IOSR Journal Of Environmental Science, Toxicology And Food Technology (IOSR-JESTFT)
ISSN: 2319-2402, ISBN: 2319-2399. Volume 2, Issue 4 (Jan. - Feb. 2013), PP 38-45
www.Iosrjournals.Org

Lead and Cadmium assay in Serum and Semen of Infertile Men attending Andrology clinic in Assiut University Hospital (Rural versus Urban)

Medhat A Saleh[1,] Emad A Taha[2]; Sahar A Ismail[2]; Hisham D Gaber[2]; Hanan A Morsi[2]; Nagwa M Ghandour[3].

Public Health and Community Medicine1, Department of Dermatology, Venereology and Andrology2; and Forensic Medicine and Clinical Toxicology Department3; Faculty of Medicine, Assiut University, Egypt.

Abstract: *Recently, environmental pollution was introduced as a contributing factor in decreasing male fecundity via deterioration of semen quality.* ***Our study aimed*** *to compare serum and seminal plasma levels of lead and cadmium in rural versus urban infertile men from Assiut governorate and also, to detect the correlations of these heavy metals with conventional semen parameters and sperm vitality.* ***Methodology*** *Seventy five infertile male patients from rural areas and 84 from urban areas in Assiut governorate were included in the study. Lead and cadmium levels in serum and seminal plasma, conventional semen analysis and sperm vitality with hypo-osmotic swelling test (HOS) were evaluated in all patients.* ***Results:*** *There was a significant increase in both serum and seminal concentrations of lead and cadmium among infertile men from urban areas in comparison to those from rural areas. No statistically significant difference could be detected in semen parameters between the 2 groups. There were significant negative correlations between serum and seminal concentrations of lead and cadmium on one hand and normal sperm morphology, progressive motility and vitality (HOS) percentages on the other hand.* ***Conclusions:*** *Infertile men from urban areas are more subject to hazardous environmental toxicants as heavy metals which may help in diagnosis of the unexplained male infertility and necessitates special attention in management of those subjects attending infertility clinics and belonging to urban areas.*

Keywords: *cadmium, heavy metals infertility, lead, semen.*

I. Introduction

Male appears more susceptible than the female to the effects of occupational or environmental exposures to reproductive toxicants (Dickman et al., 1998), and it is not surprising that environmental agents have been postulated to be contributory to deteriorating semen quality and a decline in male reproductive health (Skakkebaek et al., 2001).

Results of several studies suggest that the testis and reproductive organs may be exquisitely sensitive to cadmium, with cadmium exposure leading to profound testicular damage and irreversible infertility without affecting any other organ system (Takiguchi and Yoshihara, 2006). Cadmium and lead were found to be elevated in the seminal plasma and testes of infertile men (Benoff et al., 2000; Pant et al., 2003).

Studies provide some evidence suggesting that not only occupational exposure but also geographic variations (and thus environmental exposures) contribute to blood cadmium and lead levels (Friberg and Vahter, 1983). To the best of our knowledge no puplished studies were done in our community to reveal these heavy metals semen levels in our community.

Geographic variations (and thus environmental exposures) contribute to blood cadmium and lead levels (Friberg and Vahter, 1983). Thus it's necessary to make studies in different communities evaluating heavy metal levels, its reflection on fertile and infertile men reproductive capacity and the added effect of these agents on different genital pathologies including varicocele.

Egypt is considered one of the most polluted areas in the world (UNEP and WHO,1984, Mortada et al., 2002). The lead concentration in the atmosphere of Cairo was measured at 10 Kg/m3 in 1980 and 4.5 pg/m3 in 1984 (Nasralla M. 1984), both of which exceed World Health Organization (WHO) guidelines of 0.5 to 1 kg/m3 (UNEP and WHO, 1988).

The U.S. Environmental Protection Agency (EPA) (Office of Mobile Sources, 1991) has estimated that globally approximately 90% of the lead entering the atmosphere comes from the combustion of leaded gasoline. In Cairo, leaded gasoline has an average lead of 0.2 to 0.4 g/L added as tetra-ethyl lead, and is used without proper controls on car exhaust quality. Like leaded gasoline, leaded paints, which have inorganic lead compounds of more than 40% by weight, are also still in common use. Furthermore, there is an outdated network of lead pipes in use for the water supply, and the lead level officially allowed in Egyptian water is 0.1 mg/L, which is double the level recommended by the WHO (Amr, et al, 1991). Other topographic and environmental conditions contribute to high lead levels in the Assiut environment. For instance, industry activities.

Cadmium (Cd). is one of the metals considered to be potentially dangerous on a global level (Jarup and Akesson, 2009). Smoking, water (from galvanized pipes or water coolers) and food contamination represent the main sources of non-occupational exposure to cadmium in the general population (Zhang et al., 1999; Inhorn et al., 2008; Jarup and Akesson, 2009). Cd has an extremely long biological half-life of more than 15 years in humans with only a very small fraction is excreted and the total body content increases with age (Viaene et al., 1999; Järup and Alfvén, 2004).

Aim of the work

To evaluate difference in serum and seminal plasma levels of lead and cadmium between rural and urban infertile men from Assiut governorate. Also, to correlate these levels with conventional sperm parameters and sperm vitality.

II. Patients and Methods

Study site and patient recruitment:

Patients were recruited randomly from the Andrology clinic of Dermatology and Andrology Department, Assiut University Hospital and controls were recruited from Dermatology clinic of Dermatology and Andrology Department, Assiut University Hospital.

Sample size and study population

This study included 58 infertile men (25 from rural areas (group1) and 28 from urban areas (group2) from Assiut governorate. The 2 groups were comparable as regards age, body mass index and social status.

Inclusion criteria:

Infertile male patients having idiopathic oligo and/or astheno-zoospermia from rural (group 1) and urban (group 1) areas in Assiut governorate.

Exclusion criteria:

Patients with specific genital causes that may impair reproductive capacity as varicocele, genital infection, undescended testis…etc were excluded. Patients with other systemic diseases that may impair reproductive capacity as hepatic, renal, endocrine, autoimmune diseases…etc were excluded. Additionally, those with female factor infertility were excluded. We also excluded those with special habits or occupational exposure to heavy metals in order to limit the results of the study to the effect of basic environmental exposure.

Data collection tools and sample analysis:

History taking and clinical examination:

Each participant completed an extensive questionnaire regarding his occupation, residence, social status, diet, water source, 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.

Conventional semen analysis

All 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 WHO guidelines, 1992 (concerning sperm morphology) and 1999(concerning other semen parameters).

The evaluation included liquefaction time, PH, odour, viscosity, presence of pus or epithelial cells, sperm motility, sperm morphology, and sperm concentration. Semen findings were categorized as oligozoospermia, a sperm concentration less than 20×10^{6} /ml; asthenozoospermia, fewer than 50% progressive motile sperm; and oligoasthenozoospermia, including both criteria.

Sperm hypo-osmotic swelling (HOS) test

The hypoosmotic-swelling (HOS) test was performed according to the method described by Jeyendran et al., 1984. Briefly, 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. The percentage of HOS- reacted sperms with swollen or curled tails was calculated.

Seminal plasma metal analysis (Pant et al., 2003)

Approximately 1 ml of seminal plasma was digested twice with 5ml of an acid mixture (6HNO3: 1HClO4) in a glass tube. The residue was dissolved in 1ml of 1% HNO3 then applied to flame atomic absorption spectrophotometer (Buck model 210 VGP) with air-acetylene flame and hollow cathode lamp, lamp current (8 mA), from Chemistry Department, Faculty of Science, Assiut University for detection of cadmium and lead.

Wavelengths: Lead 283.2 nm & Cadmium 228.9 nm two determinations were made for each sample. The accuracy and precision of the analytical methods were tested with standard reference materials.

Serum metal analysis (Inhorn et al., 2008)

An approximately 2mL of whole blood were drawn from all consenting subjects and frozen for later heavy metal analysis then lysed by freezing and thawing, a known volume was digested, as semen samples, twice with 5ml of an acid mixture (6HNO3: 1HClO4) in a glass tube (Semen samples with inadequate volume were diluted and multiplied by dilution factor). The residue was dissolved in 1ml of 1% HNO3 then applied to air-acetylene flame atomic absorption spectrophotometer (Buck model 210 VGP) with hollow cathode lamp (8 mA) current, from Chemistry Department, Faculty of Science, Assiut University for detection of cadmium and lead.

Wavelengths: Lead 283.2 nm & Cadmium 228.9 nm two determinations were made for each sample. The accuracy and precision of the analytical methods were tested with standard reference materials.

Ethical consideration

Patients were invited to participate in the study on their full well and the steps and aim of the research were explained to participants before signing an informed consent. The Scientific Research Ethics Committee of Assiut Faculty of Medicine approved the study.

Statistical analysis:

Data were analyzed and expressed as mean values ± standard deviations (SD). SPSS version 16 program was used for data processing. Unpaired t-test has been used in comparison of numerical parametric data between patient and control groups. Mann-Whitney test was used in comparison of numerical non parametric data between patient and control groups. Pearson correlation test was applied to analyze correlations between different quantitative variables within each group. Values were considered significant when P values were equal or less than 0.05.

III. Results

A- Socio-demographic data of infertile patients from rural and urban areas:

Socio-demographic Variables	(Group 1) Infertile men from rural areas (n=25)	(Group 2) Infertile men from urban areas (n=28)	P-value
Age	33.68 ± 6.4	33.17± 6.05	N.S
Body mass index	24.56 ± 2.79	24.78 ± 3.37	N.S

* Mann-Whitney test

This study included 58 infertile men (25 from rural areas (group1) and 28 from urban areas (group2) from Assiut governorate. The 2 groups were comparable as regards age (33.68 ± 6.4 versus 33.17± 6.05 years respectively with no statistical difference between them), they were also comparable as regards body mass index (24.56 ± 2.79 versus 24.78 ± 3.37 kg/m 2 respectively).

B- Comparison between conventional semen parameters and sperm HOS percentage in infertile men from rural and urban areas:

Table (1): Comparison between conventional semen parameters and sperm HOS percentage from rural and urban areas

Semen variable	(Group 1) Infertile men from rural areas (n=25)	(Group 2) Infertile men from urban areas (n=28)	P-value
Semen volume (ml) Mean ± SD	2.34 ± 0.8	2.16 ± 0.8	N.S
Sperm Concentration (mil/ml)			

Mean ± SD	54.84 ± 54.30	70.75 ± 56.77	N.S
Total sperm count (mil/ejaculate) Range Median Mean ± SD	 4 – 600 80 121.8 ± 130.8	 6 –450 110 128.4 ± 98	 N.S *
Normal sperm morphology (%) Mean ± SD	 46.4 ± 15.5	 42.32 ± 21.45	 N.S
Progressive sperm motility (%) Mean ± SD	 30.44 ± 14.58	 25.46 ± 15.37	 N.S
Sperm HOS test (%) Mean ± SD	 65.12 ± 15.78	 62.07 ± 19.45	 N.S

* Mann-Whitney test

There was no statistical significant difference in semen parameters between infertile men from rural and urban areas including semen volume, sperm concentration, total sperm count, normal sperm morphology, progressive sperm motility and sperm HOS test (Table 1).

C- Comparison between serum and semen lead and cadmium concentrations in infertile men from rural and urban areas:

Table (2): Serum and semen concentrations of lead and cadmium and in infertile men from rural and urban areas

heavy metals level	(Group 1) Infertile men from rural areas (n=25)	(Group 2) Infertile men from urban areas (n=28)	P-value
Serum lead (µg/L) Mean ± SD	 21.99 ± 6.77	 27.02 ± 4.88	 <0.01
Semen lead (µg/L) Mean ± SD	 10.21 ± 4.88	 13.82 ± 4.343	 <0.01
Serum cadmium (µg/L) Mean ± SD	 5.41 ± 1.39	 6.25 ± 1.07	 <0.05
Semen cadmium (µg/L) Mean ± SD	 3.074 ± 1.18	 3.85 ± 1.24	 <0.05

* Mann-Whitney test

There were statistical significant increases in serum and seminal plasma levels of lead ($p < 0.01$ in each) among infertile men from urban areas compared to those from rural areas, and the same findings was reported as regards plasma levels of cadmium ($p < 0.05$ in each) (Table 2).

D- Correlations between serum and semen lead concentrations (in µg/L) and other semen parameters:

Table (3): Correlations between serum lead concentration and semen parameters in infertile men from rural and urban areas

Variant	r	P value
Normal sperm morphology (%)	-0.28	<0.05
Progressive sperm motility (%)	-0.5	<0.001
Sperm HOS test (%)	-0.51	<0.001

There were statistical significant negative correlation between serum concentration of lead and semen parameters in the form of (Normal sperm morphology (%) $r = -0.28$ and $p < 0.05$, Progressive sperm motility (%) $r = -0.5$ and $p < 0.001$ and Sperm HOS test (%) $r = -0.51$ and $p < 0.001$) in infertile men as shown in Tables 3.

Table (4): Correlations between semen lead concentration and semen parameters in infertile men from rural and urban areas

Variant	r	P value
Normal sperm morphology (%)	-0.66	<0.001
Progressive sperm motility (%)	-0.78	<0.001
Sperm HOS test (%)	-0.79	<0.001

There was also statistical significant negative correlation between semen concentration of lead and semen parameters in the form of (Normal sperm morphology (%) r = -0.66 and p <0.001, Progressive sperm motility (%) r = -0.78 and p <0.001 and Sperm HOS test (%) r = -0.79 and p <0.001) in infertile men as shown in Tables 4.

E- Correlations between serum and semen cadmium levels and other semen parameters

Table (5): Correlations between serum cadmium concentration and semen parameters in infertile men from rural and urban areas

Variant	r	P value
Progressive sperm motility (%)	-0.36	<0.01
Sperm HOS test (%)	-0.45	<0.01

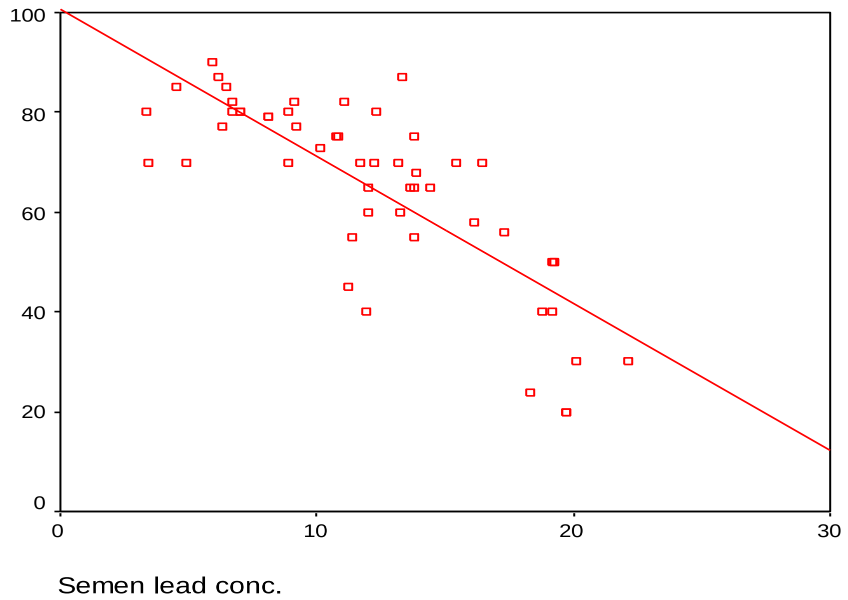
This table shows a statistical significant negative correlation between serum cadmium concentration and semen parameters in the form of (Normal sperm morphology (%) r = -0.36 and p <0.01, and Sperm HOS test (%) r = -0.45 and p <0.01) in infertile men as shown in Tables 5.

Table (6): Correlations between semen cadmium concentration and semen parameters in infertile men from rural and urban areas

Variant	r	P value
Normal sperm morphology (%)	-0.55	<0.001
Progressive sperm motility (%)	-0.66	<0.001
Sperm HOS test (%)	-0.75	<0.001

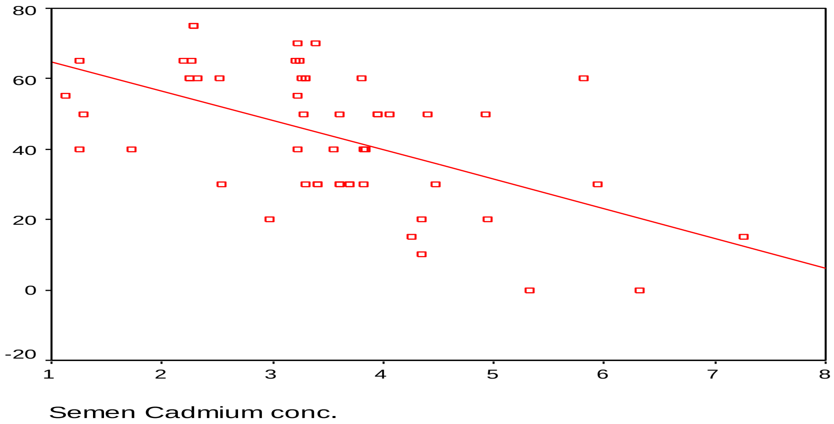
This table shows a statistical significant negative correlation between semen cadmium concentration and semen parameters in the form of (Normal sperm morphology (%) r = -0.55 and p <0.001, Progressive sperm motility (%) r = -0.66 and p <0.001 and Sperm HOS test (%) r = -0.75 and p <0.001) in infertile men as shown in Tables 6.

Fig (1): Correlations between semen lead concentration and sperm vitality in infertile men from rural and urban areas



This figures shows a statistical significant negative correlation between semen lead concentration and sperm vitality (%) (r = -0.86 and p <0.001) in infertile men.

Fig (2): Correlations between semen cadmium concentration and normal sperm morphology% in infertile men from rural and urban areas



This figure shows a statistical significant negative correlation between semen cadmium concentration and Normal sperm morphology (%) r = -0.67and p <0.001, in infertile men.

Table (7): Correlations between serum and semen lead concentration on one hand and corresponding concentrations of cadmium on the other hand

Variant	r	P value
Serum cadmium concentration	0.72	<0.001
Semen cadmium concentration	0.88	<0.001

This table shows a significant positive correlations between serum and semen lead concentrations on one hand and corresponding concentrations of cadmium on the other hand, r=0.72, 0.88 for serum and semen values respectively and p< 0.001 for each, which suggests the co-existence and/or co-exposure to the 2 heavy metals as environmental toxicants.

IV. Discussion

A positive relationship was demonstrated between low-dose environmental exposure to lead and unexplained poor sperm parameters and/or a decrease in male fertility (Saaranen et al, 1989 and Lee et al 1992). Allowable safe concentrations of toxic pollutants are still a debatable issue in developing countries where there is wide variation in human susceptibility and tolerance, which depend on general human health and other environmental factors (WHO, 1977).

Our study aimed to evaluate difference in serum and seminal plasma levels of lead and cadmium between rural and urban infertile men from Assiut governorate to spotlight on the possible environmental role of infertility in urban areas which is more polluted than the rural ones, also aimed to correlate these levels of serum and seminal plasma lead and cadmium with conventional sperm parameters and sperm vitality.

Our study included 58 infertile men (25 from rural areas (group1) and 28 from urban areas (group2). The 2 groups were comparable as regards age, body mass index and social status. The matching of the two groups in age, BMI and social class was intended to avoid confounding bias that may occur if the two groups were not comparable.

There was no significant difference in semen parameters (semen volume, sperm concentration, total sperm count, normal sperm morphology, progressive sperm motility and sperm HOS test) between infertile men from rural and urban areas in all these parameters (Table 1) This could be expected from the impaired reproductive capacity in both groups. However, there were significant increase in both serum and seminal lead and cadmium in urban group that could be attributed to the difference in residence of the two groups.

Bellinger, 1994 hypothesized that lead is not directly toxic to testicular tissue, but rather affects the androgenic control of the testis, impairing spermatogenesis and intra-testicular testosterone levels. Our results revealed that there were statistical significant increases in serum levels of lead among infertile men from urban areas compared to those from rural areas lead (P < 0.01) (Table 2). This finding is matched with several studies; one of them is Chia et al. 1992 who had demonstrated that high blood lead levels in infertile men are associated with asthenzoospermia. This may be explained by the fact that urban populations are more liable to all form of lead exposure and toxicity like traffic exposure, painting exposure and other forms of environmental lead toxicity which is not present or present in minimal concentrations in the rural side which is less polluted and less crowded than the urban side.

Pooling of fertile Egyptian men produced a mean semen lead level of 17.67pg/dL, which was about threefold higher than the values recorded in Europe [i.e., 5.2 kg/dL]. Lead levels, both in semen and in blood, were always higher in infertile population than in fertile, comparing pooled fertile cases with pooled infertile cases showed significant differences in lead levels between the two groups in blood (P< 0.0001) (Lee et al, 1992).

Burimovitz and his colleagues 1983 suggested that lead in semen is a more accurate index of the degree of reproductive exposure than lead in blood. In our study we found statistical significant increases in semen levels of **lead** among infertile men from urban areas compared to those from rural areas (P < 0.01) (Table 2). This finding is matched with Platchy et al. 1977 who highly recommended the use of lead level in semen as a biologic indicator for lead exposure.

Decreased sperm chromatin stability in men chronically exposed to lead has been demonstrated previously Wildet et al 1983. Wang et al 1992 explained that moderate occupational exposure, close to the present occupational standards, causes qualitative changes in semen parameters, including direct and indirect effects of lead on accessory genital function and sperm maturation. In a recent study involving rats (Sokol et al 1994), it was demonstrated that at 30 pg/ dl lead there is a reduction in the ability of sperm to penetrate or fertilize the egg of non exposed females in the absence of ultra structural changes. This in agreement with a study in mice by Johansson and Wide (Johansson and Wide, 1986).

In our study we found that there were a statistical significant negative correlation between serum concentration of lead and semen parameters in the form of (Normal sperm morphology (%) r = -0.28 and p <0.05, Progressive sperm motility (%) r = -0.5 and p <0.001 and Sperm HOS test (%) r = -0.51 and p <0.001) in infertile men as shown in Tables 3. this results is matched with Wyrobek et al, 1983 who suggested that the sperm morphology is the most sensitive parameter to use in studying changes in spermatogenesis caused by toxic substances. Morphology is also a good indicator for the state of the testicular germinal epithelium (Jeyendran et al 1984). Comparison of sperm count, percent abnormal forms, and percent motility demonstrated statistically significant differences based on fertility status. These changes suggested morphology as a more sensitive index to the effect of lead than other semen parameters. However, there was no effect on volume, which may be due to the absence of any effect on the vas or the prostate; this finding is contradictory to results demonstrated in experimental animals (Chowdbury et al 1984).

Furthermore, this study, to our knowledge, is one of few studies made to measure the lead levels in semen of an Egyptian population based on environmental exposure and yet recorded the highest lead level in semen in a human population. [e.g., (Emara et al, 1990 and El Samra et al 1992).Similar studies have been conducted in developed (European or Asian) countries [e.g. (Coste et al 1991, Saaranen et al 1989 and Sokol et al 1994)]. However. The difference in the levels of environmental and occupational exposure and the much more stringent standards and environmental regulations may explain, at least in part, the observed differences in levels from the current study.

As regards cadmium exposure and its effect on fertility we found the same statistical significant increases in serum and semen levels of cadmium among infertile men from urban areas compared to those from rural areas (P < 0.05) (Table 2). We also think that this deference may be attributed to the same factors of environmental pollutions that may manifest in the urban areas than the rural ones.

V. Conclusions and recommendations

Infertile men from urban areas are more subject to hazardous environmental toxicants as heavy metals which necessitates special attention in management of those subjects attending infertility clinics and belonging to urban areas.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

[1] Amr MM, Zagloul MA. Youssef MZ. Biological monitoring of lead in Egypt. Doctoral Thesis, Department of Occupational Medicine. Cairo University, Cairo. Egypt: 1991,

[2] Bellinger D. Teratogen update: lead. Teratology. 1994:50:367-73.

[3] Benoff S, Jacob A, Hurley IR (2000): Male infertility and environmental exposure to lead and cadmium. Hum Reprod Update. 6:107–21.

[4] Butrimvitz GP, Sharlip I, Lo R. Extremely low seminal lead concentrations and male fertility. Clin Chem Acta. 1983;135:229-31

[5] Chia SE, Ong C, Lee ST, Tsakak FH. Blood concentrations of lead, cadmium, mercury, zinc and copper and human semen parameters. Arch Androl. 1992;29: 177-83.

[6] Chowdbury AR, Dewan A, Gandhi DN. Toxic effects of lead on the testes of rats. Biomed Biochem Acta. 1984;1:95-100.

[7] Coste J. Mandereau L. Pessione F. Lead exposed workmen and fertility: a cohort study on 354 subjects. Eur J Epidemiol. 1991 :7: 154-g.

[8] Dickman MD, Leung CK and Leong MK (1998): Hong Kong male subfertility links to mercury in human hair and fish. Sci Total Environ; 214:165–74.

[9] Emara AM. Zagloul AM. Zayet HH. Auto-exhaust pollution in Cairo. Egypt J Occup Med. 1990:14: l-12.
[10] El Samra G. Emara H, El Sobky M. The extent of environmental pollution from motor vehicles and its biological indices. Doctoral Thesis. Department of Occupational Medicine. Cairo University: 1992.
[11] Friberg L and Vahter M (1983): Assessment of exposure to lead and cadmium through biological monitoring: results of a UNEP/WHO global study. Environ Res; 30:95–128.
[12] Inhorn M, King L, Nriagu J, Kobeissi L, Hammoud N, Awwad J, Abu-Musa A and Hannoun A (2008): Occupational and environmental exposures to heavy metals:Risk factors for male infertility in Lebanon?, Reprod Toxicol; (25): 203–12.
[13] Järup L and Akesson A. Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol. 2009 Aug 1;238(3):201-8.
[14] Järup L and Alfvén T. Low level cadmium exposure, renal and bone effects--the OSCAR study. Biometals 2004 ;17(5):505-9.
[15] Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJ (1984): Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J Reprod Fertil, 70(1):219–28.
[16] Lee S, Chia E, Ong C. Blood concentration of lead, cadmium, mercury, zinc and copper and human semen parameters. Arch Androl. 1992;29: 177-83.
[17] Mortada, W.I., Sobh, M.A., El-Defrawy, M.M., Farahat, S.E., 2002. Reference intervals of cadmium, lead, and mercury in blood, urine, hair, and nails among residents in Mansoura City, Nile Delta, Egypt. Environ. Res. Sect. A 90, 104–110.
[18] Office of Mobile Sources. Office of Air and Radiation. US Environmental Protection Agency: 1991.
[19] Pant N, Upadhyay G, Pandey S, Mathur N, Saxena DK and Srivastava SP (2003): Lead and cadmium concentration in the seminal plasma of men in the general population: correlation with sperm quality. Reprod Toxicol; 17:447–50.
[20] Plechaty MM, No11 B, Sunderman FW Jr. Lead concentrations in semen of healthy men without occupational exposure to lead. Ann Clin Lab Sci. 1977;7:515-8.
[21] Skakkebaek NE, Rajpert-De Meyts E and Main KM (2001): Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 16:972–8.
[22] Takiguchi M and Yoshihara S (2006): New aspects of cadmium as endocrine disruptor. Environ Sci; 13:107–16.
[23] UNEP and WHO (1984): Urban air pollution 1973-l 980. Global environmental monitoring systems (GEMS). New York: United Nations Environmental Program (UNEP) and Geneva: World Health organization: 1984.
[24] UNEP and WHO (1988).. Assessment of urban air quality. Report on the results of the WHO/UNEP program on health-related environmental monitoring. New York: United Nations Environnmental Program (UNEP) and Geneva: World Health Organization: 1988.
[25] Wildet K. Ellison R, Berlin M. Effects of occupational exposure to lead on sperm and semen. In Clarkson TW. Nordberg GF. Sager PR, eds. Reproductive and developmental toxicity of metals. New York: Plenum: 1983:279-300.
[26] World Health Organization (WHO). Lead. Environmental Health Criteria on Inorganic Lead. No.3; 1977.
[27] Saaranen M, Kantola S, Vanha T. Lead, magnesium, selenium and zinc in human seminal fluid: comparison with semen parameters and fertility. Hum Reprod. 1989;2:475-9.
[28] Viaene MK, Roels HA, Leenders J et al. Cadmium: a possible etiological factor in peripheral polyneuropathy. Neurotoxicology 1999; 20:7-16.
[29] Wyrobek AJ, Gordon LA, Bud&art JG, Francis MW, Kepp RW Jr, Letz G, Malling HV, Tophan JC, Whorton MD. An evaluation of 0 E. A. EL-ZOHAIRY ET AL. 151 human sperm as indicators of chemically-induced alterations of spermatogenic function. Mutat Res. 1983;115:73-148.
[30] Wang L, Ong C, Foo S. LDH-X in human seminal plasma as an indicator of the functional integrity of spermatozoa. J Am Co11 Health. 1992;41:115-9.
[31] Sokol R, Okuda H, Nagler H, Berman N. Lead exposure in vivo alters the fertility potential of sperm in vitro. Toxicol Appl Pharmacol. 1994;124:310-6.
[32] Zhang ZW, Qu JB, Watanabe T, Shimbo S, Moon CS, Ikeda M. Exposure of citizens in China and in Japan to lead and cadmium: a comparative study. Toxicol Lett. 1999, 108, 3:167-172.