

## **IMPACT OF SEMINAL LEAD AND CADMIUM ON FERTILITY CAPACITY OF MALES WITH IDIOPATHIC OLIGOASTHENOTERATOZOOSPERMIA**

*BY*

**Nagwa M. Ghandour, Emad A. Taha\*, Sohair K. Sayed \*\*,  
Ali M. Mahran\*\*\*, Medhat A. Saleh\*\*\*\* and Taymour Mostafa\*\*\*\*\***

*Departments of Forensic Medicine and Clinical Toxicology; Dermatology and Andrology\*; Clinical Pathology \*\*;  
Andrology, Venereology and Sexology\*\*\* Public Health and Community Medicine \*\*\*\*, Assiut University,  
and Andrology and Sexology \*\*\*\*\*, Cairo University, Egypt*

### **ABSTRACT**

*The aim of this study was to evaluate lead and cadmium levels in seminal plasma of men with idiopathic oligoasthenoteratozoospermia in comparison to fertile healthy controls and to correlate these levels with conventional semen parameters, sperm hypo-osmotic swelling (HOS) percentage, sperm DNA fragmentation percentage, and semen reactive oxygen species (ROS) levels. Sixty infertile male patients with idiopathic oligo and/or asthenoteratozoospermia and sixty healthy fertile men (control group) were included in the study. Lead and cadmium levels in seminal plasma, semen parameters, sperm HOS, sperm DNA fragmentation percentage and semen ROS assay were measured in all subjects. There was a significant increase in seminal lead and cadmium levels among infertile males in comparison to controls. There were significant negative correlations between seminal lead and cadmium levels on one hand and certain semen parameters especially progressive sperm motility and vitality (HOS). Importantly, significant positive correlations were noted between seminal lead and cadmium levels on one hand and sperm DNA fragmentation percentage and semen ROS level in infertile men and controls on the other hand. Thus, men with idiopathic male infertility had higher levels of lead and cadmium in their semen which correlated with impairment of sperm motility and vitality percentages and more importantly with higher sperm DNA fragmentation% and semen ROS level.*

### **INTRODUCTION**

Toxicological exposure to lead and cadmium is a public health problem due to the broad exposure to these toxic substances among the general population (González-Estecha et al., 2011). The improper disposal of household, industrial,

and hospital waste, industrial pollution, air pollution and the use of chemicals in agriculture have compromised the quality of air, water and soil (El-zohairy et al., 1995).

Globally, human fecundity appears to be on the decline, a situation that cannot

be attributed solely to an increase in contraception (Benoff et al., 2009). While the exact causes of the decline in semen quality are not yet known, environmental factors have been considered to play an important role (Li et al., 2010). Many environmental agents have been postulated to be contributory to this decline in male reproductive health (Skakkebaek et al., 2001). Studies of the effects of environmental agents on semen quality have largely focused on organic toxicants with potential endocrine disrupting activity, but heavy and transition metal endocrine disruptors may be influential as well (El-Shafei et al., 2011).

Lead (Pb) and Cadmium (Cd) are two of the known reproductive toxicants to which humans are exposed either occupationally or environmentally (Xu et al., 2003; Wirth and Mijal, 2010). Pb has been known to affect multiple organs and can affect reproduction in males and females (Anis et al., 2007; Fatima et al., 2010). Several studies suggested that the testis and reproductive organs may be exquisitely sensitive to Cd that may lead to profound testicular damage without affecting other organ systems (Takiguchi and Yoshihara, 2006). Moreover, Cd and Pb were demonstrated to be elevated in the seminal plasma and testes of infertile men with or without varicocele (Benoff et al., 2000; Benoff and Gilbert., 2001).

Abnormalities in the male genome characterized by damaged sperm DNA may be indicative for male factor infertility regardless of routine semen parameters (Sadek et al., 2011). Xu et al. (2003) demonstrated that exposure to heavy metals was associated with oxidative DNA damage in human sperm. However, most of these studies were based on occupational exposure or in smokers while few studies were concerned with the environmental exposure that may have more long term impacts on human health, particularly in a country with non-existent or unenforced occupational safety and health standards (Inhorn et al., 2008).

Male factor accounts for nearly half of all infertility cases (WHO, 2000) The term "idiopathic infertility" designates diagnosis by exclusion, after elimination of all other possible or probable causes of infertility. In idiopathic male infertility, female partner must be evidently free from any cause of infertility, semen conventional parameters are subnormal and men clinical examination reveal no specific etiology as varicocele, maldescended testes, male accessory gland infection, hypogonadism...etc (Sigman et al., 1997). These patients represent the largest group of men attending fertility clinics. They are classified according to semen findings by the terminology, of idiopathic oligo, astheno and/or teratozoospermia (Nieschlag and Leifke, 2000).

This study aimed to evaluate the level of Pb and Cd in seminal plasma of men with idiopathic oligoasthenoteratozoospermia in comparison with fertile controls; correlate these levels with conventional sperm parameters especially sperm concentration, sperm progressive motility, vitality and sperm morphology percentages; and clarify possible relation between these levels and sperm DNA damage and semen ROS levels.

### ***MATERIAL AND METHODS***

It is a case control hospital based study. It was conducted during December 2009 to December 2010.

#### **Subjects :**

Sixty oligoasthenoteratozoospermia matched men from Assuit city were recruited randomly from the Andrology Unit, of Dermatology and Andrology Department, Assiut University Hospital. Exclusion criteria included; varicocele, genital infection, undescended testis, testicular atrophy, chronic systemic diseases, female factor infertility and smoking. We meant to exclude all factors that might impair semen quality to highlight the impact of Pb and Cd on semen parameters. They were compared with 60 matched healthy men with proven fertility as controls. Among patients and controls those with special habits or occupational exposure to heavy metals were excluded.

The Scientific Research Ethics Committee of Assiut Faculty of Medicine approved the study. The steps and aim of the research were explained to participants before signing an informed consent.

The cases were subjected to history taking, clinical examination and semen analysis. In their semen, sperm hypoosmotic swelling (HOS) test, sperm DNA fragmentation, ROS, Cd as well as Pb concentrations were assessed.

#### **1- History taking and clinical examination :**

Each participant completed an extensive questionnaire regarding his occupation, residence, social status and smoking habits. Full detailed medical history was taken from all participants with special emphasis on reproductive history. They were also subjected to thorough general medical and genital examination.

#### **2- Conventional semen analysis :**

All semen samples were collected by masturbation in polypropylene containers after three to five days of sexual abstinence. After liquefaction at 37°C, conventional semen analysis was carried out according to World Health Organization guidelines (WHO, 1999). The evaluation included semen volume, sperm concentration, sperm morphology, and sperm motility; taking in consideration: liquefaction

time, pH, odour, viscosity, and presence of pus or epithelial cells.

### **3- Sperm hypo-osmotic swelling (HOS) test** (Al-Mogazy et al., 1993)

It was performed by mixing 0.1 ml sperm suspension with 1 ml hypoosmotic solution (equal parts of 150 mOsm/kg fructose and 150 mOsm/kg sodium citrate), followed by incubation for 30 minutes at 37°C. After incubation, 200-300 spermatozoa were examined by phase-contrast microscopy at 400 x. where HOS-reacted sperms with swollen or curled tails was calculated.

### **4- Sperm DNA fragmentation** (Wald et al., 2004)

The sperm DNA fragmentation index was performed on flow-cytometry model PAS DAKO-Cytomation by the kit supplied by Coulter (DNA Prep, BECKMAN-COULTER Inc. Fullerton, CA, USA) in the Clinical Pathology Department, Faculty of Medicine, Assiut University, based on the fluorescence emission from individual sperm cells after staining with propidium iodide (PI) and excitation with a 488 nm argon laser. The measurement is based upon the ability of PI to bind histochemically to DNA under appropriate staining conditions. Semen samples were diluted with phosphate buffered saline (PBS) with pH 7.4 to  $2 \times 10^6$  sperm/ml (in semen samples with sperm concentration higher than 2 mil/ml). Fifty micro liters (50  $\mu$ L) of se-

men samples were incubated in a tube with 100  $\mu$ l of lysing reagent for 15 seconds then two ml of PI were added and mixed with them and immediately after staining, tube acquisition was done by flow-cytometry. The intensity of the fluorescence emission corresponds to the DNA content. Sperm DNA fragmentation percentage was calculated after acquisition of 5000 sperms. Flowcytometry display a constant bimodal non-artifactual DNA pattern confirming the existence of two distinct populations, the main population is represented by a peak followed by a shoulder which is the marginal population. The marginal population represents a sperm group altered in the nuclear condensation (DNA fragmentation), yielding unstable chromatin which appears more stainable (Figures 1, 2).

### **5- Semen ROS level** (Wang et al., 2003)

ROS levels were measured by detecting the chemiluminescence activity using the luminol "5-amino-2, 3 dihydro-1, 4 phtalazindione" reagent ( $C_8H_7N_3O_2$ ) supplied by (MP Biomedicals, Eschwege, Germany). Liquefied semen specimens were centrifuged at 300 rpm for 7 minutes and the supernatant seminal plasma was removed and placed into sterile acid washed, metal-ion free tube, and stored frozen at -20°C for metal analysis. The pellet was washed twice with phosphate buffer saline (PBS, pH 7.4) by centrifugation at 300 g for 5 min and re-suspended in

PBS at a concentration of  $20 \times 10^6$  sperm/ml. Ten ml of luminol used as a probe was added to the aliquot. ROS levels were assessed by measuring chemiluminescence activity with an Autolam-at Luminometer (Berthold technologies, Bad-wildbad, Germany) in the integrated mode for 15 min. The results were expressed as Relative Light Unit (RLU)/20 million spermatozoa.

#### 6- Metal analysis (Pant et al., 2003)

One ml of seminal plasma was digested twice with 5 ml of acid mixture ( $6\text{HNO}_3$ :  $1\text{HClO}_4$ ) in a glass tube. Semen samples with inadequate volume were diluted and multiplied by dilution factor. The residue was dissolved in 1ml 1%  $\text{HNO}_3$  then applied to air-acetylene flame atomic absorption spectrophotometer (Buck model 210 VGP, Buck Scientific, Inc, Norwalk CT, USA) with hollow cathode lamp (8 mA current), from Chemistry Department, Faculty of Science, Assiut University for detection of Pb (wavelength 283.2nm) and Cd (wavelength 228.9nm). A sample blank was prepared with each set of samples to control for possible metals contamination from external sources. Two determinations were made for each sample. The accuracy and precision of the analytical methods were tested with standard reference materials were obtained from Sigma Chemical Co. (St.Louis, Mo, USA).

#### Statistical analysis

The data was analyzed using SPSS software package version 17 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were done in the form of mean and SD. Unpaired t-test was used to compare numerical parametric data where Mann-Whitney test was used to compare non parametric data. Pearson correlation test analyzed correlations between different quantitative variables.  $P < 0.05$  was set as statistically significant.

### RESULTS

The patients and controls were comparable as regards age ( $34.63 \pm 6.47$  vs  $32.5 \pm 6.1$  years), body mass index ( $24.27 \pm 3.09 \text{ kg/m}^2$  vs  $25.84 \pm 2.4 \text{ kg/m}^2$ ). They all belonged to the same environment. Among patients group, 46 men were presented with primary infertility with a mean duration of  $7.1 \pm 3.8$  years and 14 men were presented with secondary infertility with a mean duration of  $5.6 \pm 2.3$  years.

Semen volume, sperm concentration, normal sperm morphology progressive sperm motility, and HOS percentages showed significant decrease while sperm DNA fragmentation percentage showed significant increase in infertile men semen samples compared with the controls (Table 1). Seminal plasma lead and cadmium levels showed significant in-

crease in infertile men compared with the controls (Table 2).

In infertile oligoasthenoteratozoospermia (OAT) men, there was significant positive correlation between either seminal Pb or Cd levels with sperm DNA fragmentation percentage ( $r=0.754$ ,  $P=0.001$ ,  $r=0.898$ ,  $P=0.001$ ), seminal ROS level ( $r=0.749$ ,  $P=0.001$ ,  $r=0.891$ ,  $P=0.001$ ) (figures 3,4). On the other hand, they had significant negative correlation with sperm concentration ( $r=-0.377$ ,  $P=0.003$ ,  $r=-0.382$ ,  $P=0.003$ ), sperm motility ( $r=-0.732$ ,  $P=0.001$ ,  $r=-0.813$ ,  $P=0.001$ ), sperm vitality ( $r=-0.742$ ,  $P=0.001$ ,  $r=-0.838$ ,  $P=0.001$ ), normal sperm morphology ( $r=-0.714$ ,  $P=0.001$ ,  $r=-0.820$ ,  $P=0.001$ ).

### DISCUSSION

Several studies have indicated a worldwide decreasing trend in average sperm count and sperm quality (Li et al., 2010), raising the possibility of a causative role for environmental exposures such as heavy metals (Danadevi et al., 2003). About 25 % of infertile male patients are classified according to semen findings by the terminology, to idiopathic oligo, astheno and/or teratozoospermia (Sigman et al., 1997; Nieschlag and Leifke, 2000). At least half of these cases of idiopathic male infertility may be attributable to various environmental and occupational exposures (Akinloye et al., 2006).

Previous studies concerned with the effect of Pb and Cd on reproductive function in males focused on passive changes induced by exposure to heavy metals on histology and function of testes in animals (Cheng and Mruk, 2002; Martynowicz et al., 2005; Rodríguez-Estival et al., 2011), or semen parameters; in humans and animals (Saarenen et al., 1989; Xu et al., 1993; Kuo et al., 1997; Alexander et al., 1998; Pant et al., 2003), with fewer studies on the effect of these metals on pregnancy rate. Although blood tests are standard procedure for toxicological study of heavy metals exposure, recent data indicated that they may not adequately reveal heavy metals accumulation in the male reproductive tract. Consequently, seminal heavy metals concentrations could provide a better measure of reproductive toxicity caused by heavy metals exposure (Wu et al., 2008).

In the present study, patients with idiopathic oligo and/or asthenoteratozoospermia were thoroughly investigated to exclude any possible factor that might impair semen parameters. This allowed highlighting the impact of basic environmental exposure to heavy metals (Pb and Cd) on the reproductive function. This had been illustrated through comparison of semen parameters and levels of heavy metals in those patients versus fertile controls.

The current study revealed a significant increase in seminal Pb and Cd levels in semen of patients with idiopathic oligo and/or asthenoteratozoospermia compared with the fertile controls. This is consistent with results of different studies from different countries (Kuo et al., 1997; Alexander et al., 1998; Pant et al., 2003; Akinloye et al., 2006; Wu et al., 2008).

Semen volume was significantly lower in infertile oligo and / or asthenoteratozoospermia men compared with the controls. This might be due to the adverse effect of high level of heavy metals in seminal plasma which is secreted from prostate and seminal vesicles. Different studies reported impaired secretory function of accessory sexual glands by Pb and Cd exposure in either experimental animals (Cullen et al., 1993) or humans (Wildt et al., 1983; Xu et al., 1993; Alexander et al., 1998; Telisman et al., 2000; Akinloye et al., 2006; Acharya et al., 2008).

There were significant decreases in sperm concentration and total sperm count in patients compared to controls in this study, with a significant negative correlation found between semen Pb and Cd and sperm concentration. Kuo et al. (1997) as well as Alexander et al. (1998) observed an inverse relationship between Pb concentration and sperm concentration in lead battery workers. Pant

et al. (2003) added a significant negative correlation between seminal Pb and Cd concentration and sperm concentration in oligoasthenteratozoospermic men. Moreover, Xu et al. (2003), Akinloye et al. (2006) and Wu et al. (2008) reported a significant inverse correlation between Cd and semen quality.

Cheng and Mruk (2002), Martynowicz et al. (2005) and Rodríguez-Estival et al. (2011) pointed out that the negative impact of these metals on sperm concentration and count might be due to their deleterious effect on testicular structure and function. On the other hand, Saarenen et al. (1989) reported no association between seminal Pb or Cd concentration and sperm concentration (in healthy men of general population and in smokers respectively). Similarly, Xu et al. (1993) failed to establish a relationship between heavy metal seminal concentration and semen quality.

There was negative correlation between seminal Pb and Cd levels on one hand and normal sperm morphology percentage on the other hand. However, these correlations were significant only with semen Pb in patients. Leoni et al. (2002) reported positive correlation between Cd level in blood and abnormal sperm morphology, also Acharya et al. (2008) found a decrease in the percentage of sperm cells with normal morphology in mice injected

with Cd. Similar results were reported in another two studies on animals exposed to Cd (El-Demerdash et al., 2004; Oliveira et al., 2009).

There was a significant negative correlation between seminal Pb and Cd levels and progressive sperm motility and vitality, this is going with results of Pant et al. (2003). Leoni et al. (2002) suggested that sperm motility is a sensitive parameter to Cd toxicity. Cd was shown to disturb microtubules sliding and assembly (Kanous et al., 1993), to affect sperm mitochondrial function and structure (Oliveira et al., 2009), to compete with calcium for calmodulin binding; which is important for sperm motility resulting in decreased sperm motility (El-Demerdash et al., 2004; Schlingmann et al., 2007). Moreover, Cd may lead to premature acrosome reaction in sperms by competition for calcium binding sites (Leoni et al., 2002; Oliveira et al., 2009). Lately, Mendiola et al. (2011) reported positive association between the percentage of immotile spermatozoa and seminal plasma levels of Pb and Cd.

There was negative significant correlation between sperm vitality as measured by HOS on one hand and each of Pb and Cd on the other hand. This is consistent with prior findings of the effect of Cd on sperm viability in vitro which showed that the vitality of ram sperm was significantly

affected by exposure to Cd (Leoni et al., 2002). The oxidative stress induced by Pb and/or Cd may explain the reduction in sperm vitality (Kiziler et al., 2007; Hammadh et al., 2008).

Sperm DNA integrity evaluation and semen ROS assay are recognized as measures of sperm fertilizing capacity that may have better diagnostic and prognostic capabilities than standard sperm parameters (Sharma et al., 1999; Saleh et al., 2002). One of the main findings of this study was that there was a significant positive correlation between ROS level and the seminal plasma level of Pb and Cd. Results from previous studies support these findings (Kiziler et al., 2007; Ognjanovic et al., 2010). Pb and Cd are known to induce the production of nitric oxide (NO) and ROS; mostly superoxide anion radical ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radical (OH.); affecting sperm motility by peroxidation of membrane lipids reducing phosphorylation of axonemal proteins or by reducing ATP levels (de Lamirande and Gagnon, 1992; Droge, 2002; Hernández-Ochoa et al., 2006; Kiziler et al., 2007; Oliveira et al. 2009). Furthermore, they are associated with a decrease in components of the anti-oxidant defenses in the sperm of infertile males (Kiziler et al., 2007), and DNA damage in sperms (Ognjanovic et al., 2010).

There was a significant positive corre-



lation between sperm DNA fragmentation percentage and seminal Pb and Cd. Previously, Xu et al. (2003) suggested that oxidative DNA damage in human sperm is related to seminal Cd which is consistent with this study. However, their data did not conclusively indicate that Pb in semen induces oxidative DNA damage in human sperm. This might be due to the lower mean concentrations of Pb and Cd in seminal plasma of their patients compared to that among patients and controls of this study.

Others reported that Pb induces alterations in sperm chromatin structure in occupationally / environmentally exposed men (Wildt et al., 1983; Hernández-Ochoa et al., 2005), smokers (Hsu et al., 2009) and laboratory animals (Moriwaki et al., 2008). Only Hernández-Ochoa et al. (2006) pointed out that Pb reaches the sperm nucleus in the epididymis by binding to nuclear sulfhydryl groups from DNA-protamine complex delaying the nuclear decondensation in vitro that might be the cause for fertilization failure observed after Pb exposure. Also, these authors suggested that Pb compromise on sperm chromatin structure depends on the timing of its incorporation into sperm nuclei during spermatogenesis, epididymal maturation

or even at ejaculation.

As in case of Pb, studies also reported that Cd exposure induced sperm DNA fragmentation in animals (Moriwaki et al., 2008; Oliveira et al., 2009) and in smokers (Potts et al., 1999; Zenzes, 2000). Monsefi et al. (2010) suggested that the mechanism by which Cd increases DNA fragmentation is through inhibiting sperm chromatin condensation reflecting the defective chromatin packaging during spermiogenesis, generating ROS with oxidative DNA. However, Sergerie et al. (2000) showed that presence of Cd in semen or blood was not related with increased sperm DNA fragmentation.

It is concluded that infertile oligoasthenoteretozospermia men had higher levels of seminal Pb and Cd correlated with impaired seminal variables, semen ROS level and sperm DNA fragmentation. This may reflect a higher concentration in the reproductive tract of those men and an increased susceptibility of impaired fertility capacity secondary to basic environmental exposure to heavy metals. Further long-term studies in larger-sized random population are needed to evaluate the effect of heavy metals and other environmental toxins on male reproductive capacity.

**Table (1):** Comparison between semen variables in the investigated groups.

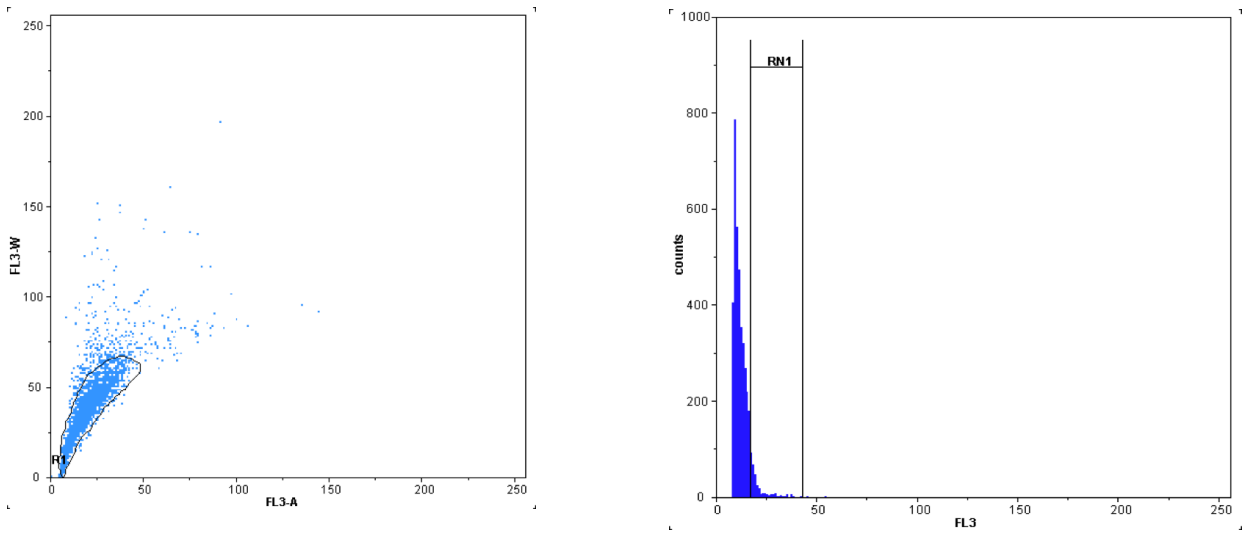
Semen variables	Infertile patients (n=60)	Control group (n=60)	P-value
Semen volume (ml)			
Range	1 - 4	2 - 4	
Mean $\pm$ SD	2.08 $\pm$ 0.84	2.52 $\pm$ 0.53	<0.05
Sperm Concentration (mil/ml)			
Range	2 - 150	20 - 220	
Median	20	65	<0.01*
Mean $\pm$ SD	53.4 $\pm$ 56.02	78.83 $\pm$ 53.51	
Normal sperm morphology (%)			
Range	0 - 60	50 - 77	
Mean $\pm$ SD	34.83 $\pm$ 12.51	63.53 $\pm$ 6.46	<0.001
Progressive sperm motility (%)			
Range	0 - 40	40 - 65	
Mean $\pm$ SD	23.17 $\pm$ 11.7	53.33 $\pm$ 7.23	<0.001
Sperm HOS test (%)			
Range	20 - 75	60 - 90	
Mean $\pm$ SD	56.77 $\pm$ 14.64	79.77 $\pm$ 7.04	<0.001
Sperm DNA fragmentation (%)			
Range	6.8 - 25.5	3.5 - 11.4	
Mean $\pm$ SD	13.95 $\pm$ 6.210	6.24 $\pm$ 1.85	<0.001
Semen ROS level (**RLU)			
Range	1229 - 7600	180 - 898	
Mean $\pm$ SD	3062 $\pm$ 1625	359.83 $\pm$ 217.66	<0.001

\* Mann-Whitney test

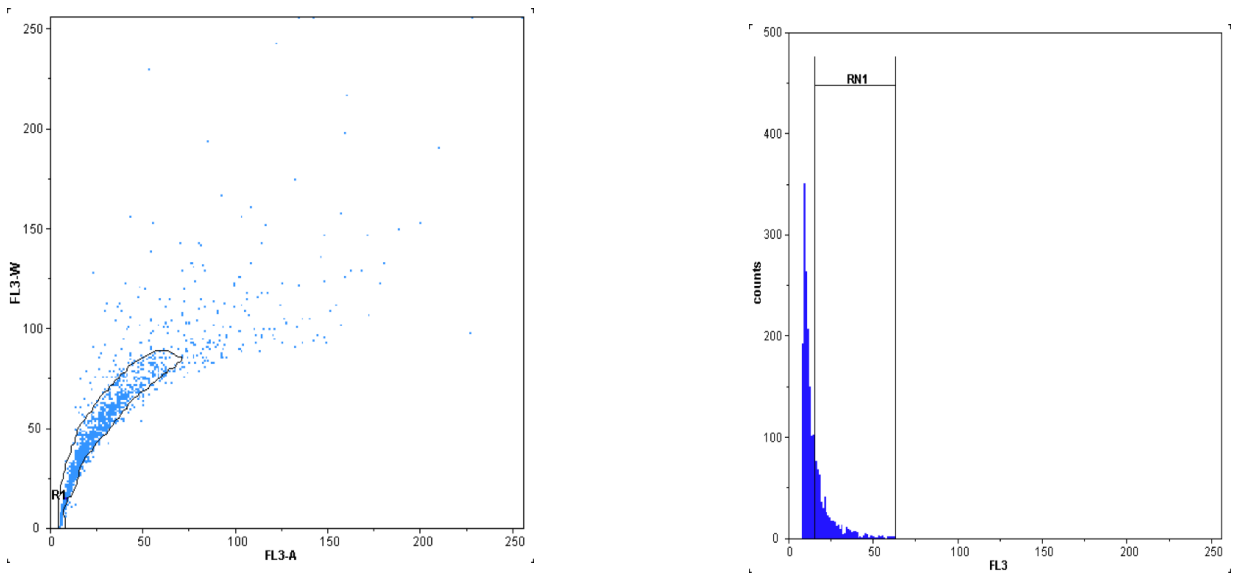
\*\* RLU = Relative Light Unit

**Table (2) :** Semen lead and cadmium levels in the investigated groups.

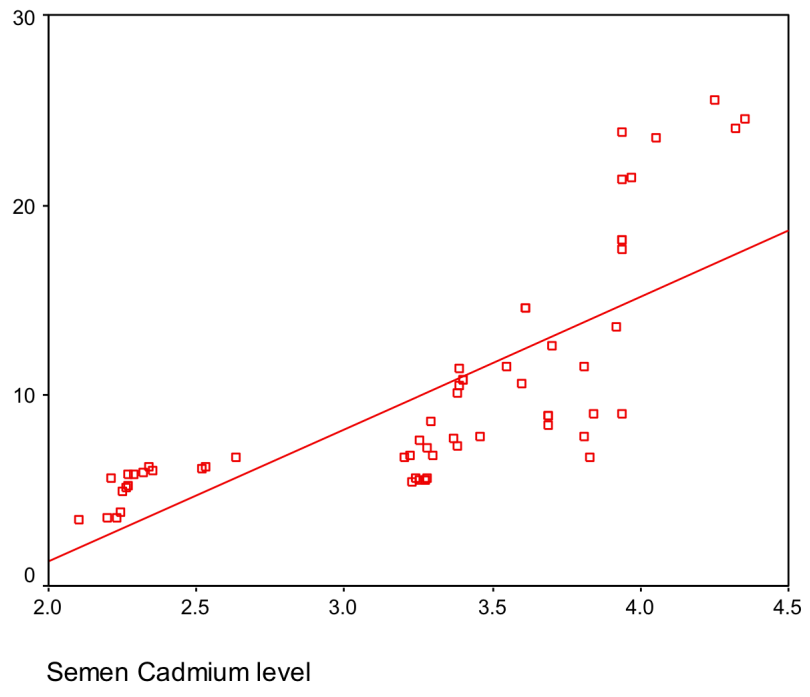
Semen heavy metals level	Infertile patients (n=60)	Control group (n=60)	P-value
Semen lead ( $\mu\text{g/L}$ )			
Range	32 - 60	17 - 28	
Mean $\pm$ SD	44.4 $\pm$ 8.6	21.7 $\pm$ 2.6	<0.001
Semen cadmium ( $\mu\text{g/L}$ )			
Range	3.2 - 4.3	2.1 - 3.4	
Mean $\pm$ SD	3.77 $\pm$ 0.294	2.774 $\pm$ 0.514	<0.001



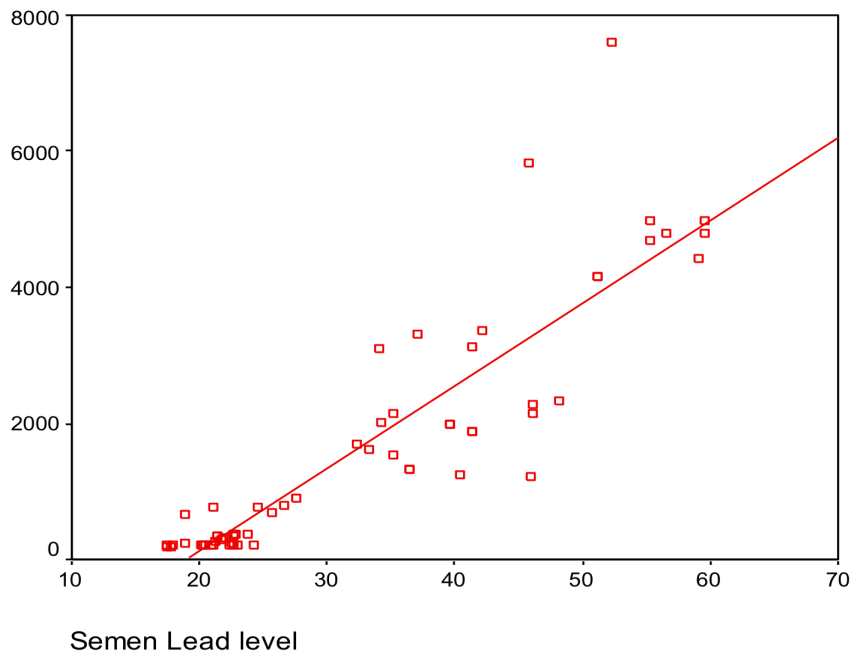
**Figure (1):** Flow cytometer scatter plot (left) and histogram (right) of a control subject.  
- Sperm DNA fragmentation percentage in this control subject was 5.3%



**Figure (2):** Flow cytometer scatter plot (left) and histogram (right) of an infertile subject.  
- Sperm DNA fragmentation % in this infertile man was 16 %.



**Figure (3):** Correlation between semen Cadmium level and sperm DNA fragmentation % in infertile men.



**Figure (4):** Correlation between semen lead and ROS levels in infertile men.

## REFERENCES

- Acharya, U. R.; Mishra, M.; Patro, J. and Panda, M. K. (2008) : "Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium". *Reprod. Toxicol.*, 25(1):84-88.
- Akinloye, O.; Arowojolu, A.; Shittu, O. and Anetor, J. (2006) : "Cadmium toxicity: a possible cause of male infertility in Nigeria". *Reprod. Biol.*, 6(1):17-30.
- Alexander, B. H.; Checkoway, H.; Faustman, E. M.; van Netten, C.; Muller, C. H. and Ewers, T. G. (1998) : "Contrasting associations of blood and semen concentrations with semen quality among lead smelter workers". *Am. J. Ind. Med.*, 34(5):464-469.
- Al-Mogazy, H. M.; Al-Khadra, S.; Kenawy, G.; Al-Zeftawy, A.; Mostafa, T. and Attia, A. (1993) : "Correlation between human sperm hypo-osmotic swelling test and other semen parameters". *J. Clin. Lab. Anal.*,7(4): 243-246.
- Anis, T. H.; ElKaraksy, A.; Mostafa, T.; Gadalla, A.; Imam, H.; Hamdy, L. and Abu el-Alla, O. (2007) : "Chronic lead exposure may be associated with erectile dysfunction". *J Sex Med.*,4(5):1428-1436.
- Benoff, S. and Gilbert, B. (2001) : "Varicocele and male infertility", part I : preference. *Hum. Reprod. Update*,7(1):47-54.
- Benoff, S.; Hauser, R.; Marmar, J.; Hurley, I.; Napolitano, B. and Centola, G. (2009) : "Cadmium concentrations in blood and seminal plasma : correlations with sperm number and motility in three male populations (infertility patients, artificial insemination donors, and unselected volunteers)". *Mol. Med.*,15 (7-8):248-262 .
- Benoff, S.; Jacob, A. and Hurley, I. (2000) : "Male infertility and environmental exposure to lead and cadmium". *Hum. Reprod. Update*, 6(2):107-121.
- Cheng, C. Y. and Mruk, D. D. (2002) : "Cell junction dynamics in the testis: sertoli-germ cell interactions and male contraceptive development". *Physiol. Rev.*,82 (4):825-874.
- Cullen, C.; Singh, A.; Dykeman, A.; Rice, D. and Foster, W. (1993) : "Chronic lead exposure induces ultrastructural alterations in the monkey seminal vesicle". *J. Submicrosc. Cytol. Pathol.*, 25(1): 127-135.
- Danadevi, K.; Rozati, R.; Reddy, P. P. and Grover, P. (2003) : "Semen quality of Indian welders occupationally exposed to nickel and chromium". *Reprod. Toxicol.*,17(4):451-456.
- de Lamirande, E. and Gagnon, C. (1992) : "Reactive oxygen species and

human spermatozoa. I. Effects on the motility of intact spermatozoa and on sperm axonemes". *J. Androl.*, 13(5):368-378.

**Droge, W. (2002)** : "Free radicals in the physiological control of cell function". *Physiol. Rev.*, 82(1): 47-95.

**El-Demerdash, F. M.; Yousef, M. I.; Kedwany, F. S. and Baghdadi, H. H. (2004)**: "Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and beta-carotene". *Food Chem. Toxicol.*, 42 (10):1563-1571.

**El-Shafei, M. D.; Mostafa, M. E. and Mostafa, T. (2011)** : "Oestrogen receptors in the developing rat prostate". *Andrologia*, 43(2): 94-99.

**El-Zohairy, E. A.; Youssef, A. F.; Abul-Nasr, S. M.; Fahmy, I. M.; Salem, D.; Kahil, A. K. and Madkour, M. K. (1995)** : "Reproductive hazards of lead exposure among urban egyptian men". *Reprod. Toxicol.*, 10(2):145-151.

**Fatima, P.; Debnath, B. C.; Hossain, M. M.; Rahman, D.; Banu, J. Rahman, M. W. and Begum, S. A. (2010)**: "Relationship of blood and semen lead level with semen parameter". *Mymensingh Med. J.*, 19(3): 405-414.

**González-Estechea, M.; Trasobares, E.; Fuentes, M.; Martínez, M. J.; Cano, S.; Vergara, N.; Gaspar, M. J.; González-Revaldería, J.; Barciela, M. C.; Bugarín, Z.; Fernández, M. D.; Badía, P.; Pintos, C.; González, M.; Guillén, J. J.; Bermejo, P.; Fernández, C. and Arroyo, M.; PESA Group. (2011)** : " Blood lead and cadmium levels in a six hospital employee population. PESA study, 2009". *J Trace Elem. Med. Biol.*, 25 ( 1):S22-S29.

**Hammadeh, M. E.; Al Hasani, S.; Rosenbaum, P.; Schmidt, W. and Fischer Hammadeh, C. (2008)** : "Reactive oxygen species, total antioxidant concentration of seminal plasma and their effect on sperm parameters and outcome of IVF/ICSI patients". *Arch. Gynecol. Obstet.*, 277(6): 515-526.

**Hernández-Ochoa, I.; García-Vargas, G. López-Carrillo, L.; Rubio-Andrade, M.; Morán-Martínez, J.; Cebrián, M. E. and Quintanilla-Vega, B. (2005)** : "Low lead environmental exposure alters semen quality and sperm chromatin condensation in northern Mexico". *Reprod. Toxicol.*, 20(2): 221-228.

**Hernández-Ochoa, I.; Sánchez-Gutiérrez, M.; Solís-Heredia, M. J. and Quintanilla-Vega, B. (2006)** : "Spermatozoa nucleus takes up lead during the epididymal maturation altering chromatin condensation". *Reprod. Toxicol.*,21(2):171-178.

Hsu, P. C.; Chang, H. Y.; Guo, Y. L.; Liu, Y. C. and Shih, T. S. (2009) : "Effect of smoking on blood lead levels in workers and role of reactive oxygen species in lead-induced sperm chromatin DNA damage". *Fertil. Steril.*, 91(4): 1096-1103.

Inhorn, M. C.; King, L.; Nriagu, J. O.; Kobeissi, L.; Hammoud, N.; Awwad, J.; Abu-Musa, A. A. and Hannoun, A. B. (2008): "Occupational and environmental exposures to heavy metals : Risk factors for male infertility in Lebanon?". *Reprod. Toxicol.*, 25(2): 203-212.

Kanous, K. S.; Casey, C. and Lindemann, C. B. (1993) : "Inhibition of microtubule sliding by Ni<sup>2+</sup> and Cd<sup>2+</sup> : evidence for a differential response of certain microtubule pairs within the bovine sperm axoneme". *Cell.Motil.Cytoskeleton*, 26(1):66-76.

Kiziler, A. R.; Aydemir, B.; Onaran, I.; Alici, B.; Ozkara, H.; Gulyasar, T. and Akyolcu, M. C. (2007) : "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.*, 120(1-3):82-91.

Kuo, H. M.; Wang, C. S. and Lai, J. S. (1997) : "Semen quality in workers with long term lead exposure: a preliminary study in Taiwan". *Sci. Total Environ.*, 204(3): 289-292.

Leoni, G.; Bogliolo, L.; Deiana, G.; Berlinguer, F.; Rosati, I. Pintus, P. P.; Ledda, S. and Naitana, S. (2002) : "Influence of cadmium exposure on in vitro ovine gamete dysfunction". *Reprod. Toxicol.*, 16(4):371-377.

Li, D. K.; Yan, B.; Li, Z.; Gao, E.; Miao, M.; Gong, D.; Weng, X.; Ferber, J. R. and Yuan, W. (2010) : "Exposure to magnetic fields and the risk of poor sperm quality". *Reprod. Toxicol.*, 29(1):86-92.

Martynowicz, H.; Andrzejak, R. and Medras, M. (2005) : "The influence of lead on testis function". *Med. Pr.*, 56(6): 495-500.

Mendiola, J.; Moreno, J. M.; Roca, M.; Vergara-Juárez, N.; Martínez-García, M. J.; García-Sánchez, A.; Elvira-Rendueles, B.; Moreno-Grau, S.; López-Espín, J. J.; Ten, J.; Bernabeu, R. and Torres-Cantero, A. M. (2011) : "Relationships between heavy metal concentrations in three different body fluids and male reproductive parameters: a pilot study". *Environ. Health*, 10(1): 6-13.

Monsefi, M.; Alaei, S.; Moradshahi, A. and Rohani, L. (2010) : "Cadmium-induced infertility in male mice". *Environ. Toxicol.*, 25(1):94-102.

Moriwaki, H.; Osborne, M. R. and Phillips, D. H. (2008) : "Effects of mixing

metal ions on oxidative DNA damage mediated by a Fenton-type reduction". *Toxicol. In Vitro*, 22(1): 36-44.

**Nieschlag, E. and Leifke, E. (2000)** : Empirical therapies for idiopathic male infertility. In : *Andrology Male Reproductive Health and Dysfunction*, Nieschlag, E., and Behre, H.M. ,2<sup>nd</sup> ed., Springer, Berlin, Heidelberg, New York, p.p. 327-336.

**Ognjanovic, B. I.; Markovic, S. D.; Ethordevic, N. Z.; Trbojevic, I. S.; Stajin, A. S. and Saicie, Z. S. (2010)** : "Cadmium-induced lipid peroxidation and changes in antioxidant defense system in the rat testes: protective role of coenzyme Q (10) and vitamin E". *Reprod. Toxicol.*, 29 (2): 191-197.

**Oliveira, H.; Spanò, M.; Santos, C. and Pereira, Mde. L. (2009)** : "Adverse effects of cadmium exposure on mouse sperm". *Reprod. Toxicol.* ,28(4):550-555.

**Pant, N.; Upadhyay, G.; Pandey, S.; Mathur, N.; Saxena, D. K. and Srivastava, S. P. (2003)** : "Lead and cadmium concentration in the seminal plasma of men in the general population: correlation with sperm quality". *Reprod. Toxicol.*, 17(4): 447-450.

**Potts, R.J.; Newbury, C.J.; Smith, G.; Notarianni, L. J. and Jefferies, T. M. (1999)** : "Sperm chromatin damage asso-

ciated with male smoking". *Mutat. Res.*, 423(1-2): 103-111.

**Rodríguez-Estival, J.; Taggart, M.A. and Mateo, R. (2011)**: "Alterations in vitamin A and E levels in liver and testis of wild ungulates from a lead mining area". *Arch. Environ. Contam. Toxicol.*, 60(2): 361-371.

**Saarenen, M.; Kantola, M.; Saarikoski, S. and Vanha-Pettula, T. (1989)** : "Human seminal plasma cadmium: comparison with fertility and smoking habits". *Andrologia*, 21(2):140-145.

**Sadek, A.; Almohamdy, A. S.; Zaki, A.; Aref, M.; Ibrahim, S. M. and Mostafa, T. (2011)**: "Sperm chromatin condensation in infertile men with varicocele before and after surgical repair". *Fertil. Steril.*,95(5): 1705-1708.

**Saleh, R. A.; Agarwal, A.; Nelson, D. R.; Nada, E. A.; El-Tonsy, M. H.; Alvarez, J. G.; Thomas, A. J. Jr. and Sharma, R. K. (2002)**: "Increased sperm nuclear DNA damage in normozoospermic infertile men: a prospective study". *Fertil. Steril.* , 78(2): 313-318.

**Schlingmann, K.; Michaut, M. A.; McElwee, J. L.; Wolff, C. A.; Travis, A. J. and Turner, R. M. (2007)** : "Calmodulin and CaMKII in the sperm principal piece: evidence for a motility-related calcium/



calmodulin pathway". *J. Androl.*, 28(5): 706-716.

**Sergerie, M.; Ouhilal, S.; Bissonnette, F.; Brodeur, J. and Bleau, G. (2000) :** "Lack of association between smoking and DNA fragmentation in the spermatozoa of normal men". *Hum. Reprod.*, 15(6):1314-1321.

**Sharma, R. K.; Pasqualotto, F. F.; Nelson, D. R.; Thomas, A. J. Jr. and Agarwal, A. (1999) :** "The reactive oxygen species-total antioxidant capacity score is a new measure of oxidative stress to predict male infertility". *Hum. Reprod.*, 14(11): 2801-2807.

**Sigman, M.; Lipshultz, L. and Howards, S. (1997) :** Evaluation of the subfertile male. In: *Infertility in the Male*, Lipshultz, L. I. and Howards, S. S. (eds), Mosby-Year book, St. Louis, USA. p.p. 173-177.

**Skakkebaek, N. E.; Rajpert-De Meyts, E. and Main, K. M. (2001) :** "Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects". *Hum. Reprod.*, 16(5):972-978.

**Takiguchi, M. and Yoshihara, S. (2006) :** "New aspects of cadmium as endocrine disruptor". *Environ. Sci.*, 13 (2):107-116.

**Telisman, S.; Cvitkovic, P.; Jurasovic, J.; Pizent, A.; Gavella, M. and Rocic, B. (2000):** "Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men". *Environ. Health Perspect.*, 108(1):45-53.

**Wald, M.; Lewin, L. M.; Soffer, Y.; Oschri, Y.; Shochat, L.; Vigodner, M. and Goldan, R. (2004) :** "Evaluation of sperm samples from infertile men by flow cytometry". *Harefuah*, 143(1): 22-25.

**Wang, X.; Sharma, R. K.; Gupta, A.; George, V.; Thomas, A. J.; Falcone, T. and Agarwal, A. (2003) :** "Alterations in mitochondria membrane potential and oxidative stress in infertile men: a prospective observational study". *Fertil. Steril.*, 80(2):844-850.

**Wildt, K.; Eliasson, R. and Berlin, M. (1983) :** Effects of occupational exposure to lead on sperm and semen. In: *Reproductive and Developmental Toxicology of Metals*, Clarkson, T. W., Nordberg, G. F. and Sager, P.R., 1st edition. Plenum Press, New York, p.p. 279-299.

**Wirth, J. J. and Mijal, R. S. (2010) :** "Adverse effects of low level heavy metal exposure on male reproductive function". *Syst. Biol. Reprod. Med.*, 56(2):147-167.

**World Health Organization (1999) :**

WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction, 4<sup>th</sup> edition. Cambridge University Press, Cambridge.

**World Health Organization (2000) :** WHO manual for the standardized investigation, diagnosis and management of the infertile male. Rowe, P.J.; Comhaire, F.H.; Hargreave, T. B. and Mahmoud, A.M.A. 1<sup>st</sup> edition of Cambridge University Press, Cambridge.p.p.1-3.

**Wu, H. M.; Lin-Tan, D. T.; Wang, M. L.; Huang, H. Y.; Wang, H. S.; Soong, Y. K. and Lin, J. L. (2008) :** "Cadmium level in seminal plasma may affect the pregnancy rate for patients undergoing infertility evaluation and treatment". *Reprod. Toxicol.*, 25(4):481-484.

**Xu, B.; Chia, S.; Tsakok, M. and Ong, C. N. (1993) :** "Trace elements in blood and seminal plasma and their relationship to sperm quality". *Reprod. Toxicol.*,7(6):613-618.

**Xu, D. X.; Shen, H. M.; Zhu, Q. X.; Chua, L.; Wang, Q. N.; Chia, S. E. and Ong, C. N. (2003) :** "The associations among semen quality, oxidative DNA damage in human spermatozoa and concentrations of cadmium, lead and selenium in seminal plasma". *Mutat. Res.*, 534(1-2): 155-163.

**Zenzes, M. T. (2000) :** "Smoking and reproduction: gene damage to human gametes and embryos". *Hum. Reprod. Update*, 6(2):122-131.

## مردود مستنوى الرصاص و الكادميوم فى السائل المنوى على خصوبة الرجال ذو ضعف عدد و حركة الحيوانات المنوية الغير معروف السبب

المشتركون فى البحث

د. نجوى محمود غندور  
د. سهير كامل سيد\*\*  
د. مدهت عربى صالح#  
د. عماد عبد الرحيم طه\*  
د. على محمد مهران\*\*\*  
د. تيمور مصطفى##

من أقسام الطب الشرعي والسموم الإكلينيكية، الأمراض الجلدية و التناسلية والذكورة\*، الباثولوجيا الإكلينيكية\*\*،  
التناسلية و الذكورة و أمراض الجنس\*\*\*، الصحة العامة و طب المجتمع# - كلية الطب - جامعة أسيوط.  
قسم أمراض الذكورة و الجنس## كلية الطب - جامعة القاهرة.

تهدف الدراسة إلى تقييم مستوى الرصاص و الكادميوم فى السائل المنوى على خصوبة الرجال ذو ضعف عدد و حركة الحيوانات المنوية الغير معروف السبب و مقارنتهم بالأصحاء و علاقة هذا المستوى بالقياسات الأخرى للسائل المنوي وحركة و حيوية الحيوانات المنوية و نسبة تناقص التورم التناضحى فى الحيوان المنوى و كذلك نسبة التكسير فى الحامض النووى و مركبات الاكسجين النشطة . قامت الدراسة على ٦٠ مريض من الرجال ذو ضعف عدد و حركة الحيوانات المنوية الغير معروف السبب و مقارنتها بعدد ٦٠ رجل من الأصحاء. و نتج عن هذه الدراسة وجود زيادة واضحة فى مستوى الرصاص و الكادميوم فى السائل المنوي لهؤلاء المرضى بالمقارنة بالأصحاء. و تبين وجود علاقة عكسية بين مستوى الرصاص و الكادميوم فى السائل المنوي والقياسات الأخرى للسائل المنوي وحركة و حيوية الحيوانات المنوية و نسبة تناقص التورم التناضحى فى الحيوان المنوى ولكن وجدت علاقة متزايدة بين مستوى الرصاص و الكادميوم فى السائل المنوي ونسبة تكسير الحامض النووى و مستوى مركبات الاكسجين النشطة فى السائل المنوي.