La contaminación ambiental y ocupacional por plomo y sus efectos en la salud reproductiva masculina, evidencia de daño al ADN

Occupational and environmental contamination by lead and its effects on male reproductive health, evidence of dna damage

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Resumen

La relación que existe entre los factores ambientales y la salud nos conduce a considerar dentro de la problemática del medio ambiente a aquellos trastornos que provienen de la contaminación del aire, el suelo y el agua, así como los resultados del abuso de drogas, productos químicos y agentes físicos potencialmente dañinos. Se multiplican las amenazas a la salud cuando ciertos agentes habitualmente presentes en el medio ambiente llegan a alcanzar concentraciones superiores a las permisibles por las normas de salubridad internacionales; o bien, se afecta el equilibrio natural cuando el hombre produce masivamente diversos compuestos químicos, derivados del creciente desarrollo de la tecnología, dando lugar a una constante acumulación de contaminantes.

En dicho contexto, la actividad industrial del hombre también produce sustancias cuyas aplicaciones repercuten benéficamente en su calidad de vida, pero, infortunadamente, muchas veces traen consigo gran cantidad de residuos dañinos que ponen en riesgo el medio ambiente.

Palabras Clave: contaminación ambiental, plomo, salud, adn.

Abstract

The relationship between environmental factors and health leads us to consider within the environmental problems those disorders stemming from air, soil and water pollution, as well as the results of drugs abuse, potentially harmful physical agents and chemical products. The threats to health multiply when certain commonly present agents manage to reach concentrations above the permissible by international safety standards; alternatively, affects the natural balance when the man produces massively different chemical compounds, derived from the growing

development of technology, giving rise to a steady accumulation of contaminants. In this context, the human industrial activity also produces substances whose applications beneficently impact their quality of life, but, unfortunately, often bring with them lots of harmful residues which endanger the environment.

Key Words: environmental pollution, lead, health, DNA.

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Introduction

Among the adverse effects of these pollutants is damage to the genetic material, making this a toxicological risk which has not been appreciated enough despite its impact on the health of affected individuals and their descendants. The damage induced by these agents can be at the level of somatic and/or germ cells. When the mutagenic effect takes place in somatic cells it produces changes in genetic information, with the emergence of potentially malignant cells in the individual. Alternately, if the genetic damage occurs in the germ cells, this manifests in malformations and physiological effects, altering the reproductive capacity and/or quality of the species. The most aggressive waste for the environment, according to the Agency for environmental protection (EPA in United States) and the European Economic Community, are heavy metals, appearing (Pb) lead and its compounds between the first nine of the classification. Other metals, such as arsenic (As) and cadmium (Cd) are also within the more aggressive, as well as zinc (Zn), considered highly aggressive in special situations (De Blas, 1991). It was noted that high levels of lead are associated with nephrotoxic, neurotoxic effects, alterations in hematological and reproductive function.

In the Comarca Lagunera, industrial and agricultural development has significantly deteriorated the environment of the region. The life quality has been affected in the aspects mentioned above, being our main interest establishing the possible relationship between the pollutants, primarily the Pb, and infertility in males exposed by its activity to such an environment.

La Laguna region, located in the central and northern Mexico comprises 11 municipalities (urban, suburban and rural areas), divided between the states of Durango and Coahuila. While this region has developed very significantly in the agricultural and industrial sectors; its orographic conditions have decreased the quality of life of its inhabitants. The factors associated with the origin of these problems have not been fully described, much less analyzed the processes at the

cellular and molecular level. Some causes of environmental degradation in the region are already known; for example, milk production and meat and fodder, which are among the largest in the country. Both have killed groundwater in the lower part of the closed basin of the lake, even contaminating drinking water with arsenic; Furthermore, the location of the world's fourth largest in the center of Torreon, Coahuila, main producer of silver in the world and in Latin America gold, lead and zinc smelter has caused an impressive contamination, which has been gravely accentuated in recent months by the high emission of heavy metals like lead into the environment. These factors have caused many problems in the health and quality of life of the inhabitants of this region.

One problem of primary interest is the ability of human reproduction. The reproductive system in humans is specifically vulnerable to the serious environmental problems that impact the environment, arising from the growth and development of peoples. However, too many reproductive damage in humans are due to endemic causes of order in the environment, and which generally influence political and socioeconomic cultural, religious.

To justify this type of work shows that individuals who have been exposed to heavy metals such as Pb, have damage to their DNA, they have poor sperm quality, with a number of anomalies in the evaluation of semen quality or a combination of several of these. Therefore, it is imperative and a priority to establish first the effect of Pb association with spermatogenic cells that initiate the process and the parameters of reproductive quality.

On the other hand, it is urgent to provide data linking exposure factors with genetic damage, as this region shows a high rate of congenital and genetic malformations. The sperm DNA integrity is essential for the accurate transmission of genetic information and, therefore, for the good health of future generations. It is also important to include the comet assay as an additional parameter spermatobioscopy. The aim of this paper is to show the results of research conducted in the Department of Cell Biology and Ultrastructure the Center for Biomedical Research of the Faculty of Medicine of the Autonomous University of Coahuila, Torreon Unit, in collaboration with other related departments damage DNA comet assay using sperm cells and sperm quality of individuals occupationally exposed to lead.

BACKGROUND

Spermatogenesis. The male reproductive system basically comprises the testes, ducts (epididymis and vas deferens), the accessory glands (seminal vesicles, prostate and bulbourethral glands) and penis (Guyton and Hall, 1996). In testis Leydig cells, which are stimulated by luteinizing

hormone (LH) (secreted by the pituitary) for the synthesis of testosterone (T) they are located. Sertoli cells, cells that secrete growth factors and providing physical support to the germ cells to develop, in addition, Sertoli cells are stimulated by follicle stimulating hormone (FSH) to synthesize and provide nutritional elements are also located that they are necessary for the development of sperm. In testicular germ cells are also located, which by the process of spermatogenesis divide and differentiate into spermatozoa (Guyton and Hall, 1996). Spermatogenesis is a cyclic process which has a duration of 74 days in man.

This process consists of 3 phases:

Successive mitotic divisions of spermatogonia to yield spermatocytes (espermacitogénesis).

Meiosis of spermatocytes to give rise to haploid (meiosis) spermatids.

Morphological differentiation of spermatids into spermatozoa (spermiogenesis).

During spermiogenesis, round spermatids perform morphological changes include loss of cytoplasm, acrosome formation, tail growth and development of a condensed nucleus with replicational activity, diminished transcriptional and repair (Guyton and Hall, 1996).

In the final phase of spermiogenesis is performed chromatin condensation, in which process the basic protamines rich in cysteine and arginine, packaged DNA of the sperm nucleus (Balhorn, 1982). Replacing the protamines histones somatic 85-90%, so the mature sperm remains minimal histones (10-15%) (Gatewood et al, 1987). Chromatin, besides being condensed, it is stabilized by the formation of disulfide bridges between the cysteine residues of the protamines, when the sperm passes through the epididymis (Bedford and Calvin, 1971). With chromatin condensation and stabilization thereof, acquires the sperm fertilization potential (Saowaros and Panyim, 1979; Manfredi et al, 1986). When the process of ejaculation occurs, sperm are expelled from the epididymis and vas deferens and immediately the prostate and seminal vesicles excrete their contents to contribute to the volume of seminal fluid (Setchell and Brooks, 1988). The seminal fluid secreted by the prostate is rich in zinc (Zn), citric acid and acid phosphatase, while the fluid secreted by the seminal vesicles containing fructose and high molecular weight proteins (MPAP) (Arver, 1982; Bjorndahl et al, 1991). A very important aspect that prostate zinc is incorporated sperm nucleus and maintains reduced to thiol groups of protamines not forming disulfide bridges state; thus, this Ion maintains normal chromatin condensation (Kvist, 1980ª).

Testicular function. The testicle or male gonad is stimulated by the synthesis and stimulation of LH and FSH gonotrofinas having a nightly secretion rhythm, sleep-related initially. These hormones stimulate the testis to start his two functions: ESTEROIDIOGÉNESIS: predominance in the

production of androgens, favoring the appearance of secondary sexual characteristics, such as growth and development of the penis and testes, pigmentation and appearance of roughness on scrotum, growth of pubic and auxiliary hair from various body regions including the face, start sex drive, deepening of the voice, growth of the larynx, muscle development, onset of baldness front line, ability to penile erection, ejaculation etc., and contribution of growth hormone (GH) which stimulates liver production mainly type I insulin growth factor, this acting on the growth plates, causing the characteristic peak of growth at puberty; the other function is GERMINAL: multiplication and maturation of germ cells starts, known as spermatogenesis cycle. During the ejaculatory event, which is dependent on testosterone, semen is ejected to be placed in the female genital tract so that fertilization occurs and, thus, preserve the species (Ayala, 1995).

Biology of sperm. Sperm formation starts in spermatogenesis, which is hormonally regulated; Luteinizing (LH) hormone acts on Leydig cells and regulates the production of androgens, follicle stimulating hormone (FSH) acts on the Sertoli cell and its role in spermatogenesis (differentiation of spermatids) during the first event, for maintenance of spermatogenesis is not essential testosterone. (Bujan, 1988).

Sperm maturation. Mammalian sperm acquire the ability to fertilize the egg and motility to pass through the epididymis, they experience changes in the form of metabolic pathways by binding to the zona pellucida. Advances in research have shown that mature sperm motility is regulated by calcium ions, cyclic AMP, adenosine and intracellular pH and that these factors interact with each other (Ayala, 1995).

LEAD

Distribution and accumulation. Lead is not homogeneously distributed in the body, but is distributed in several interrelated compartments: 1) blood lead (PbS), which contributes 1% of the body burden with a high percentage of red blood cells, represents an exhibition recent since the average life of PbS is only 36 days, 2) lead in soft tissues such as the kidney and nervous system which is responsible for most of the toxic effects of lead and 3) bone lead, which is the main component in the body burden, representing 95% in adults and 70% in children. Lead in bone represents the difference between the cumulative absorption in all sources of lead exposure and total excretion of the same, ie, it represents a chronic exposure. (ATSDR, 1999). The distribution of Pb in the sexual organs is still unknown, but it has been observed that Pb accumulates in seminal

vesicles, in prostate, testis and epididymis (Johansson and Wide, 198; Oldereid 1993, Batra et al., 1998).

Toxic effects of lead exposure. Lead is the most ubiquitous metal and is detectable in virtually all ecosystems and in all biological systems; according to a study conducted in Latin America and the Caribbean, exposure to lead is still a health problem in the region, unfortunately there is very little literature available to assess the extent of the problem of lead exposure in our country related to the problem reproductive.

The main concern is to eradicate child population exposure to this metal, although it has been recognized as a health problem also for adults, particularly in industry. Lead exposure in Latin America includes mining, smelting and refining, production and use of glazed ceramics, reuse and recycling of batteries and in countries where leaded gasoline is still used, this is a source of environmental exposure.

Latin America (particularly Mexico and Peru) accounts for 14% of world production of lead. In Mexico there are some major mines and one of the largest primary smelters located in the northeast of the country (more than 100 000 tonnes), plus some secondary smelters (Romieu et al., 1997).

The toxic effects of lead are observed in almost all organs and many biochemical processes, among which we mention the central and peripheral nervous system, kidney, heme synthesis, cardiovascular, reproductive and gastric system (Goyer, 1996). Despite the lack of studies on human, animal studies show that some inorganic lead compounds are potent human carcinogens, especially to the kidney. Critical or sensitive effects occur in pediatric populations and involve the central nervous system, whereas in adults with excessive occupational exposure neuropathies and / or chronic renal disease are presented, although the effect may be more critical hypertension. Early biochemical alterations were demonstrated in the hematopoietic system.

Lead inhibits two enzymes of the biosynthetic pathway of heme, the dehydratase of delta-aminolevulinic acid (ALAD) and ferrochelatase; the effects on heme synthesis provide biochemical indicators of lead exposure in the absence of detectable effects. The bioavailability and toxic activity seem to lead regulated by its interaction with proteins with high affinity for the same, both rich in hydrogen group such as carboxyl group (Goering, 1993).

The activity of many enzymes including enzymes heme biosynthetic route (Quintanilla-Vega et al., 1995), constituting as nuclear proteins of sperm protamines (Quintanilla-Vega et al., 1999a) and enzymes calmodulin dependent (Goering, 1993), are inhibited by lead affinity for sulfhydryl and

carboxyl groups of the cysteine residues and aspartic and glutamic acid, respectively. The affinity of the protein also appears to lead to determine susceptibility by exposure. You can mention the erythrocyte protein described participating in the "protection" to lead toxicity (Raghavan et al., 1980; Calderon-Salinas et al., 1991), the ALAD (Wetmur et al enzyme. 1998) and brain proteins (Quintanilla-Vega et al., 1995b) and kidney (Smith et al., 1998) that may play a role in individual susceptibility to lead. Lead is the main contaminant in near related to the refining and smelting industries in the same areas, but other metals are also present as contaminants, including cadmium, arsenic and zinc.

Toxic effects on male reproduction by lead exposure. The male reproductive system is very vulnerable to physical and chemical exposures, but the specific role of environmental and occupational factors in the etiology of male infertility is not well established. The association between occupational exposure to lead and reproductive failures was the first observation associated adverse reproductive effects from occupational exposures (Lancranjan et al., 1975). However, it was not until the early nineties that paternal exposure potential in the development of the progeny received attention. Lead is known to alter both male and female reproductive function, although the mechanisms are not completely clear. The effects of lead on reproduction and development are dependent on sex and seem to involve multiple sites in the hypothalamus-pituitary-gonadal axis, causing altered hormone levels (Sokol, 1987) and changes in the characteristics and function of sperm, even a blood lead concentrations below the limit accepted by work environments (Lerda, 1992, Hernandez-Ochoa et al, 2001, Moran-Martinez et al, 2004;.. Hernandez-Ochoa et al., 2005).

Table 1. Studies show the association of lead and its effect in sperm quality

Estudio	Resultado	Referencia
Trabajadores de fábrica de baterías, técnicos, oficinistas (23 vs 74µg/dl)	↓ motilidad ↓ concentración	Lancranjan *et al*., 1975
Trabajadores de fábrica de baterías (23 vs 49 µg/dl)	↓ volumen y motilidad ↓ concentración	Lerda *et al*., 1992
Trabajadores de fábrica de baterías (22vs79 µg/dl)	↓ concentración de espermatozoides sin daño endócrino	Assenato *et al*., 1987
Trabajadores industriales (10-37 µg/dl)	↓ motilidad, viabilidad y concentración sin daño endócrino	Telisman *et al*., 2000
Trabajadores de una fundidora de Pb (17 vs 73µg/dl)	↓ Testosterona ↑ LH	Rodamilans *et al*., 1988
No específica (17-77µg/dl)	↓ LH ↑ FSH	McGregor *et al*., 1990
Trabajadores de una fábrica de baterías (9.6-77µg/dl)	↑ LH-FSH ↓ Testosterona/ años de ocupación	Ng *et al*., 1991
Trabajadores de fábrica de baterías	↓ motilidad	Viskum S. et al 1999
Trabajadores de una fundidora de Pb (15-40 µg/dl)	↓ Motilidad ↓ Concentración total	Alexandre BH et al 1996

Although the harmful effects of lead on male reproduction have been known for decades (Winder, 1993), their mechanisms of action remain contradictory. While some studies suggest a direct effect on the testicles, causing a decrease and impaired sperm production, morphology and motility (Assenato et al., 1987; El-Zohairy et al., 1996; Lerda, 1992), other studies show evidence of effects on the central area in the hypothalamus-pituitary axis, causing a hormonal disorder (Gustafson et al., 1989). There is also controversy in animal studies to assess the adverse effects of lead. While routine parameters to evaluate the quality of semen (concentration, motility and morphological changes) are affected in most cases, some authors present results support the hypothesis that lead has toxic effects in the hypothalamus axis -pituitaria (Sokol, 1987). Although there is no consistent association between the incidence of spontaneous abortions and paternal exposure to lead has been shown in some studies, an increased risk of abortions when the parent is exposed before conception (Lindbohom et al., 1991). Although it is unclear parental involvement in the problems of reproduction in animals results clearly show a decrease in the ability of lead-treated sperm to fertilize the egg (Sokol et al, 1994) and / or implantation loss the fertilized egg (Wide L. Johansson, 1986).

Genetic damage. Published studies indicate that exposure to xenobiotics can cause changes in sexual behavior and contribute to the incidence of subfertility, infertility, stillbirths, fetal growth retardation, intrauterine fetal damage, birth defects and ovarian or testicular failure. The extent to which the atmosphere adversely affects the human reproductive health (; Giorlandino et al., 1998 Jacobs, 1992) is known. In recent years, great attention has been focused on the potential of a wide range of xenobiotics to interact and alter the genetic homeostasis (Lahdetie et al., 1999). Moreover, in the final phase of spermatogenesis takes place chromatin condensation, in which process the basic arginine and cysteine-rich proteins packaged DNA of the sperm nucleus (Balhorn, 1982), replacing the Somatic in 85-90%, leaving a minimal amount of histones (10-15%) (Gatewood et al., 1987). With chromatin condensation and stabilization thereof by the formation of disulfide bridges between the cysteine residues of the protamines, sperm fertilization potential acquired during passage through the epididymis (Saowaros and Panyim, 1979; Manfredi et al., 1986). On the other hand, in the process of ejaculation, sperm are expelled from the epididymis and vas deferens; and prostate and seminal vesicles secrete their contents contribute to the volume of seminal fluid (Stachell and Brooks, 1988). The liquid prostate is rich in zinc, this is incorporated into the nucleus of the sperm, reducing the thiol groups of cysteine residues of the protamines not forming disulfide bridges and, therefore, maintains normal chromatin condensation (Kvist, 1980ª). Therefore, the amount of Zn bound to these protamines is regarded as the index of the chelating ability of seminal fluid and as a measure of bioavailability of free Zn that can be incorporated into sperm (Bjorndahl et al, 1991).

Sperm DNA damage. Increasingly, the sperm DNA integrity is recognized as an independent measure of its quality. The sperm DNA integrity is vital in initiating and maintaining a pregnancy to term in vivo and in vitro. Routine semen analysis does not identify defects in sperm chromatin architecture. The evaluation of the integrity of DNA in sperm, besides the systematic study of the seminal parameters, could provide additional information about the quality of sperm. This could alleviate the social and emotional problems associated with failed attempts at assisted reproduction techniques. Infertility affects 15-20% of couples, and in about half of cases, is of male origin. Semen analysis in which concentration, pH, volume, motility and normal sperm morphology are measured, and remains the most important clinical test to predict infertility, revealed no sperm defects that affect the integrity of the male genome. Much evidence indicate a negative correlation between alterations in both in vivo and in vitro organization of genomic DNA material and sperm fertilization potential. This emphasizes the fact that the stability of the DNA, which is

capable of descondensarse at the right point in the process of fertilization, is one of the criteria to be taken into account when considering whether a sperm is fertile or not. (Lopes S, et al., 1998; Aitken R, Krausz C, 2001). Patients may have normal Semen and remain infertile, since the source of infertility may be due to the presence of an abnormal sperm DNA, that factor is not measured systematically. DNA integrity in sperm can be considered as an independent and parameter indicative of its quality. (Sun JG et al., 1997).

2.3.2 What is normal sperm DNA? Sperm DNA is organized so as to maintain the stable compact chromatin. This organization of the DNA not only allows that is very well packaged genetic material to be transferred to eggs, but also ensures that DNA is delivered in a chemical such fitness and contributing to the development of the embryo by the genetic information more accessible. The fertile sperm has a stable DNA which is able at the appropriate time descondensarse the fertilization process and transmit the DNA flawless. (Ward WS et al., 1991).

Origin of sperm DNA damage. The origin of the lesions in the DNA of sperm may be due to multiple causes, such as the presence of a disease, drug use, high fever, elevated testicular temperature, air pollution, smoking, varicoceles, hormonal factors, or advanced age. The molecular mechanisms involved in these lesions is still under intense investigation. The main mechanisms considered are: abnormal sperm DNA packaging during spermatogenesis. (Carrell DT L. and Liu, 2001) (C. Cho et al., 2003)

Abnormal sperm DNA packaging. This could result in abnormal packing defects in the conformation of chromatin and DNA fragmentation in the sperm. For the packaging of the sperm chromatin occurs, the activity of endogenous nucleases that cut DNA and liguen during protaminación is necessary (Sakkas D., et al., 1999). These cuts provide a release of torsional stress that helps the chromatin packaging during the change of histones by protamines. Abnormalities in the control of this process could result in gaps DNA unrepaired. These changes would occur before spermiation.

DNA fragmentation in the sperm. Among all parameters that are studied to determine sperm quality, there is one that has attracted considerable interest in recent years: the fragmentation of the DNA molecule in the male gametes (FAG). This parameter has a significant interest since the transfer of the whole molecule intact DNA from the sperm to the egg, it is essential to get the gestation of a normal individual. The cells of eukaryotic organisms have in their core a long DNA molecule in which all the information necessary for the development of the individual is collected. Any error in its structure, for example, the loss of continuity in the present DNA breaks, will lead to difficult problems. In its natural state, this long DNA molecule is broken because, in the nucleus of

every cell, genetic information is compartmentalized into discrete elements is known as chromosomes. Therefore, the nuclear DNA has a number equivalent to double strand breaks, or "biologically correct breakage" discontinuities. The break ends are sealed by structures of DNA and proteins that meet specific mission to empower each chromosome, telomeres. That is, a diploid nucleus, where DNA has been replicated to generate two new cells then must show 4XN "biologically correct breaks", where n = number of chromosomes of the species in question. An example, in the case of humans, where each core has 46 chromosomes, if these are replicated 184 exist "biologically correct breaks" or telomeres 184 per cell. Only one extra double-strand break, not stabilized by repair mechanisms in any chromosome DNA anywhere, may involve problems to the cell presenting it. In other words, the double-strand breaks that occur and persist in DNA are strong determinants of chromosome stability in subsequent cell divisions and if these are present in sperm, can be transmitted at the time of fertilization. (Gonsálvez J. et al., 2007)

Assessment of genetic damage. In this regard, some strategies for measuring metal exposure and genetic damage to sperm cells, are by evaluating the appearance of problems of sperm chromatin decondensation and the percentage of teratospermia in the study samples. The chromatin condensation during spermatogenesis is given to the result of the morphological characteristics of the sperm head and this suggests that sperm nuclear morphology is also an indicator or associated with the organization of chromatin, looks strongly linked to fertility (Ward and Coffey 1999). The susceptibility of sperm nuclear DNA induction of toxic or acid denaturation is inversely related to the structural integrity of the chromatin (Evenson et al., 1980).

BACKGROUND REGIONAL. Preliminary studies by the group of the Autonomous University of Coahuila (Moran Martinez. 1998) in individuals residing in the vicinity of the metallurgical area of Torreon, Coahuila, found that these individuals have higher lead levels in seminal fluid and lower percentage of motility and viability of sperm in a control group. Similarly, zinc levels in the seminal fluid were significantly higher, suggesting that an increase in zinc concentration in semen may also alter its quality (Moran Martinez et al, 1999a;. Moran Martinez et al. 1999b). On the other hand, in the pilot study in areas with endemic regional hydroarsenicism in the Laguna region, described by Moran Martinez et al., (1999, 1993), it was found that arsenic concentrations in urine were significantly higher when compared with control group. The subjects in this group exposed to arsenic were of poor quality in concentration, motility and viability of sperm (Moran Martinez et al., 1993). Also measuring some enzymes such as acid phosphatase in seminal fluid of these

subjects was significantly modified in the exposed group when compared with the control group (Moran Martinez et al., 1999). There are other important factors study or analysis in our region, which contribute significantly to the deterioration of reproductive health, such as: a) pregnancy in adolescents (10-23 years old), b) sexually transmitted diseases, c) prevalence of congenital diseases. In Mexico, 2.4 of every 100 births are associated with congenital malformations. In the state of Coahuila is exceeded the national average with 3.4 to 100. The most common malformations are defects of the neural tube, d) high incidence of genitourinary malformations e) infertility (Leke et al., 1993). This is indicative of the need to develop a strategy of comprehensive study to provide solutions and prevention guidelines to the problems presented.

MATERIALS AND METHODS

Study design

A first study group (GNE), was not exposed to lead. For this, a cross-sectional epidemiological study where participants were those individuals living in the three major cities of Laguna Region (in Torreon Coahuila, Gomez Palacio and Lerdo in Durango State) was performed. Individuals included were residents of the colonies that are considered low exposure areas, according to their location in or against the prevailing winds in the region. To determine the number of samples to study the results of the preliminary study in the same region by Moran-Martinez et al were taken into account., In 1998, where 21 individuals of high exposure and low exposure part 27 with a sperm motility 49% and 67% respectively. According to the mean difference in this parameter found semen quality, the number of sample calculated was 76 individuals, of whom took 15 samples of frozen for the determination of DNA damage in sperm. The choice of participants for this first group was by invitation, making home visits in selected colonies. Within the inclusion criteria were considered: the granting of informed consent to participate in the study consent, a minimum of 2 years of residence in place, denying habits of smoking or drinking alcohol and having no activities in areas of potential exposure other pollutants, such as farming or cement or cromadoras industries.

A second study group (GE), was formed by the workers of Peñoles metallurgical plant; Whereas there are provisions to prevent participation in any study by the employer to the workers of the plant, the number of males was subject to the work of persuasion and highlighting the importance of the results obtained with the participation of workers in the investigation. Workers were invited personally by home visits; They should have at least 2 years of working within the plant, to

consent in writing and denying habits of smoking or drinking alcohol. The number of sample calculated was 67, of whom 22 were taken to determine DNA damage.

Sampling and storing biological samples. Each individual participant provided a blood sample and a semen sample within a period of time not exceeding one week. Blood samples, taken by venipuncture (approximately 5 mL) and obtaining serum were stored at 20C until used for the determination of heavy metals. Semen samples obtained by masturbation (with 3 to 7 days of sexual abstinence or not ejaculation) and maintained at 37 ° C for analysis. Subsequently, 1 million cells of each sample was taken by centrifugation seminal fluid was separated from cells and stored at -70 until use for the determination of heavy metals in these two compartments, the remaining sample was stored at -70 ° C.

Semen analysis. It was performed within the first hour after ejaculation following the guidelines of the World Health Organization (WHO, 1992), evaluating various parameters including liquefaction, consistency (viscosity), ejaculate volume, pH, sperm concentration, motility, sperm morphology and viability.

Liquefaction. First determine liquefaction, aspirating sample with a Pasteur pipette observing the length of the filament. In a normal sample semen comes into small droplets, in the case of a delayed liquefaction (over 20 min.), The drop forms a filament greater than 2 cm in length or remains coagulated.

Volume and Ph. When the sample completely liquefied, the volume in 15 mL Falcon tubes is measured. The pH is measured with a pH test strip.

Concentration. Sperm concentration was determined by the method hematocytometer. A 1:20 dilution is prepared by mixing 50 uL of sperm with 950 mL of a solution of 5% NaHCO3 in 35% formaldehyde. For samples containing less than 20 x 106 spermatozoa / mL, a 1:10 dilution was used, while for samples containing more than 100 x 106 spermatozoa / mL, a 1:50 dilution was used. On each side of hemacytometer (Neubauer 0.1 mm, Hausser Scientific, Gaithersburg, MD, USA) were placed 10 mL of the previously homogenized mixture was allowed to stand for 7 min to ensure sedimentation of sperm and then was held cell counts by phase-contrast microscopy (Olympus model BX40 microscope). Sperm concentration was performed in duplicate with coefficient of variation (CV) <7%.

Motility. Se evalúa por medio de un equipo analizador de motilidad (Motion Analysis Corporation, Cell Track Mod. VP 110, Sta. Rosa, CA, EUA). La muestra se coloca en el microscopio (Olympus BH-2, con contraste de fases, Japan) anexo al equipo y la imagen será detectada por el analizador de imagen. Se evaluarán de 5 a 10 campos para tomar la lectura final. La determinación se realizará

en el Departamento de Biología Celular y ultraestructura del Centro de Investigación Biomédica de la Facultad de Medicina de la Universidad Autónoma de Coahuila.

Viabilidad. by exclusion method Eosin and 0.5% in physiological solution, which is based on the dead cells whose plasma membranes are damaged allow entry of the dye is assessed fresh. 100 sperm were evaluated by light microscopy and live sperm (uncolored) from the dead (colored) they differed.

EXPOSURE ASSESSMENT

Determination of Pb in blood. Determining blood Pb voltammetry was performed using a coulometer anode (Anodic Stripping Volammetry, Mod. B 3010, ESA Inc. Chelmsford, MA, USA).

Determination of Pb in seminal fluid. The quantitative evaluation of the concentrations of Pb in seminal fluid was performed in a atomic absorption spectrophotometer Beijing-Elmer 5100 (Perkin-Elmer, Norwalk, CT, USA) equipped with graphite furnace (HGA-600), Zeeman background correction (Perkin-Elmer 5100), a hollow lamp (Perkin-Elmer, Mod. Lumina) cathode and an autosampler (Perkin-Elmer AS-60).

Assessment of damage (comet assay)

Preparation of lamellae. Prepare agarose normal melting point to 5%, adding to the slide 175 ul of agarose normal melting point per slide, and cover it immediately with your finger and let it dry.

Fix cells. Add 75 ul per slide LMP agarose in an Eppendorf tube with a cell sample, (semen) 100,000 cells, mix and place the slides previously already regulate 5% agarose, and apply a coverslip cooling for 5 min., Remove carefully coverslip and immersing the foil in lysis solution at 4 ° C.

Celular.2.5 lysis MNaCl, 100 mM disodium EDTA, 10mM Tris base, 8% DMSO, Triton X-100 0.8%) at 4 ° C at least 2 hours. The following steps are carried out under yellow light: Carefully remove the foil from the lysis solution.

Electrophoresis. Place it in a horizontal electrophoresis chamber at 4 ° C, covering the slide with electrophoresis buffer 300 mM EDTA 1 mM NaOH (pH 10) and leave for 20 minutes in unwinding (unwinding). After 20 minutes turn the electrophoresis chamber with 23 volts 300 milliamperes for 20 minutes. Once off the power supply, carefully remove the slides of the electrophoresis chamber.

Neutralization. Drain the excess foil electrophoresis buffer and place the slides into a neutralizing buffer Koplin with 0.4 M Tris pH 7.5, leave for 5 minutes, drain and dip the slide in Koplin with absolute alcohol for 5 minutes, carefully remove and leave dry off.

Subsequently stained with ethidium bromide and observed in the fluorescence microscope.

RESULTS

Unexposed group (GNE)

Characteristics of the study population. The general characteristics of the study population are shown in Table 1. Body mass ranges established by Perez de Gallo and Marvan Laborde (1996) (wasting: <20 normal: 20-25 overweight and> 25), indicated that 76.5% of the population was overweight. Regarding smoking habits, drug and alcohol, 72% were smokers and 28% non-smoking and only 65% of smokers remained the habit at the time of the interview; 91% drank alcoholic beverages, mainly beer. Finally, to determine caffeine consumption was assumed that a portion of soda or coffee containing the same amount of caffeine.

Tabla 2.- Características generales del GNE

Característica	Sujetos estudiados
Edad (años) [a]	34 ± 8 (21-54)
Peso (kg) [a]	81.6 ± 16.7 (52-125)
Talla (m) [a]	1.71 ± 0.062 (1.55-1.93)
Índice de Quetelet [a]	27.8 ± 5.4 (17.6-57.4)
Emaciación (%)	2.9
Normal (%)	20.6
Sobrepeso (%)	76.5
Fumadores (%)	72.0
Fumadores actuales (%)	65.3
Consumidores de bebidas alcohólicas (%)	91.2
Consumidores de drogas (%)	5.9
Consumo de cafeína [b]	2.07 ± 1.98 (0 - 12)

[a] Arithmetic mean ± standard deviation. b average daily consumption of one serving of cola drinks and / or soda taste and / or diet soda and / or coffee Indica. The ranges are shown in brackets. n=15.

Quality of semen. Table 2 shows the parameters of semen quality is. When comparing the averages of each parameter semen quality with those established by the WHO (1992) shows that are within normal; however, part of the population had some altered parameters such as motility with the highest percentage of individuals with abnormalities (44%), followed by the morphology (35%), the pH was the least affected (1%).

Table 3. Semen quality parameters in individuals GNE

Parámetro	Media ± DE	Rango	% Anormalidad [a]
Motilidad (%)	51.8 ± 21.3	4-87	44
Viscosidad*	-	-	35
Morfología (%)	62.6 ± 17	13-87	32
Volumen (mL)	2.7 ± 1.3	0.7-6.4	21
Viabilidad (%)	67.4 ± 16.5	17-94	12
Concentración ($X10^6$ células/mL)	104.6 ± 78.5	10-360	11
PH	8.2 ± 0.3	7.5-9	1

[a] It shows the percentage of the population that presented semen quality parameters below normal. Normal values according to WHO (1992): Motility> 50% phones; morphology> 50% normal forms; volume> 1.5 mL; viability> 50% living; sperm concentration> 20 x 106 cells / mL; pH 7.2-8.5. * This setting does not mean it has value and range for the way in which it assesses.

Assessment of exposure to Pb. Mean levels PbS was 9.21 ± 3.22 mg / dL with a range of 1.9-24.4 mg / dL. The average was within the allowable limit (10 ug / dL) according to the CDC (1991); however, a wide range of concentrations, where 40% of subjects showed PbS concentrations> 10 mg / dL was observed.

Association between levels of PbS and impact indicators. To analyze the associations between indicators of exposure (independent variables) and effect (dependent variables), the bivariate analysis or simple linear regression. PbS levels were not significantly associated with semen quality parameters as shown in Table 3. Similarly, no correlation between the concentration of PBS (9.21 mg / dL) and Pb found in seminal fluid (4.13 µgPb / dL), p> 0.05; r = 0.2148.

Table 4. Bivariate analysis between the quality of semen and PbS in the GNE

Parámetro	β	P	r^2
Motilidad (%)	-0.633	0.271	0.0183
Morfología (%)	0.1125	0.808	0.0009
Viscosidad	-0.0101	0.437	0.0092
Volumen (mL)	-0.0428	0.227	0.0220
Viabilidad (%)	-854.87*	0.882	0.0003
Concentración (10^6 células/mL)	-0.0264[a]	0.238	0.0210

El valor de β se presenta * al cubo y [a] en logaritmo.

Exposed group (GE)

Characteristics of the study population. The average time of work in the smelter workers was 13.05 ± 6.80 years (range 2-27 years). The general characteristics of the population for this study group are shown in Table 6.

Table 6. General characteristics of the study population in the GE

Característica	Sujetos estudiados
Edad (años) [a]	37.51 ± 92 (19-61)
Peso (kg) [a]	84.1 ± 20.2 (51.9-104)
Talla (m) [a]	1.76± 0.058 (1.59-1.98)
Índice de Quetelet [a]	29.5 ± 4.8 (19.3-61.1)
Emaciación (%)	3.0
Normal (%)	22.3
Sobrepeso (%)	64.8
Fumadores (%)	64.8
Fumadores actuales (%)	52.6
Consumidores de bebidas alcohólicas (%)	88.3
Consumidores de drogas (%)	6.6
Consumo de cafeína [b]	3.13 ± 1.77 (0 - 14)

[a] Arithmetic mean ± standard deviation. b average daily consumption of one serving of cola drinks and / or soda taste and / or diet soda and / or coffee Indica. The ranges are shown in brackets. n=22.

Quality of semen. In Table 7 the semen quality parameters are shown. When comparing the averages of each parameter semen quality with those established by the WHO (1992), we note that some of the parameters evaluated showed abnormal values. For example, a significant reduction was observed in the number of sperm cells of all workers (average 46.74X106 cells / ml). Moreover, the 8.57% and 17.14% of participants had azoospermia and oligospermia respectively. The 31.42% of the subjects had a decrease in sperm viability assessment. Total motility showed a decrease of 37.42%. An important result was found in the progressive motility. In fact, 71.42% of the workers presented this type of problem, the average of progressive motility in these workers

was 20.24 ± 17.17 µ / s (normal range 25 µ / s) (Table 8). Another parameter that was presented abnormal morphology data (64%), the pH was the least affected (5% of abnormality).

Table 7. semen quality parameters in individuals occupationally expuestos

Parámetro	Media ± DE	Rango	% Anormalidad [a]
Motilidad (%)	62.58 ± 32.4	8 – 95	62
Viscosidad*	-	-	49
Morfología (%)	48.3 ± 12	13 – 87	64
Volumen (mL)	2.7 ± 1.3	0.7 - 6.4	26
Viabilidad (%)	68.58 ± 18.2	17 – 94	28
Concentración (10^6 células/mL)	46.74 ± 41.2	10 – 360	38
PH	8.3 ± 0.3	7.5 – 9.5	5

[a] It shows the percentage of the population that presented semen quality parameters below normal. Normal values according to WHO (1992): Motility> 50% phones; morphology> 50% normal forms; volume> 1.5 mL; viability> 50% living; sperm concentration> 20 x 106 cells / mL; pH 7.2-8.5. * This setting does not mean it has value and range for the way in which it assesses.

Sperm motility. The assessment of cell motility and speed realized in the Multi-Analyser for sperm motility, showed statistical difference when comparing the GE and GNE (Table 8). In effect, the linear speed in the GNE was 69.3 ± 10.4 and 12.9 ± 38.7 in the GE ($p < 0.01$). Progressive motility was 29.1 ± 6.2 µ / s in GNE and 17.3 ± 4.8 µ / s in GE ($p < 0.05$).

Table 8. Comparison of the rates in sperm motility in the GNE and GE.

TIPO DE MOTILIDAD	GNE (µ/s)	GE (µ/s)	VALORES NORMALES*	P
VSL (Progresión)	29.1±6.2	17.3±4.8	25.0µ/s	<0.001
VCL (Velocidad de trayectoria)	62.4±15.4	58.7±18.2	40.0µ/s	>0.05
LIN (Linearidad)	69.3±10.4	38.7±12.9	40µ/s	<0.001
ALH (Movimiento lateral de cabeza)	4.3±1.3	4.1±2.0	3.0µ/s	>0.05
VAP (promedio de trayectoria)	42.8±9.2	18.3±3.9	35.0µ/s	<0.001

* valores estandarizados Motion Analysis.

Assessment of exposure to Pb. The mean levels of PbS was 40.64 ± 15.75 mg / dL with a range of 12.8 ± 73 mg / dL. In our country there is no rule governing this type of metal in the human body in occupational exposure. In the United States the Standard establishes a ceiling for PbS 40 mg / dL (CDC (1991). Based on this data, 57.14% of workers had levels of mercury above 40 mg / dL. On the other hand, PbS and Pb concentrations in semen showed statistically significant when compared to the GNE and GE, as shown in Table 9 differences.

Table 9. Concentration of lead in blood and semen in subjects exposed and not occupationally exposed to Pb

MUESTRA	GNE	GE
SEMEN	2.76 ± .7734 µg/dL	4.13 ± 0.7015 µg/dL*
SANGRE	9.21 ± 3.22 µg/dL	40.64 ± 15.75 µg/dL**

*p< 0.05; **p< 0.001

Association between levels of PbS and impact indicators. To analyze the associations between indicators of exposure (independent variables) and effect (dependent variables), the bivariate analysis or simple linear regression. PbS levels were significantly associated with some of the semen quality parameters as shown in Table 10. A significant correlation was found between the GE PbS concentration (40.64 ± 15.75 mg / dL) and concentration Pb in seminal fluid (4.13 ± 0.7015 mg / dL) ($p < 0.001$; $r = 0924$) (Figure 1).

Table 10. bivariate analysis between semen quality and levels of lead in GE

Parámetro	β	P	r^2
Motilidad (%)	-0.128	0.017	0.0281
Morfología (%)	0.0924	0.020	0.0113
Viscosidad	-0.1132	0.518	0.0213
Volumen (mL)	-0.0352	0.149	0.0380
Viabilidad (%)	-432.61*	0.349	0.0112
Concentración (10^6 células/mL)	-0.0301[a]	0.043	0.0112

El valor de β se presenta * al cubo y [a] en logaritmo. *P<0.05*

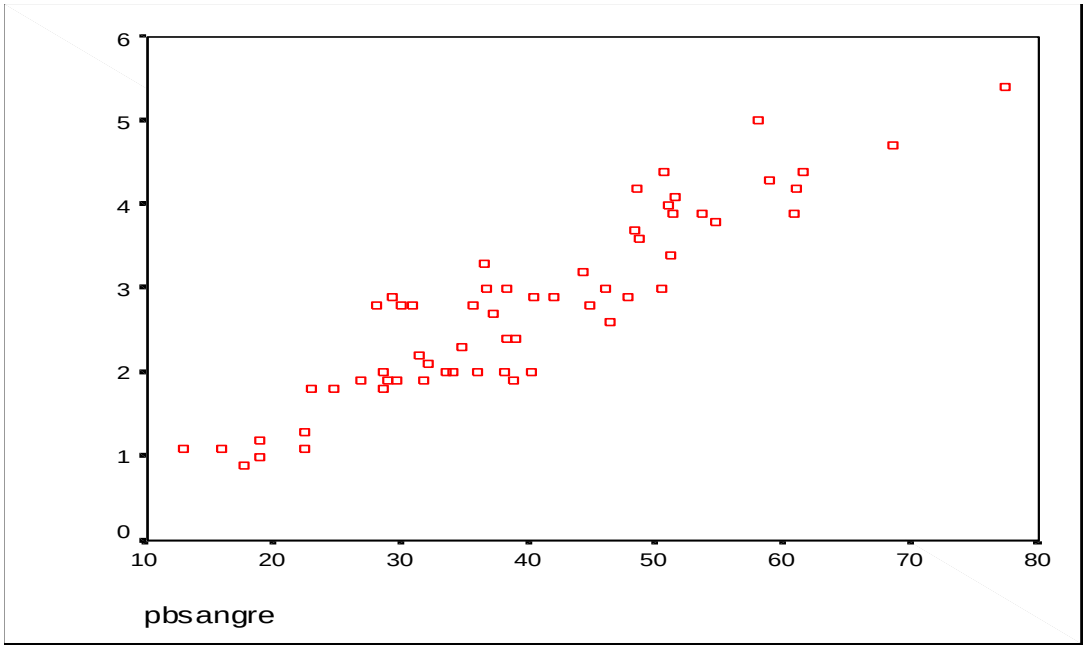


Figure 1. Correlation between blood lead concentration and Pb in seminal fluid (µg/dL) in occupationally exposed individuals (p<0.05; r =0.573).

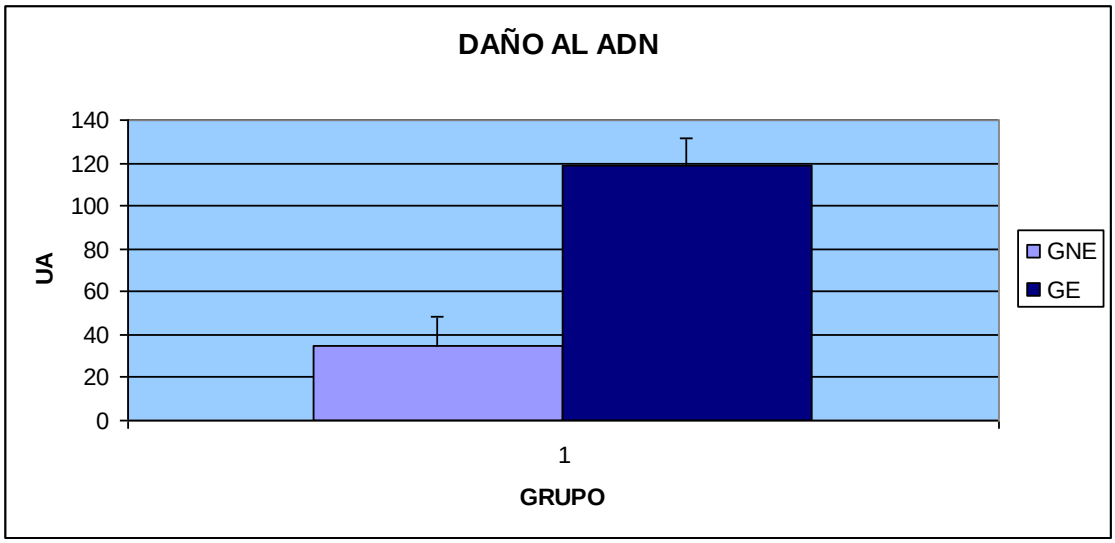
Sperm morphology. The average found for the determination of teratospermia in the study groups are shown in Table 11. The determined morphological characteristic deficient in the evaluation was in the middle part of the spermatozoa (mean cell defects part) significant differences in group comparisons. The feature known as cytoplasmic droplet was the most predominant defect. GE GNE = 28.7% and 20% (p <0.05). Another important findings was found to feature macrocéfalas sperm cells meeting a significant difference between the GNE = 11.2% and = 15% (p <0.5).

Table 11. Comparison of sperm morphology in the study groups

FORMA CELULAR	GNE	GE
NORMAL	62.6±17	48.3±12*
MACROCÉFALO	11.2%	15%
MICROCÉFALO	5 %	7%
DEFECTOS DE LA PARTE MEDIA	20 %	28.7%*
DOBLE CABEZA	1 %	1%
DOBLE COLA	0 %	0%

*p<0.05

Association between levels of PbS and DNA damage in the groups studied. Regarding the statistical analysis of the overall levels of DNA damage in sperm cells between the study groups with a highly significant p> .0001 (Figure 2) was found.



The individual values of the levels of DNA damage are shown by subjects occupationally exposed to Pb and GNE respectively in Tables 12 and 13.

Table 12. Global DNA fragmentation and levels of damage in sperm cells GNE. Total Shown average of evaluated cells and standard deviation of the average total DNA damage.

0	I	II	III	IV	TOTAL	FG
70	20	10			100	40
74	20	6			100	32
75	21	4			100	29
77	22	1			100	24
69	24	7			100	38
80	20				100	20
58	23	14	5		100	66
73	17	7	3		100	40
77	20	3			100	26
77	18	5			100	28
78	14	8			100	30
80	15	5			100	25
66	16	10	8		100	60
72	21	7			100	35
71	23	6			100	35
					promedio	35.2
					ds	12.79062156

FG= Fragmentación global de ADN

Table 13. Global DNA fragmentation and levels of damage in sperm cells of GE. It shows the total assessed cells and mean ± standard deviation of the average total DNA damage.

0	I	II	III	IV	TOTAL	FG
28	39	20	10	3	100	121
29	37	19	14	1	100	121
28	32	40			100	112
25	31	35	9		100	128
24	40	22	11	3	100	129
27	39	29	5		100	112
27	37	30	6		100	115
26	26	42	6		100	128
30	37	32	1		100	104
36	30	30	4		100	102
38	30	25	7		100	101
31	33	22	10	4	100	123
33	26	24	10	7	100	132
34	40	16	10		100	102
35	41	15	9		100	98
29	39	21	8	3	100	117
28	35	22	15		100	124
27	33	15	20	5	100	143
29	32	19	16	4	100	134
28	37	33	2		100	109
30	32	15	20	3	100	134
					Media	118.52381
					DS	12.8203707

FG= Fragmentación global de ADN

DISCUSSION

The comet assay for genotoxicity in a test well established, now takes place mainly with somatic cells of different organs to detect genotoxic activity of potential carcinogens. It is considered useful for monitoring test positive or inconclusive results of in vitro tests and for the assessment of local genotoxicity test, however, the comet assay also has the potential for detection of genotoxicity in germ cells and it can be used to demonstrate the ability of a substance or its (s) metabolite (s) to interact directly with the genetic material of germ cells. These results are important for the classification of germ cell mutagens, for example, in the context of the "Globally Harmonized System of Classification and Labelling of Chemicals (GHS). The literature contains the results of in vitro studies, ex vivo and in vivo, with regard to the evaluation of genotoxicity in germ cells, only in vivo studies are important, but other studies provided important information on

various aspects of the methodology. Many studies of comet assay with human sperm have made in the context of male infertility and assisted fertilization, the various aspects of trial modifications used are discussed.

Measuring the effects of DNA by the comet assay sperm requires additional steps for chromatin decondensation, many different modifications of alkaline and neutral comet assay in use without a standard protocol established yet. High levels of background variables and the effects of DNA were reported and there is still need for standardization and validation of the comet assay and sperm. Some human biomonitoring studies with human sperm were released, but it seems premature to use these data for hazard identification and classification of chemicals, however, standard alkaline conditions in the in vivo Comet assay can be easily adapted to cell research in the reproductive organs. However, studies to standardize and validate these methods are necessary before the comet assay can be usefully applied in assessing the risk of germ cell mutagens. In this research, the percentage share of subjects who were included in the same stage of sampling was good because the turnout was 76% overall for the study groups (GNE = 65%, GE = 71 %). In some references consulted, the participation rate for this type of study where the donation of the semen sample is low is required. Indeed, in a study of populations of workers where the possibility of attracting more participants is, Robins et al., (1997) reported a 64% stake in a plant used batteries in a cross sectional study in South Africa. . On the other hand, Viskum et al, (1999) reported a response rate of 18% in a prospective study employed a battery factory of the republic of Denmark; The Group of Alexander et al., (1988) reports a participation rate of 13% of a smelter workers, this study was cross-sectional kind in Canada.

Evaluation of lead exposure. To study the environmental part, the cities of Torreon, Coahuila. and Gomez Palacio and Lerdo, Durango. (Control group) are located in the center of the northeastern region of Mexico and are the main urban area of the Laguna region with over 800,000 inhabitants. This zone has several sources of contamination Pb, for example a smelter above the heavy vehicular traffic. The metal is located in the city of Torreon, Coahuila, which is immersed in the urban area, and includes a smelter Pb, refinery Pb and Silver and a refining plant electrolytic Zn, producing in 2003.: 129.712 tons from 218.457 tons of Pb and Zn (report Industrias Penoles, 2004). Several studies have shown evidence of Pb pollution in the city of Torreon for decades. Albert and García (1977) determined the concentrations of Pb in hair of children from 5 different regions of Mexico including: a) north of Mexico City, b) the city of Puebla, Puebla, c) the City of Matamoros, Coah., which served as a control, d) the city of Torreon, Coahuila, and e) its

twin city Gomez Palacio, Durango. Pb concentrations in samples from the control region were the lowest (4.2 mg / g) and Pb concentrations found in the hair of children in cities of Mexico, Puebla and Gomez Palacio were 12.1, 17.7 and 12.8 mg / g, respectively. In contrast, in the city of Torreon the mean value was 55.1 g / g with a maximum of 220 mg / g. Also, Hernandez-Serrano (1982) evaluated blood lead concentrations (PbS) of medical students residents of Torreon, Coahuila. and reported that 10% (9/90) of PbS students presented values above 25 mg / dl. Moreover, Calderon-Salinas et al. (1996) conducted a study in 98 children (7-12 years) living within 1 km of distance to the metallurgical complex. The average concentration of PbS found was 17.3 mg / dl. Meanwhile, Benin et al. (1999) conducted a study in 1 km radius of the metallurgical area of Torreon, Coahuila. and they reported levels of Pb powder (17.9-48.84 mg / g) above the levels established so as not contaminated sites, as well as high concentrations of As and Cd. Recently, the group of Garcia-Vargas et al., (2001) evaluated the levels of Pb powder 3 primary schools located at various distances (8100 m, 1750 m and 650 m) of the metallurgical area of the city of Torreon, Coah., finding Pb levels above the values established by the EPA (1997). In our study, referring to the part of environmental exposure (GE) the average level of PbS found in the adult male population residing mainly in the cities of Torreon and Gomez Palacio (n = 74) was 9.21 mg / dL. This value is slightly below the limit set by the CDC (10 mg / dL), however, he found 40% of participants subjects with values above it. These results indicate that environmental pollution by Pb in this part of our region has remained, since the average level of PbS (lead exposure indicator) is on the threshold of the maximum permitted limit; therefore risks in susceptible populations increase. Similarly, Garcia-Vargas et al., (2001) in his study of children in the region, showed that the metal area of Torreon, Coah., Remains a major source of environmental pollution risk to children's health, mainly. The effects of Pb toxicity are very diverse and their shape or course of action in the human difficult to establish since this metal enters the body and accumulates. Therefore, assessment of the concentration of Pb in blood, is considered one of the best biomarkers to measure exposure to this metal (Flegal and Smith, 1995). They have been reported in a variety of jobs which have been associated concentrations of Pb in blood with an adverse effect on semen quality. The best known effects establish an impact on sperm concentration, cell motility in sperm viability in an increased occurrence of problems teratospermia and semen volume (Lancranjan et al, 1975;. Wildt et al. 1983; Cullen et al., 1984; Fisher-Fischbein et al., 1987; Lerda, 1992, Chia et al., 1992; Alexander et al, 1996).. In these studies the variables that qualify sperm quality affected after displaying an average concentration of 40 mg / dL of blood Pb. However, in other jobs with lower levels of Pb adverse effects on semen quality is. Indeed, concentrations> 25,> 24 and> 10 ug

/ dL describe the effect (Sallmén et al., 2000; GENART et al., 1992; Selevan et al., 1984). Workers in a smelter Pb, Pb range of blood found was 15-69 g / dL, and no statistically significant association with semen quality parameters (Alexander et al., 1996). Likewise, Plechaty et al, (1977).; Xu et al., (1993), found no statistical difference between the concentration of Pb in blood (average of 15 mg / dL) and sperm quality parameters. These references allude to a certain concentration of Pb in blood, which does not seem to directly affect sperm quality, which suggests that it is possible to have a sufficient concentration accumulated in the male reproductive tract to cause an effect, for this reason is possible to establish that the use Pb as a reference element and its minimum to generate a biological effect on the human concentration is about debate. From another perspective, the evaluation of Pb concentration in semen apparently provides a more accurate to establish an effect of this metal on the male reproductive system and may represent a more accurate in this type of exposure (way way Plechaty et al. , 1977). Thus, the assessment of the concentration of Pb in sperm would be a more effective way to evaluate these effects as bioaccumulation from the bloodstream into the reproductive tract is limited by the blood-testis barrier (Dym and Fawcett, 1970) blood-epididymal (Hoffer and Hinton, 1984). However, most of the work in which Pb is determined semen has not found a direct association between the metal and the characteristics of sperm quality (Stachel et al., 1989; Jockenhövel et al, 1990;. The -Zohairy et al., 1996; Robbins et al., 1997; Alexander et al., 1998; Viskum et al, 1999).. In other studies, and contrary to what has been said in previous works, there are studies where a clear adverse effect on semen characteristics Pb shown even at low concentrations. For example, some mentioned effect asthenospermia and teratospermia. In our study, comparing the concentration of Pb in semen from the GNE and GE, a significant difference when comparing the respective concentrations was found. Our results were similar to those described by Saaranen et al (1987), which reported the level of Pb in infertile men (3.2 ug / dL) and fertile men (1.7 ug / dL). On the other hand, Jockenhövel et al., (1990) reported a significant difference in semen in subjects exposed to lead, results in fertile men of 1.3 ug / dL versus 5.6 ug / dL in infertile men. These works serve to mean that exposure to low levels of Pb in sperm affect semen quality; Burimovitz et al., (1989) suggest that Pb in semen is the main indicator of damage to the reproductive level, even more than the exposure assessment of Pb in blood. Our results are similar to those described by Wyrobeck et al., 1983; Butrimovitz et al., 1999; Robbins et al., 1997; Lerda et al., 1992; Chia et al., 1990), where teratospermia and asthenospermia variables are primarily affected, suggesting that sperm morphology is a major variables used as the sensitive indicator spermatogenic level problems caused by toxic substances. The teratospermia is one of the main parameters for

evaluating the functionality of the germinal epithelium. This can be explained because the main contact time between the Pb and germ cells occurs at this stage. Here, the structural aspects of sperm development, can be irreparably damaged and consequently cause physiological deficiencies. Germ cells are protected by Sertoli cells until the sperm has matured (spermiation) in this process is more difficult than harm (blood-testis barrier) by toxic substances, however, as mentioned, Pb, for example occurs , it can be transported through the seminiferous tubules, epididymis and logically in secretions of the prostate and seminal vesicles. Comparison of sperm count, percentage of abnormal forms, viability and motility in this study, statistical differences based on the conditions of fertility among the groups analyzed sample. In a study conducted on laboratory animals with exposure to Pb Chowdhury et al., (1984) observed effects of metal associating a testicular atrophy in these animals. Moreover, Kaushal et al. (1996) observed a significant decrease of young spermatids and spermatozoa in rats treated with different concentrations of Pb. Also, the group of Saxena et al., (1987) noted changes in spermatogenesis degradation Leydig cells in rats treated with this toxin. Moreover, sperm motility can be altered as a consequence of testicular damage, epididymal or by the presence of an agent in seminal fluid (Sipes et al., 1997). In our study on the environmental aspect, motility was not associated with the Pb concentration in seminal fluid, suggesting that this parameter sperm quality was affected before the accessory glands secrete their content, that is, during development in the testicle, or, during its passage through the epididymis. However, concentrations of Pb in blood in the occupationally exposed group were associated with those found in the seminal fluid, which allows us to think that this partnership is clear to these factors of sperm quality in subjects who formed the group of workers metallurgical. In this group, unlike that found in the part of environmental exposure, it is clearly observed an effect of metal by the differences found by comparing the values with the exposed group of labor way. The dysmotility by lead in sperm, may be the result of inhibition of the activity of mitochondria, which are located in the middle of the sperm (Eddy, 1988). In another study in vitro by the group Kanwar et al., (1988), they observed an inhibition of sperm motility after exposure to Pb, as a result of inhibition of certain key enzymes in the metabolism of carbohydrates, some as glycogen phosphorylase, glucose-6-phosphatase, ATPase-dependent Mg2 + and lactic dehydrogenase and succinic acid. In fact, a deficiency in motility, mainly linear in the EG was adversely altered to be evaluated by the method proposed in our work.

Sperm quality. Recently, Bonde et al., (1998) reported that the probability of pregnancy is increased with increasing the concentration above 40 million / ml, suggesting that the criteria established by WHO should be used with caution and some males with sperm counts above the lower normal range (20 million / ml) by WHO subfertile can be defined. Moreover, the sperm concentration in the EG was 46.7 million / ml. In addition, there is evidence that in some parts of the northern hemisphere, sperm quality (sperm concentration, as the main factor) is reduced during the summer (Gyllenborg et al., 1999). Experiments with rhesus monkeys suggest that such seasonal variations can be induced by the endogenous biological clock is reset annually by changes in daylength (Levine, 1999). Another environmental factor that could partly explain this seasonal relationship, are temperature fluctuations (Sood et al., 1993). However, Levine et al., (1992) demonstrated in subjects who were under climate controlled conditions, the summer heat is not averse to male reproductive capacity. On the other hand, there are other reports that support described by Levine et al., (1999), ie, no significant changes or trends observed in sperm parameters during times of the year (Mallddis et al., 1991). In our study, no significant decreases in sperm concentration in GNE were found, even taking into account that this stage of the study was developed in the months from June to August, the hottest months in the Laguna region and where the average temperature was 40.6 ° C (for those months), just as at this time, the subjects were under the natural variations of the photoperiod in this region, where the length of daylight hours for the summer solstice was 13.36 hours light (CENIDs -RASPA-INIFAP, 2001). Then there are the stages of sampling for the GE conducted in the months of October to December. In this group the concentration of sperm / ml was most affected and gave other sperm parameters, suggesting that more than the influence of hours / light or room temperature reduction in sperm quality is due to exposure to heavy metals. Our affected parameters (viability, mobile and stationary life forms life forms, and sperm motility) are late sperm characteristics acquired during sperm maturation in the testis and its passage through the epididymis. Therefore, the secondary biological effect of exposure to metal may be acute or subacute. It is also important to mention that chronic exposure to metals may mark the emergence of these deficiencies in sperm quality of these subjects (GE), and the results suggest that damage to the germinal epithelium is gradual and possibly cumulative. In addition, sperm motility perhaps secondary to the damage that can result in exposure to metals on the musculoskeletal system of the sperm placed in a point of analysis this effect to this partnership. Indeed, one possible modification is suggested both mitochondrial axonema available in the flagellum and metabolism for energy conversion. To enrich the previous comments on this physiological aspect caused by metals, it is important to note that there have

not established specific and clear mechanisms that explain the decreased sperm motility. Two possible scenarios have been considered: one is in the process of ATP production, which involves enzymes that can be covalently phosphorylated in one or more amino acid residues (Ser, Tri, Tyr or His). Also, the phosphorylation and dephosphorylation of ATP are catalyzed by a variety of protein kinases and phosphatases and deregulation of one of these two groups of enzymes can catalyze uncontrollable manner ATP hydrolysis (Murray et al., 1997). Both enzymatic alterations reduce the content of ATP in the cell; in the case of sperm, this will decrease the amount of energy available for motility. The second hypothesis established to explain the decline in sperm motility in GE is that any structural alterations in the musculoskeletal system therefore bring sperm alterations in sperm motility. In our study no evaluations of ultra-structure of the middle of the sperm, which would yield more information in this regard were made, as many of the flaws in motility is inadequate mitocrondrial available in this part of the cell. Moreover, in the GE significant deficiencies both in the teratospermia they were found (above the normal value, 50% or more of morphologically normal cells) and a decrease of total motility, as well as linear and progressive (50% or moving more cells) (WHO, 1992). This may explain the latter case as if set as structural damage, sperm cell motility may be due to injury associated with poor efficiency in fuel metabolism molecules such as fructose, mannose and glucose metals, among others, and poor morphology that prevailed in these most affected groups.

DNA fragmentation. The alterations in the genetic material may further include chromosomal aneuploidy in sperm nuclear decondensation both as DNA breaks. Recently, the sperm DNA integrity is being recognized as an independent measure of quality; and it has shown that this can affect fertility in vivo and in vitro (Muriel et al. 2006a). The cause of infertility in men with normal semen parameters could be related to the presence of abnormal DNA in sperm.

The evaluation of the integrity of DNA in sperm, besides the systematic study of the seminal parameters, could provide additional information about the quality of the sperm, which could be helpful in guiding the infertile couples. The results of this study concerning DNA fragmentation in the sperm argue that there is a significant increase in the incidence of sperm cells with broken DNA strands in men exposed to lead occupationally, after evaluating the samples processed by this essay. In a study with rats exposed to different heavy metals, including lead, the effect of metalloids (Pb, As and Cd), on DNA compaction it was evaluated in primary spermatocytes. In this study spermatocytes damage associated with the concentration of Pb administered found (Nava-Hernánez et al., 2009). Previous studies using these animals and lead models have demonstrated

damage to testicular level and a decrease in the concentration of stem cells, ie, the direct damage in the seminiferous tubules, considered a dynamic part of the testicles where occurs the mitotic division (Massanyi et al., 2007). The administration of Pb causes a significant decline in fertility, cell kinetics studies show a decrease in spermatids and mature sperm (Batra et al., 2004). Nava Hernández et al., 2009, mentioned that the Pb, Cd and As are directly toxic to primary spermatocytes, causing DNA damage. DNA integrity is proper as a mechanism ensuring the paternal genome playback (Cordelli et al., 2003). The DNA damage in immature germ cells may impair fertility and cause abnormal results, as spontaneous abortions, genetic diseases and increased incidence of cancer (Brinkworth, 2000;. Spano et al, 2000; Coddington et al. 2004). The origin of the potential damage and the mechanism of DNA fragmentation in germ cells is not clear, it can take place directly in the mature sperm as a result of endogenous exposure (reactive oxygen species) or exogenous to mutagens (chemicals or radiation) in developing d spermatogenesis (Aitken et al., 1998; Ahmadi and Ng, 1999; Alvarez et al., 2002, Sakkas et al., 2002). Sergerie et al. (2005) described the association between snuff and no sperm DNA fragmentation in fresh semen samples from healthy men (not infertile); while Saleh et al. (2002a) they studied this association in infertile men and reported that differences between smokers and nonsmokers were not statistically significant. However, they cautioned that the semen samples of infertile men smokers had high levels of oxidative stress (OS) compared to nonsmokers. This strengthens the results of this study and reinforces the hypothesis that the control exercised by some detrimental effect factors that affect the male gametes could be hidden in the ejaculate. . Muratori et al, (2000) described that to some extent DNA fragmentation was positively associated with abnormal sperm morphology and associated with defects in the queue; but they found a negative correlation between DNA broken and progressive mobility. It based on previous work, which was recorded rates of fertilization and embryo quality some indicators were related to the extent of sperm DNA fragmentation after swim-up (Muriel et al 2006a.); together with the results of the current study, we can conclude that the way to determine DNA fragmentation seems evident that the rates should be evaluated after processing by swim-up.

Relationship between fragmentation and semen qualityIn humans, it found a strong association between abnormal semen parameters and nuclear DNA breaks in ejaculated sperm. The fragmentation of the genetic material of the sperm is higher in patients diagnosed with oligoteratoastenozoospermia. (Huang et al. 20) evaluated this phenomenon using the TUNEL assay, appreciating the DFI (DNA fragmentation index) was significantly higher in patients with

abnormal semen parameters. In our study, this trend is clearly observed in the exposed group to present a higher proportion of DNA fragmentation, although GE was not analyzed in relation to blocks with higher concentrations of Pb, the trend shown in grades breaks and ratio. Gandini et al., Studying the relation between apoptosis and semen parameters, also showed an increase in fragmentation was associated with a decrease in sperm concentration and motility. This correlation was also observed morphology. Gametes showing breaks in their DNA had small heads and amorphous. In our work the degree of apoptosis was not assessed but clearly shows a degree of sperm infeasibility above 30, which indicates possible association. Also, the data found in our study showed a change in the proportion of normal forms as well as that found in the middle of the GO. However, the data obtained by Larson-Cook et al., Showed that DFI was not always related to semen parameters. Only 30% of men with a higher than 27% DFI had astenozoospermia and / or oligozoospermia. Although in most cases the fragmentation concerns seminal abnormalities is very important to note that 8% of men with normal sperm parameters (infertility of unknown origin) also present in DNA fragmentation.

Relationship between sperm DNA fragmentation and sperm fertilizing potential. There have been several studies that show the relationship between sperm DNA integrity and fertility. These studies show that infertile men have a higher fraction of sperm with DNA breaks, and is intended to establish a cutoff above which would be unfavorable prognosis. In Europe and the United States two extensive studies on the relationship between the results of the SCSA technique and fertilizing ability were carried out independently. Both showed that a DNA fragmentation index (DFI) than 30-40% is incompatible with in vivo fertility. Regardless of concentration, sperm motility and morphology, they had established a significant correlation between the SCSA, COMET and TUNEL techniques to human sperm, therefore, data obtained by a technique could be compared with those obtained by the other two. Recently, Sergerie et al., Using the TUNEL found practically the same cut points Evenson et al. This measured the sperm DNA fragmentation in a group of 66 infertile men (40.9 ± 14.3) and 47 fertile males (13.1 ± 7.3), establishing a cutoff of 20%. Chohan et al., Reached the same conclusion by comparing a group of 60 infertile men with a group of seven donors through three techniques (SCSA, TUNEL and SCD). Here, the fertility ratio in the design workers was not set properly. However, a delay was observed in this factor in some of the participants of 6, 8 or even 12 months in achieving pregnancy. Comparing molecular techniques highlighted in this paper or this comparison is made, which is a good strategy because it would

give more information on the mechanisms of potential ways or mechanisms of DNA damage in these works.

CONCLUSIÓN

The results of this study show an adverse effect of heavy metals such as Pb on the secretion of immature germ cells, sperm quality and genetic damage, the latter estimated by the degree of morphological deficiencies and level of DNA fragmentation of sperm cells . In summary, occupational and endemic to different metals such as Pb exposure can alter the process of spermatogenesis. The concentrations of metals both in seminal plasma and within the same cell can be good indicators for evaluating effects on sperm quality. Finally, this study is the first in which an effect on semen quality, sperm morphology and fragmentation associated heavy metals (Pb) in the Laguna region described. Both DNA fragmentation and cell morphology represent two important variables to determine possible genetic damage, ie, direct modifications of metal exposure and different stages of genesis and development of human sperm.

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