
- [25] Lin TI, Morales MF. Application on the one-step procedure for measuring inorganic phosphate in the presence of proteins the actomyosin ATPase system. *Anal Biochem* 1977;77:10.
- [26] Kapoor D, Jones TH. Smoking and hormones in health and endocrine disorders. *Eur J Endocrinol* 2005;152:491–9.
- [27] Abdalla AM, Al-Kaabba AF, Saeed AA, Abdulrahman BM, Raat H. Gender differences in smoking behavior among adolescents in Saudi Arabia. *Saudi Med J* 2007;28:1102–8.
- [28] Al-Fayez SF, Salleh M, Ardawi M, Zahran FM. The relationship between carboxyhaemoglobin levels and hematological values in sheesha smokers. *Trop Geograph Med* 1989;40:115–23.
- [29] Al-Nuaim AR. High prevalence of metabolic risk factors for cardiovascular diseases among Saudi population, aged 30–64 years. *Int J Cardiol* 1977;62:227–35.
- [30] Al-Saleh IA. Lead exposure in Saudi Arabia and its relationship to smoking. *Biometals* 1995;8:243–5.
- [31] Al-Senaidey AM, Al-Zahrany YA, Al-Faqeeh MB. Effects of smoking on serum levels of lipid peroxides and essential fat-soluble antioxidants. *Nutr Health* 1997;12:55–65.
- [32] Al-Tamimi TM, Al-Bar A, Al-Suhaimi S, et al. Lung cancer in the eastern region of Saudi Arabia: a population based study. *Ann Saudi Med* 1996;16:3–11.
- [33] Amer MH. Epidemiological aspects of oesophageal cancer in Saudi Arabian patients. *King Faisal Spec Hosp Med J* 1985;5:69–78.
- [34] Bener A, Al-Frayh A, Ozkaragoz F, Al-Jawadi TQ. Passive smoking effects on wheezy bronchitis. *Ann Saudi Med* 1993;13:222–5.
- [35] Hakim JG, Osman AA, Kler TS, Tajuddin MR, Rahman M, Kyaw MT. Acute myocardial infarction in a region of Saudi Arabia—the Gizan experience. *Saudi Med J* 1991;12:391–6.
- [36] Schultz H, Ezzat A, Allam A, Gray A. Smoking and health pp. New insights and recent developments. *Ann Saudi Med* 1998;18:1–5.
- [37] Smith CJ, Livingston SD, Doolittle DJ. An international literature survey of “IARC Group I carcinogens” reported in mainstream cigarette smoke. *Food Chem Toxicol* 1997;35:1107–30.
- [38] Saaranen M, Kantola M, Saaroski S, Vanha-Perttula T. Human seminal plasma cadmium pp. comparison with fertility and smoking habits. *Andrologia* 1989;21:140–5.
- [39] Kiziler AR, Aydemir B, Onaran I, et al. High levels of cadmium and lead in seminal fluid and blood of smoking men are associated with high oxidative stress and damage in infertile subjects. *Biol Trace Elem Res* 2007; 120:82–91.
- [40] Chia SE, Xu B, Ong CN, Tsakok FM, Lee ST. Effect of cadmium and cigarette smoking on human semen quality. *Int J Fertil Menopausal Stud* 1994;39:292–8.
- [41] Keck C, Bramkamp G, Behre HM, Muller C, Jockenhovel F, Nieschlag E. Lack of correlation between cadmium in seminal plasma and fertility status of non-exposed individuals and two cadmium-exposed patients. *Reprod Toxicol* 1995;9:35–40.



- [42] Telisman S, Jurasovic J, Pizent A, Cvitkovic P. Cadmium in the blood and seminal fluid of non-occupationally exposed adult male subjects with regard to smoking habits. *Intl Arch Occup Environ Health* 1997; 70:243–8.
- [43] Telisman S, Cvitkovic P, Jurasovic J, Pizent A, Gavella M, Rocic B. Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc and copper in men. *Environ Health Perspect* 2000; 108:45–53.
- [44] Triphan J, Aumüller G, Brandenburger T, Wilhelm B. Localization and regulation of plasma membrane  $\text{Ca}^{2+}$ -ATPase in bovine spermatozoa. *Eur J Cell Biol* 2007; 86:265–73.
- [45] Breitbart H, Rubinstein S, Nass-Arden L. The role of calcium and  $\text{Ca}^{2+}$ -ATPase in maintaining motility in ram spermatozoa. *J Biol Chem* 1985; 252:60:11548–53.
- [46] Jimenez-Gonzalez C, Michelangeli F, Harper CV, Barratt CL, Publicover SJ. Calcium signalling in human spermatozoa pp. a specialized ‘toolkit’ of channels, transporters and stores. *Hum Reprod Update* 2006; 12: 253–67.
- [47] Murugavel P, Pari L. Diallyl tetrasulfide modulates the cadmium-induced impairment of membrane bound enzymes in rats. *J Basic Clin Physiol Pharmacol* 2007; 18:37–48.
- [48] Passia D, Hilscher B, Abel J, Haider SG, Hilscher W, Redi C. Metallothionein synthesis in liver and kidney and enzymohistochemical changes in the testis induced by cadmium treatment. *Acta Histochem Suppl* 1985; 31:159–65.
- [49] Tokushige A, Higashino H, Searle BM, et al. Cadmium effect on the Na,K-ATPase system in cultured vascular smooth muscle cells. *Hypertension* 1984; 6:20–6.
- [50] Visser GJ, Peters PH, Theuvenet AP. Cadmium ion is a non-competitive inhibitor of red cell  $\text{Ca}^{2+}$ -ATPase activity. *Biochim Biophys Acta* 1993; 1152:26–34.
- [51] Powell SR. Antioxidant properties of zinc. *J Nutr* 2000; 130:1447s–54s.
- [52] Frederickson CJ. Neurobiology of zinc and zinc containing neurons. *Int Rev Neurobiol* 1989; 31:145–238.
- [53] Chia SE, Ong CN, Chua LH, Ho LM, Tay SK. Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men. *J Androl* 2000; 21:53–7.
- [54] Oldereid NB, Thomassen Y, Purvis K. Seminal plasma lead, cadmium and zinc in relation to tobacco consumption. *Int J Androl* 1994; 17:24–8.
- [55] Al-Bader A, Omu AE, Dashti H. Chronic cadmium toxicity to sperm of heavy cigarette smokers pp. immunomodulation by zinc. *Arch Androl* 1999; 43:135–40.
- [56] Eggert-Kruse W, Zwick EM, Batschulat K, et al. Are zinc levels in seminal plasma associated with seminal leukocytes and other determinants of semen quality. *Fertil Steril* 2002; 77:260–9.
- [57] Eberle J, Schmidmayer S, Erben RG, Stangassinger M, Roth HP. Skeletal effects of zinc deficiency in growing rats. *J Trace Elem Med Biol* 1999; 13:21–6.
- [58] Abou-Shakra FR, Ward NI, Everard DM. The role of trace elements in male infertility. *Fertil Steril* 1989; 52:307–10.
- [59] Wong WY, Flik G, Groenen PM, et al. The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men. *Reprod Toxicol* 2001; 15:131–6.