

# Sperm Chromatin Structure, Semen Quality and Lead in Blood and Seminal Fluid of Infertile Men

NJ Awadalla<sup>1</sup>, M El-Helaly<sup>1</sup>, M Gouida<sup>2</sup>,  
R Mandour<sup>3</sup>, M Mansour<sup>4</sup>

## Abstract

**Background:** Exposures to lead above the threshold value of 50–60 µg/dL have been linked to diminished semen quality parameters. Worldwide, the lead exposure has been diminished during the last years. Therefore, it has become of a great concern to examine the effects of lead exposures on semen quality at low levels of exposure.

**Objective:** To evaluate the effect of low level (<20 µg/dL) blood lead on semen quality and sperm chromatin structure.

**Methods:** A cross-sectional study was conducted on 29 men with primary infertility attending the outpatient clinic of infertility in Mansoura University Hospital, Egypt, from March to May 2010. Semen quality parameters and sperm flow-cytometry analysis were compared between two groups of infertile men with blood lead level (BLL) above, and below 20 µg/dL, respectively.

**Results:** The mean BLL in the studied subjects was 20.08 µg/dL. 45% of the studied men had BLL ≥20 µg/dL. Non-significant reduction in sperm count, impaired sperm motility and altered sperm morphology were observed in those with BLL ≥20 µg/dL compared to those with BLL <20 µg/dL. Concerning semen flow-cytometry analysis, percentage of haploid sperms was significantly lower among men with BLL ≥20 µg/dL (78%) compared to that among those with BLL <20 µg/dL (87%). A positive significant correlation was observed between BLL and percentage of diploid sperms. The chromatin condensation was however, negatively correlated with BLL ( $p < 0.05$ ).

**Conclusion:** Semen quality of men with primary infertility does not have any correlation with BLL at the cutoff value of 20 µg/dL. However, even at this low level, a significant decrease in haploid sperm counts and chromatin condensation was observed.

**Keywords:** Flow-cytometry; Lead poisoning; Semen analysis; Chromatin; Infertility; Fertility

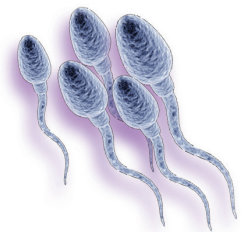
## Introduction

There is growing concern about the substantial decrease in sperm density over the last five decades in general population worldwide.<sup>1,2</sup> Possible explanations for the decrease in semen quality include increased stress, lifestyle, and a

variety of endocrine altering chemicals in the environment that can be linked to decreased male reproductivity, as supported mainly by animal studies.<sup>3,4</sup>

Lead is a highly toxic metal for humans and other mammals. It is ubiquitous in the human environment and accumulates in the human body over life time, includ-

<sup>1</sup>Department of Public Health and Preventive Medicine, <sup>2</sup>Genetic Unit, <sup>3</sup>Toxicology Unit, Emergency Hospital, and <sup>4</sup>Department of Dermatology and Andrology, Faculty of Medicine, Mansoura University, Egypt.



Correspondence to Nabil Joseph Awadalla, MD, Occupational and Environmental Medicine, Department of Public Health and Preventive Medicine, Mansoura University, Mansoura, Egypt.  
Tel: +2-012-404-4308  
Fax: +2-50-223-4092  
E-mail: njgirgis@yahoo.co.uk

**TAKE-HOME MESSAGE**

- Lead is a highly toxic metal for humans and other mammals.
- Lead has been linked to impaired semen quality (decreased number, motility, and altered morphology of sperms).
- Exposure to lead causes oxidative DNA damage, perturbed acrosome reactions, lowered prostate secretory function, and possible decreases in fertility rates.
- Working with lead-based paints and leaded gasoline increase the risk of lead exposure.
- Smokers and blue collar workers were more susceptible to lead exposure.
- Semen quality of men with primary infertility does not have any correlation with blood lead level at the cutoff value of 20 µg/dL.

ing prenatal period. Apart from numerous sources of occupational exposure to lead as in lead smelting and refining,<sup>5</sup> lead battery manufacture,<sup>6</sup> production and use of lead-based paints<sup>7</sup> and production of lead-glazed pottery and crystal glass,<sup>8</sup> the most important environmental sources are food, especially seafood from metal-polluted areas, unsafe potable water, air from gasoline in dense traffic areas, lead-based paints of housing, smoking habits from tobacco.<sup>9</sup>

Lead has been linked to impaired semen quality; many studies have reported decreased number, motility, and altered morphology of sperms<sup>9-11</sup> and also oxidative DNA damage, perturbed chromatin condensation, altered acrosome reactions, and decreased prostate secretory function as well as increased frequency of sponta-

neous abortion and many other adverse outcomes of pregnancies fathered by workers exposed to lead,<sup>12-14</sup> and possible decreases in fertility rates.<sup>15</sup>

Although, the adverse effects of lead on the reproductive functions are not controversial, the threshold at which these effects could occur, is still under investigation. While, most studies point to a no-adverse effect level of 40–50 µg/dL in blood,<sup>10,16</sup> new studies employing more refined and advanced techniques to measure male reproductive function may challenge this prevailing view.<sup>17</sup>

During the past two decades, increased attention has been paid to potentially adverse effects of occupational and environmental exposures on human reproductive function. The concern that such effects may occur at relatively low blood lead levels (BLLs) was one of the principal considerations in the process of promulgating occupational health and safety standards on exposure limits for inorganic lead in the US.<sup>18,19</sup> So, it has become of a great concern to examine the effects of lead exposures on semen quality at lower levels. We therefore, conducted the present study to examine the association between low BLL and any adverse effects on semen quality and sperm chromatin structure, among infertile men.

**Patients and Methods****Study design and population**

A cross-sectional study was conducted on those men with primary infertility who attended the outpatient clinic of Andrology at Mansoura University Hospital, Egypt, from March to May 2010 for fertility evaluation. The study was approved by the Ethics Committee of Mansoura Faculty of Medicine. Written informed consent was taken from each subject included in the study. The inclusion criteria includ-

ed primary infertility, absence of female factor for infertility, absence of medical and surgical causes of infertility such as diabetes mellitus, urinary tract infection, sexually-transmissible diseases, a history of chemotherapy or radiotherapy, varicocele, undescended testes, small testes or testicular injury.

### Methods

All recruited patients completed a specially-designed questionnaire composed of questions about the patient's demographic data including age, gender, residence place, marital status, smoking habits and occupation. Also, the questionnaire included questions about the presence of female causes of infertility, medical and surgical causes of infertility and sexually-transmissible diseases. Medical examination of all patients was carried out by an andrology specialist to exclude the possibilities of medical and surgical causes of infertility. Moreover, laboratory assessments including fasting blood glucose and urinalysis, and Doppler examination of both testes were carried out for all participants.

### Semen analysis

All participants were asked to collect their semen at the Andrology clinic laboratory by masturbation into a sterile plastic specimen cup. Subjects were instructed to abstain from ejaculation for at least three days prior to sampling. All semen samples were processed and analyzed by computer-aided semen analyzer (CASA, version 10 HTM-IVOS; Hamilton Thorne Research, Beverly, Mass, USA). Each semen sample was liquefied for at least 20 minutes, but no longer than one hour prior to semen analysis. Volume, pH, sperm concentration per mL, sperm motility, sperm morphology (Morphological Index) and sperm viability were examined according to the World Health Organization (WHO)

guidelines for the examination of human semen.<sup>20</sup>

To measure both sperm concentration and motility, a minimum of 200 sperm cells from at least four different fields were analyzed from each specimen. "Motile sperm" was defined according to the WHO grade as 'a' grade sperm (rapidly progressive with a velocity  $\geq 25$  mm/s at 37 °C) and 'b' grade sperm (slow/sluggish progressive with a velocity  $\geq 5$  mm/s but,  $< 25$  mm/s). "Progressive motile sperm" was defined as grade 'a' sperm.<sup>20</sup> Concerning sperm morphology, at least two slides were made for each fresh semen sample. The resulting thin smear was allowed to air dry for one hour before staining with the Diff-Quik staining kit (Dade Behring AG, Dudingon, Switzerland). Morphological assessment was performed with a Nikon microscope using an oil immersion 100 $\times$  objective (Nikon Company, Tokyo, Japan). A minimum of 200 sperm cells was counted from two slides prepared for each specimen. Strict scoring criteria were used to classify men as having normal or subnormal morphology, according to Kruger, *et al.*<sup>21</sup>

### Blood and seminal fluid lead analysis

Spermatozoa were separated from seminal plasma by centrifugation at 600 g for 10 min. Seminal plasma was re-centrifuged at 100 000 g for two hours; the supernatant seminal fluid was used for the analysis of minerals. Venous blood samples were collected into trace element-free tubes and centrifuged at 1000 g for 10 min. All precautions were taken to prevent any environmental contamination of serum or semen samples during the collection and laboratory work. The polyethylene tubes used in the analyses were washed with 5% nitric acid and rinsed with deionized water as a precaution against contamination. Samples were stored at -20 °C until the assay. Analyses were done using the graph-

ite furnace technique with Zeeman background correction (Zeeman 5000 atomic absorption spectrophotometer, HGA 500; Perkin Elmer, Norwalk, CT, USA) as described earlier.<sup>22</sup>

**Flow-cytometry analysis**

One mL of semen sample diluted with phosphate buffer solution (PBS) in a round bottom tube was taken and washed twice with PBS before fixation. The sample was fixed immediately with 1 mL ice-cold absolute alcohol drop by drop with gently shaking and preserved at +4 °C until staining. Staining with propidium iodide for DNA ploidy was carried out according to the procedure described by Vindelov.<sup>23</sup> For measuring the de-condensation, another sample was stained using sodium dodecyl sulphate and processed according to Hacker-Klom, *et al.*<sup>24</sup> Flow-cytometric analysis was aimed at measuring the following parameters: cellular debris percentage in sub-haploid region (<1 mL), mature haploid spermatozoa percentage at 1-mL peak, haploid round spermatids percentage at 1-mL peak, diploid spermatozoa percentage in 2-mL peak, chromatin condensation percentage and chromatin

de-condensation percentage were analyzed using FACS caliber flow-cytometer (Becton Dickinson, Sunnyvale, CA, USA).

**Statistical analysis**

The participants were divided into two groups according to their BLL: BLL ≥20 µg/dL (high BLL) and BLL <20 µg/dL (low BLL). Data were analyzed using SPSS® ver 11 for Windows®. Data were examined for normal distribution using one-sample Kolmogorov-Smirnov test. Qualitative data were presented as number and percentage; quantitative data were presented as mean±SD. Both groups were compared regarding the socio-demographic data, semen plasma lead level, parameters of semen quality and flow-cytometry findings using χ<sup>2</sup> test for qualitative variables and *Student's t* test, and Mann-Whitney U test for parametric and non-parametric quantitative variables, respectively. Correlation between semen and blood lead levels and other parameters were measured using Pearson's correlation coefficient for parametric data and Spearman's rank correlation for non-parametric. A p value <0.05 was considered statistically significant.

**Table 1:** Socio-demographic criteria of the studied infertile men according to their blood lead level (BLL)

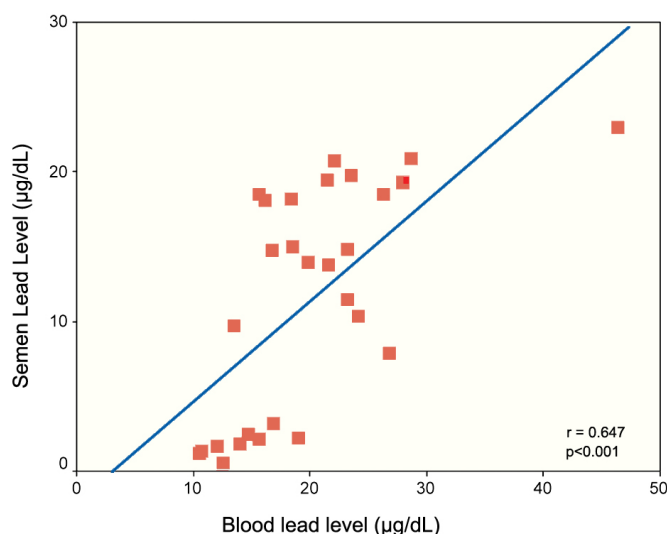
Sociodemographic	BLL <20 µg/dL n=16	BLL ≥20 µg/dL n=13	p value	Total n=29
Age	28.8±3.1	29.9±5.1	0.48	29.2±4.2
Residence: rural/urban	11/5	9/4	1.00	20/9
Current smoker	6 (38%)	9 (69%)	0.09	15 (52%)
Job categories:				
Farmers	6 (38%)	2 (15%)		8 (28%)
Blue collar workers*	7(44%)	6 (46%)	0.31	13 (45%)
White collar workers	3 (19%)	5 (39%)		8 (28%)

\*Mechanics, painters and carpenters

## Results

Although, 100 men were attending the clinic for infertility evaluation during the study period, only 29 men fulfilled the inclusion criteria and accepted to share in the study. None of the controls agreed to give semen sample, as they considered themselves as fertile men with no need for semen analysis. The mean±SD blood and semen lead levels in 29 participants were 20.08±7.26 (range: 10.40–46.36) µg/dL and 11.40±7.53 (range: 0.40–22.83) µg/dL, respectively. Socio-demographic criteria of the studied patients stratified according to their BLL are presented in Table 1. No significant differences were observed in distribution of age and residence place between the two studied groups. Although the frequency of smokers and blue collar workers were higher in those who had high BLL compared to those with low BLL, the difference was not statistically significant.

The mean±SD semen fluid lead in those who had high BLL (16.02±4.94 µg/



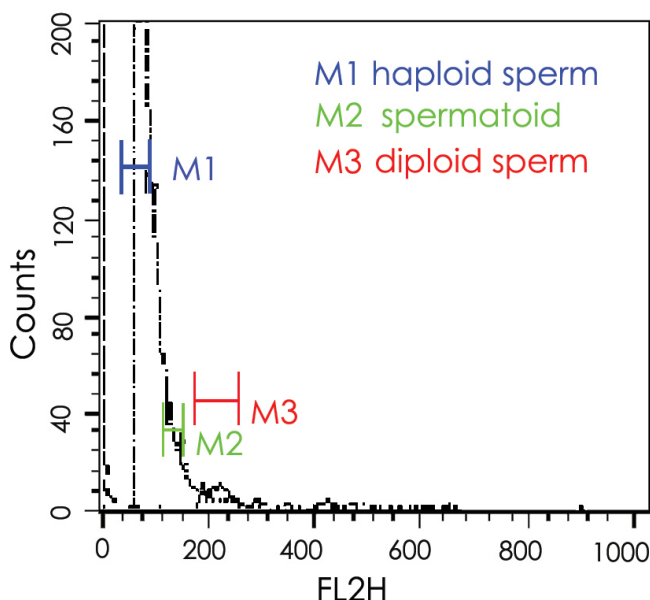
**Figure 1:** Scatter plot of seminal plasma and blood lead level in the studied infertile men.

dL) was significantly ( $p=0.002$ ) higher than those with low BLL ( $7.64 \pm 7.27$  µg/dL). There was a significant positive correlation ( $r = 0.647$ ;  $p < 0.001$ ) between blood and semen lead levels (Fig 1).

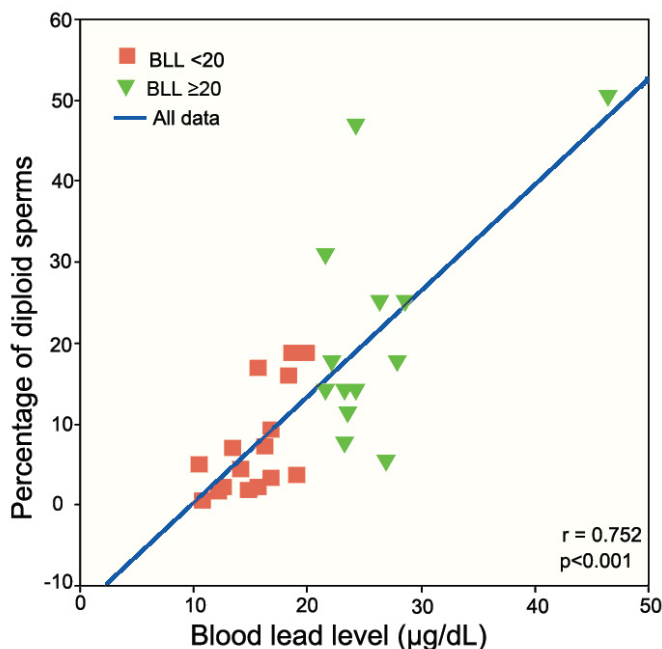
Semen quality parameters are presented in Table 2. Although sperm count was

**Table 2:** Semen quality parameters in the studied infertile men according to their blood lead level (BLL).

Semen quality parameters	BLL <20 µg/dL n=16	BLL ≥20 µg/dL n=13	p value
Volume (mL)	3.75±1.5	4.37±1.8	0.32
Density (×10 <sup>6</sup> /mL)	58.35±58.0	43.93±36.4	0.68
Motility:			
Class a (%)	25.05±16.6	21.28±15.7	0.541
Class b (%)	19.12±7.2	17.22±10.3	0.567
Class c (%)	14.04±11.8	8.02±6.0	0.108
Class d (%)	41.79±24.3	53.46±25.4	0.219
Morphology index	15.35±6.6	12.70±9.1	0.374
Oligospermia	2 (13%)	5 (39%)	0.19
Teratospermia	6 (38%)	6 (46%)	0.71
Athenospermia	4 (25%)	7 (54%)	0.14



**Figure 2:** DNA histogram for one studied semen sample.



**Figure 3:** Scatter plot of BLL and percentage of diploid sperms.

lower in cases with high BLL than those with low BLL, the difference was not statistically significant. In terms of motility, frequencies of those with grade 'a,' 'b' and

'c' were lower in men with high BLL compared to cases with low BLL; grade 'd' was more frequent in the former group. These differences however were not statistically significant. Frequencies of oligo-, terato- and atheno-spermia were higher in those with high BLL compared to men with low BLL. However, the differences were not statistically significant.

Flow-cytometry parameters in relation to BLL are presented in Table 3 and Figures 2, 3 and 4. The mean±SD percent of haploid sperms in high BLL group ( $87.30 \pm 21.8\%$ ) was significantly ( $p < 0.05$ ) higher than those with low BLL ( $78.32 \pm 13.9\%$ ). The mean±SD percent of diploid sperms in men with high BLL ( $7.32 \pm 6.5\%$ ) was significantly ( $p < 0.001$ ) lower than low BLL group ( $21.68 \pm 13.9\%$ ). Furthermore, percentage of diploid sperms had significant positive correlations with both BLL ( $r = 0.75$ ;  $p < 0.001$ ) and semen lead level ( $r = 0.631$ ;  $p < 0.001$ ). The mean chromatin condensation was reduced significantly in those with high BLL compared to low BLL group ( $p < 0.05$ ). Chromatin condensation had significant negative correlations with BLL ( $r = -0.390$ ;  $p < 0.05$ ) and semen lead level ( $r = -0.401$ ;  $p < 0.05$ ). Frequencies of spermatid, debris and apoptosis as well as mean chromatin de-condensation were not significantly different between low and high BLL groups.

## Discussion

The mean BLL in the studied subjects was  $20.08 \mu\text{g/dL}$ . A significant proportion (45%) of the studied men with primary infertility had  $\text{BLL} \geq 20 \mu\text{g/dL}$ . Lead is probably interfering with male reproductive function; it prolongs the time to conception (TTC) by actions at several sites and levels. In contrast to several studies that observed no adverse effects of lead on male reproductive functions below the blood level of  $40\text{--}50 \mu\text{g/dL}$ ,<sup>10,16</sup> a recent

**Table 3:** Flow-cytometry parameters of the studied infertile men with according to their blood lead level (BLL).

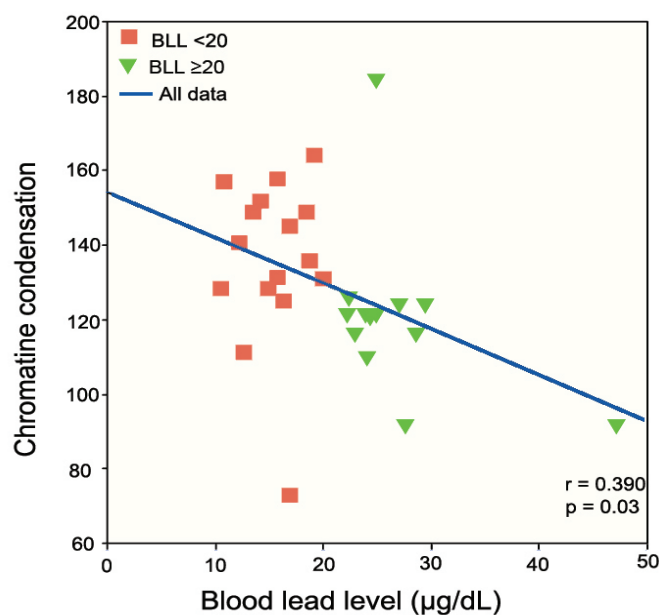
Semen	BLL <20 µg/dL	BLL ≥20 µg/dL	p value	Total
Haploid sperm	87.30±21.8	78.32±13.9	0.009	83.28±18.98
Spermtid	1.00±3.4	3.23±9.1	0.779	2.00±6.59
Diploid sperm	7.32±6.5	21.68±13.9	0.001	13.76±12.63
Debris	11.37±4.9	12.95±16.0	0.475	12.07±11.16
Apoptosis	6.57±7.7	11.47±19.7	0.880	8.77±14.33
Chromatin condensation	135.90±21.9	121.80±22.3	0.004	129.58±22.88
Chromatin de-condensation	146.68±20.2	138.02±23.5	0.156	142.80±21.82

study challenged this prevailing view.<sup>17</sup> Additionally, the health effects of lead exposure could be exacerbated by inadequate nutrition, which can result from low-income living. It has been shown that vitamin C plus thiamine, as well as thiamine in combination with zinc or vitamin E, counteract some of the lead-induced toxic effects in experimental studies.<sup>25,26</sup>

The present study showed a significant decrease in chromatin condensation and a significant increase in diploid sperms detected by flow-cytometry in those with high BLL compared to low BLL group. The condensed nature of sperm chromatin protects the genetic integrity during sperm journey through male and female reproductive tracts. Consequently, human spermatozoa in which chromatin is not completely condensed are reported to have a low percentage of fertilization<sup>27</sup>—they failed to fertilize the ovum, even when they are directly injected into the ovum.<sup>28</sup>

It has been proposed that lead may either affect DNA synthesis in precursors of the spermatozoa or interfere with the normal replacement of nuclear histones by cysteine-rich protamines during sperm

chromatin condensation. In an experimental animal study, lead has been shown to bind firmly with thiol groups present on cysteine residues in protamines—a protein that exerts a protective function on DNA.<sup>29</sup> Moreover, in humans zinc contributes to sperm chromatin stability and



**Figure 4:** Scatter plot of BLL and chromatin condensation.

binds to protamine 2. Lead competes with zinc and binds human protamine 2 causing conformational changes in the protein.<sup>30</sup> This decreases the concentration of DNA protamine 2 binding which probably leads to alterations in sperm chromatin condensation.<sup>10</sup> Changes in sperm chromatin structure by increased *in situ* denaturation is strongly correlated with the presence of sperm DNA strand breaks<sup>31</sup> which is found to be associated with reduced fecundity in humans and lead-related subfertility.<sup>32</sup> Our results was also in accordance with Telisman, *et al*,<sup>9</sup> who found that even moderate exposure to very low level of lead (BLL  $<4 \times 10^{-5}$   $\mu\text{g}/\text{dL}$ ) can considerably reduce reproductive capacity in men. This appears to be at least partly mediated through their interference with zinc metabolism.

The frequency of smokers and blue collar workers were higher among those with high BLL than low BLL group. Smoking is one of the important non-occupational sources for lead exposure.<sup>9</sup> Concerning occupational exposure, working with lead-based paints and leaded gasoline increase the risk of lead exposure.<sup>33</sup>

In the present study, BLL was positively correlated ( $p < 0.01$ ) with semen fluid lead level. This supports other studies used BLL as an indicator for semen fluid lead burden. This is also, in accordance with Friedman and Claudio (2007)<sup>34</sup> who stated that the BLL is an accurate environmental screening and diagnostic test that has greatly facilitated efforts to identify individuals and groups with lead poisoning and to monitor their improvement after the source of lead has been identified and eliminated. Moreover, Telisman, *et al*,<sup>9</sup> found that the blood indicators of individual exposure to lead and cadmium may be better than seminal fluid indicators in terms of their correlation with indices of decreased semen quality in men.

Our results revealed no significant de-

crease in any of the semen quality parameters, at BLL of 20  $\mu\text{g}/\text{dL}$ . However, these results may be affected by the low participation and cross-sectional study design. Jensenet, *et al*,<sup>17</sup> reported that considering the potential for selection bias in cross-sectional studies on semen quality, longitudinal studies with sufficient exposure assessment are of interest, although in practice, conduction of such longitudinal studies are difficult.<sup>17</sup> The decrease in sperm count, impairment in sperm motility and alterations in sperm morphology were insignificantly higher in participants with BLL  $\geq 20$   $\mu\text{g}/\text{dL}$  compared to those with BLL  $< 20$   $\mu\text{g}/\text{dL}$ . This is in accordance with, several cross-sectional studies that have linked exposure to inorganic lead with reduced semen quality above the level of 40–50  $\mu\text{g}/\text{dL}$ .<sup>10,16</sup>

The limitations of the present study were the relative small sample size, its cross-sectional design, and lack of a comparison group.

Overall, we found no significant reduction in semen quality among the studied individuals at the BLL of 20  $\mu\text{g}/\text{dL}$ . However, a significant decrease in sperm haploid sperm and chromatin condensation were detected at this low level of exposure.

**Conflicts of Interest:** None declared.

## References

1. Giwercman A, Carlsen E, Keiding N, Skakkebaek NE. Evidence for increasing incidence of abnormalities of the human testis: a review. *Environ Health Perspect* 1993;**101 Suppl 2**:65-71.
2. Swan SH, Elkin EP, Fenster L. Have sperm densities declined? A reanalysis of global trend data. *Environ Health Perspect* 1997;**105**:1228-32.
3. Safe SH. Environmental and dietary estrogens and human health: is there a problem? *Environ Health Perspect* 1995;**103**:346-51.
4. Toppari J, Larsen JC, Christiansen P, *et al*. Male



- reproductive health and environmental xenoestrogens. *Environ Health Perspect* 1996;**104 Suppl 4**:741-803.
5. Alexander BH, Checkoway H, van Netten C, *et al*. Semen quality of men employed at a lead smelter. *Occup Environ Med* 1996;**53**:411-6.
  6. Ravichandran B, Ravibabu K, Raghavan S, *et al*. Environmental and biological monitoring in a lead acid battery manufacturing unit in India. *J Occup Health* 2005;**47**:350-3.
  7. Department of Housing and Urban Development Environmental Protection Agency. Lead; requirements for disclosure of known lead-based paint and/or lead-based hazards in housing. Fed Regist. March 6:61:24CFR Part 35, 40CFR Part 745. **1996**.
  8. McCann M, Barazani G, eds. Health hazards in the arts and crafts. Washington, DC: Society for Occupational and Environmental Health; **1980**.
  9. Telisman S, Cvitkovic P, Jurasovic J, *et al*. 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.
  10. Bonde JP, Joffe M, Apostoli P, *et al*. Sperm count and chromatin structure in men exposed to inorganic lead: lowest adverse effect levels. *Occup Environ Med* 2002;**59**:234-42.
  11. Hjollund NH, Bonde JP, Jensen TK, *et al*. Male-mediated spontaneous abortion among spouses of stainless steel welders. *Scand J Work Environ Health* 2000;**26**:187-92.
  12. Lancranjan I, Popescu HI, O GA, *et al*. Reproductive ability of workmen occupationally exposed to lead. *Arch Environ Health* 1975;**30**:396-401.
  13. Lindbohm ML, Sallmen M, Anttila A, *et al*. Paternal occupational lead exposure and spontaneous abortion. *Scand J Work Environ Health* 1991;**17**:95-103.
  14. Nordstrom S, Beckman L, Nordenson I. Occupational and environmental risks in and around a smelter in northern Sweden. V. Spontaneous abortion among female employees and decreased birth weight in their offspring. *Hereditas* 1979;**90**:291-6.
  15. Gennart JP, Buchet JP, Roels H, *et al*. Fertility of male workers exposed to cadmium, lead, or manganese. *Am J Epidemiol* 1992;**135**:1208-19.
  16. Joffe M, Bisanti L, Apostoli P, *et al*. Time To Pregnancy and occupational lead exposure. *Occup Environ Med* 2003;**60**:752-8.
  17. Jensen TK, Bonde JP, Joffe M. The influence of occupational exposure on male reproductive function. *Occup Med (Lond)* 2006;**56**:544-53.
  18. US Department of Labor Occupational Safety and Health Administration. Occupational exposure to lead: final standard. Fed Regist. 43:52952-53014, 54353-54616 and 46: 60758-60776 1981.
  19. Occupational Safety and Health Administration. OSHA Occupational Standards. Permissible exposure limits. Lead. 29 CFR, 1910.1025. Washington, DC: Occupational Safety and Health Administration. **1985**.
  20. World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction*. New York, : Cambridge University Press, **1999**.
  21. Kruger TF, Acosta AA, Simmons KF, *et al*. Predictive value of abnormal sperm morphology in in vitro fertilization. *Fertil Steril* 1988;**49**:112-7.
  22. Saaranen M, Suistomaa U, Kantola M, *et al*. Lead, magnesium, selenium and zinc in human seminal fluid: comparison with semen parameters and fertility. *Hum Reprod* 1987;**2**:475-9.
  23. Vindelov LL. Flow microfluorometric analysis of nuclear DNA in cells from solid tumors and cell suspensions. A new method for rapid isolation and straining of nuclei. *Virchows Arch B Cell Pathol* 1977;**24**:227-42.
  24. Hacker-Klom UB, Gohde W, Nieschlag E, Behre HM. DNA flow cytometry of human semen. *Hum Reprod* 1999;**14**:2506-12.
  25. Dhawan M, Kachru DN, Tandon SK. Influence of thiamine and ascorbic acid supplementation on the antidotal efficacy of thiol chelators in experimental lead intoxication. *Arch Toxicol* 1988;**62**:301-4.
  26. Flora SJ, Singh S, Tandon SK. Thiamine and zinc in prevention or therapy of lead intoxication. *J Int Med Res* 1989;**17**:68-75.
  27. Hammadeh ME, Zeginiadov T, Rosenbaum P, *et al*. Predictive value of sperm chromatin condensation (aniline blue staining) in the assessment of male fertility. *Arch Androl* 2001;**46**:99-104.
  28. Rosenbusch BE. Frequency and patterns of premature sperm chromosome condensation in oocytes failing to fertilize after intracytoplasmic sperm injection. *J Assist Reprod Genet* 2000;**17**:253-9.
  29. Foster WG, McMahon A, Rice DC. Sperm chromatin structure is altered in cynomolgus monkeys with environmentally relevant blood lead levels. *Toxicol Ind Health* 1996;**12**:723-35.

30. Quintanilla-Vega B, Hoover DJ, Bal W, *et al.* Lead interaction with human protamine (HP2) as a mechanism of male reproductive toxicity. *Chem Res Toxicol* 2000;**13**:594-600.
31. Aravindan GR, Bjordahl J, Jost LK, Evenson DP. Susceptibility of human sperm to in situ DNA denaturation is strongly correlated with DNA strand breaks identified by single-cell electrophoresis. *Exp Cell Res* 1997;**236**:231-7.
32. Spano M, Bonde JP, Hjollund HI, *et al.* Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. *Fertil Steril* 2000;**73**:43-50.
33. National Research Council (U.S.). Committee on Lead in the Human Environment. *Lead in the human environment : a report / prepared by the Committee on Lead in the Human Environment, Environmental Studies Board, Commission on Natural Resources, National Research Council.* Washington, D.C., National Academy of Sciences, **1980**.
34. Washington, DC: Commission on Natural Resources, National Research Council, National Academy of Sciences, **1980**.
35. Friedman G, Claudio L. Disparities in Environmental and Occupational Health. In: Rom W, ed. *Environmental and Occupational Medicine.* 4th ed, Lippincott Williams & Wilkins, **2007**: 175.

### Editorial Freedom at *The IJOEM*

*The IJOEM* is an international peer-reviewed journal which will publish articles relevant to epidemiology, prevention, diagnosis, and management of occupational and environmental diseases. It will also cover work-related injury and illness, accident and illness prevention, health promotion, health education, the establishment and implementation of health and safety standards, monitoring of the work environment, and the management of recognized hazards. *The IJOEM* adheres to the World Association of Medical Editors (WAME) Policy on “The Relationship between Journal Editors-in-Chief and Owners” available at [www.wame.org/resources/policies#independence](http://www.wame.org/resources/policies#independence). More specifically, the Editor-in-Chief has editorial independence and as such has full authority over the journal’s editorial content including how and when information is published. Editorial decisions are based solely on the validity of the work and its importance to readers, not on the policies or commercial interests of the owner.

*The IJOEM* is the official journal of the National Iranian Oil Company (NIOC) Health Organization. The NIOC Health Organization—established as an independent entity—provides health and medical services to the population, including to NIOC employees and their families. Neither the NIOC nor the NIOC Health Organization interferes in the evaluation, selection or editing of individual articles, either directly or by creating an environment in which editorial decisions are strongly influenced.