Effects of chronic lead exposure on zinc concentration and spermatic parameters in Wistar rats

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ABSTRACT

Objective: Many xenobiotics such as solvents, pesticides and heavy metals, are suspected of being responsible for the decline in male fertility observed during the last century. Within this framework, to we evaluate the effects of lead on the male fertility of young male Wistar rats pubescent.

Methods: Ten young male Wistar rats of 90 ± 5 days old and of an average body weight of 250 ± 20g were randomly divided into two groups (n = 5 in each group): the control group received distilled water and the experimental group received the lead acetate solution (0.3%). After 90 days, the rats were euthanized followed by a quick removal of the testes and epididymis. These organs were immediately used in different experiments: The testes for the evaluation of spermatic parameters and epididymis for assays of heavy metals by atomic Absorption Spectroscopy.

Results: These results indicate a highly significant increase in the concentration of lead and a significant reduction of zinc in the rats exposed compared to the control rats.

In addition, the increase in lead concentration and the decrease in zinc concentration resulted in a significant decrease in sperm parameters (vitality, motility, density and morphology) in the exposed rats compared with the control rats.

Conclusion: This study shows that in adult rats, chronic exposure to lead induces changes in spermatic parameters, leading to a decrease in male fertility.

KEY WORDS: Lead
Zinc
Spermatic parameters
male fertility

Introduction

Recent epidemiologic data conclude that approximately 15 % of couples are confronted with difficulties to have children and proceed to consult a doctor for this problem [1]. According to the World Health Organization (WHO), in approximately half of these cases, a male factor is in question [2] and issues of quantitative and/or qualitative deteriorations of spermatozoa generally present [3]. Moreover, 30 to 40 % of infertile men have high levels of ROS (Reactive Species of Oxygen) in their seminal liquid [4]. The assumption privileged for one decade has supposed that natural or synthetic substances, present in the environment, could act on the hormonal systems and may be at the origin of the various anomalies observed in the males of many animal species. Thus, many research groups were interested in the potential agents toxic effects of various chemical and physical of our environment on the human fertility [5]. Among the accused pollutants, appear many xenobiotics such as the solvents, pesticides and heavy metals to quote only those. They are suspected to be responsible for the fall of the male and female fertility observed during this last century [6].

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These environmental substances that have the ability to mimic or antagonize the action of natural hormones attract the attention of the scientific community, eager to establish a bond between the exposure to such compounds and the appearance of the noxious effects observed on the reproduction. Thus, it appeared significant to us to singularly specify the toxicity of heavy metals such as lead, given the abundance of its sources (like its distribution in water pipelines, industrial pollution, automobile traffic…) and the

possible bioconcentration of this metal in seminal plasma [7] or the follicular liquid [8]. The studies primarily of clinical and epidemiologic order, carried out in workmen exposed to lead during their work and in police officers or taxi drivers, put in obviousness a fall of the female fertility and number of pregnancies, a reduction in the male fertility associated with a reduction in the sperm number and motility and a reduction of the volume of the seminal liquid [9]. Moreover, Konan et al**.** [10] highlighted the presence of lead in the semen of the patients consulting for male infertility at the Pasteur Institute of Cote d'Ivoire. However, the conclusions of these studies are often contradictory because of the heterogeneity of the studied populations (smoking, food practices, genetic inheritance…) and of their environment, in which many factors can act in synergy [11]. In addition, the studies carried out *in vitro* do not make it possible to explain the mechanism of action of these environmental pollutants on fertility, many international experts [12] considering the results of this research agree they do not integrate the complex mechanisms of hormonal regulation of the organism. Moreover, concerning medical ethics, we have difficulty returning molecular studies relating to the human fertility [13]. The mice or the rats appear in this case like excellent models to apprehend the problems of fertility in mankind because the installation of the spermatogenesis through puberty to an adult are similar in these rodents with that of a man [13]. Within this framework, to evaluate the effects of lead on the male fertility of young male rats of Wistar stock pubescent, they were subjected, during 90 consecutive days, with an exposure to lead by ingestion.

Materials and methods

Animals and treatment

Ten young male Wistar rats of 90 ± 5 days old and of an average body weight of 250 ± 20g were obtained from the Ecole Normale Supérieure of Abidjan animal facility. These animals were housed at the Pasteur Institute animal care facility in plastic cages and a cycle of day/night was maintained (approximately 12 hours of light and 12 hours of darkness) in a ventilated animal room. The rats were acclimated for 14 days to their new environment before the treatment and had free access to sterile distilled water and sterilized standard food. All the animals were handled in accordance with the guidelines and protocols approved by the Care and Use of Animals Committee of Cote d'Ivoire.

The rats were randomly divided into two groups (n = 5 in each group): the control group received distilled water and the experimental group received the lead acetate solution. The 0.3 % of lead solution was prepared by dissolving 7.5 g of lead acetate in 2.5 L of distilled water and one mL of 5 N HCl was added [14]. The experiment was conducted for 90 days and during that period, any unusual symptom were recorded daily. After 90 days, the rats were euthanized followed by a quick removal of the testes and epididymis. These organs were immediately used in different experiments according to their different protocols.

Evaluation of the sperm vitality

To evaluate the vitality of the spermatozoa, twenty microliters of the 0.9% of NaCl with the incised epididymis was placed on a microscope slide followed by the addition of 5 µl of physiological eosin solution. The two solutions were mixed and after 30 seconds of incubation, the mixture was observed under an optical microscope (Olympus Japan) with a 400X enlargement. Vitality was appreciated by observing the heads of the spermatozoa. The head of dead spermatozoa are colored pink while the live spermatozoa have white heads. The percentage of live spermatozoa was obtained by randomly counting a total 200 spermatozoa on various fields, dead and live spermatozoa combined.

Evaluation of the sperm motility

Just after the quick removal, the epididymis was immediately incised and placed in a 15 mL falcon tube containing 10 mL of 0.9 % NaCl already incubated at 36ºC [15]. To appreciate the sperm motility, twenty microliters of this solution was immediately placed on a microscope slide and directly observed using an upright microscope Olympus JAPAN (X 400). The mobile and immobile Spermatozoa were quickly counted randomly in five fields and the percentage of the mobile Spermatozoa was calculated using the method described by Prasad et al**.,** [16].

Counting of the sperm numbers

The sperm count was performed according to the method described by Shinshi [17]. Immediately after euthanizing the rats, the tail of the left epididymis of each rat was removed, subsequently incised and placed in suspension in a 10 ml of 0.9% NaCl already maintained at 36ºC. The resulting mixture was then incubated at 4ºC for one hour. Sperms were then observed using 20 µL of this mixture under an optical microscope. The number of spermatozoa per ml was estimated according Prasad et al. [16].

Evaluation of the sperm abnormalities

Sperm morphology was assessed using eosin stain [18]. Twenty µL of the epididymis solution prepared as previously described was spread out on a microscope slide using another slide. The smear was air dried for 15 minutes at room temperature. The slide was then immersed in a salted solution of alcoholic eosin (0.5 - 0.9%). The slide was dried again and then observed under an optical microscope.

Preparation of the testicular homogenate

The left testis of each rat was removed and placed in normal saline and the tunica albugenia removed. The testis were homogenized with potassium phosphate buffer (0.1 M, pH 6.8) in mortar on an ice tray. After centrifugation at 10,000 g for 20 minutes at 4°C, the supernatant was recovered and aliquoted in Eppendorf tubes and then stored at -20°C for the quantification of metals in the testes.

Metals analyses (lead and zinc) in the testicular homogenate

The lead and zinc were assayed at the Institute National Polytechnique Houphouët-Boigny by Flame Atomic Absorption Spectrometry using a Varian AA20 device as described Gbétoh et al. [19]. The previously thawed samples were digested using a solution of hydrochloric acid (0.1M) in specific assay tubes so that their concentration was within the calibration range. The Air-Acetylene flame at 3000°C was used for the atomization of the samples. The reading wavelengths of lead and zinc were respectively 217 nm and 214.8 nm. The detection limit was 0.001mg /L, ie 1µg /mL. The concentration of lead and zinc was determined by means of calibration curves of each metal ion, from standard solutions with respective concentrations of 0.5; 1 and 2mg/L. These solutions were prepared from 1000 ppm multi-standard solutions. The assay in a given sample was performed in triplicate.

Statistical analysis

The statistical analyses were carried out by using the software Graph Pad Prism 5 Demo. The results are presented in the form of average ± SEM. The test of Student and the test of Annova were used for the comparison of the averages. A value of p <0.05 was regarded as significant. The significant statistical differences are announced in the tables and the figures by a star (*), the statistical differences very significant by two stars (* *) and the highly significant statistical differences by three stars (***).

Results

Effects of lead on the spermatic parameters

According to the results reported in Table 1, lead induces a significant decrease in spermatic parameters (vitality, motility, density and morphology) in the exposed rats compared to the control rats.

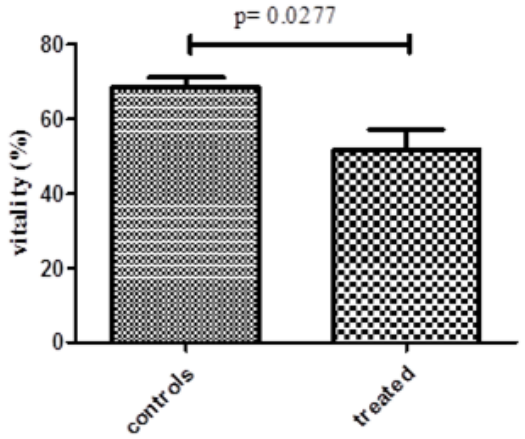
Table 1. Spermatic parameters of controls and treated animals.

Animals	Vitality	Motility	Sperm count	Abnormalities
Control	68.40%± 2.77	76%±2.36	$85.08.10^6$±3.39	4.60%±0.67
Treated	51.60 ±5.63*	57.6±3.31**	$51.4.10^6$±13.43*	13%±1.04***

Effects of lead on the sperm vitality

After 90 days of intoxication, a significant decrease in the number of live spermatozoa is observed in the treated rats compared to the non-treated control rats. The results indicate that live spermatozoa decreased (p=0.0277) from 68.40% ± 2.77 in the controls to 51.60% ± 5.63 in rats treated with lead acetate (Figure 1).

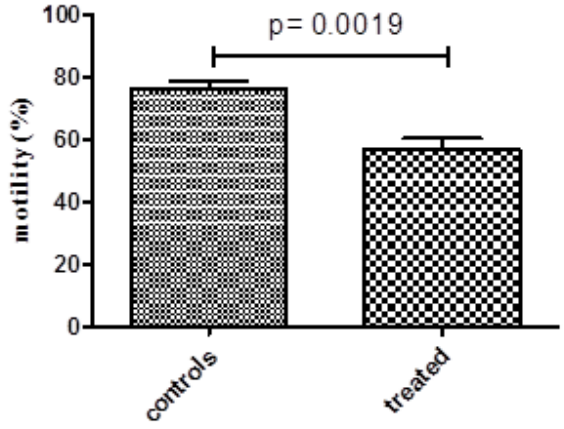
Figure 1. Effect of lead on sperms vitality. The results indicate that live spermatozoa decreased (p=0.0277) from 68.40% ± 2.77 of the controls rats to 51.60% ± 5.63 of rats treated.



Effects of lead on the sperm motility

The effect of lead on the sperm motility revealed an important reduction and a very significant decrease (p = 0.0019) in the number of mobile spermatozoa compared to the control non-treated rats. The results show a decrease in the percentage of mobile spermatozoa from 76% ± 2.36 in control rats to 57.60% ± 3.31 in exposed rats (Figure 2).

Figure 2. Effect of lead on sperms motility. Lead induced a significant decrease(0.0019) in the percentage of mobile spermatozoa from 76% ± 2.36 in control rats to 57.60% ± 3.31 in exposed rats.



Effects of lead on sperm density

In the experimental group, the sperm count was significantly low ($51.40.10^6$ ± 13.43/mL) compared to the control group ($85.08.10^6$ ±3.39 /mL). A high density of sperms (Figure 3A) was observed in the control rats compared to the exposed rats (Figure 3B). The results obtained revealed a significant decrease (p=0.038) in the number of spermatozoa in the exposed rats (Figure 3C).

Figure 3. Effect of lead on sperm counts. The density of spermatozoa decreased from 85.8.106 ± 3.39/mL in the controls rats (Figure 3A) to 51.40.106 ± 13.43/mL in the exposed rats (Figure 3B). The results revealed a significant decrease (p = 0.038) in the number of spermatozoa in the exposed rats compared to the controls (Figure 3C).

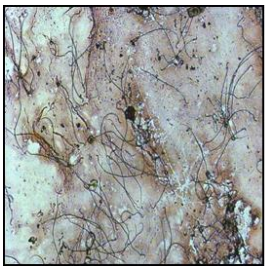


Figure 3A

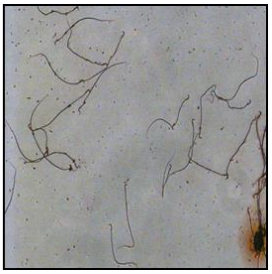


Figure 3 B

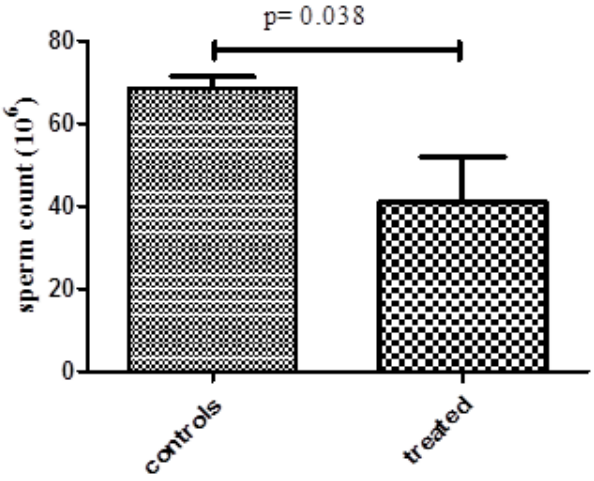


Figure 3C

Effects of Lead on sperm morphology

Lead intoxication increased the incidence of abnormal sperms. A significant increase in total sperm shape abnormality was recorded in animals exposed to lead acetate over control groups (p=0.0001). Head and tail abnormalities were observed in the rats treated with the solution of lead acetate. The average rate of the anomalies passed from 4.6% ±0.67 in the control group to 13%±1.04 in the exposed rats (Figure 4A, 4B and 4C).

Figure 4. Morphological changes in rat sperms. Figure 4A shows the spermatozoa in the control rats and figure 4B shows spermatozoa in the exposed rats. Lead intoxication increased the incidence of abnormal sperms in exposed rats compared to control rats (p = 0.001). Tail (1) and head (2) abnormalities of the sperms were observed in the rats treated (Figure 4B). The average rate of the anomalies passed from 4.6% ±0.67 in the control group to 13%±1.04 in the exposed rats.

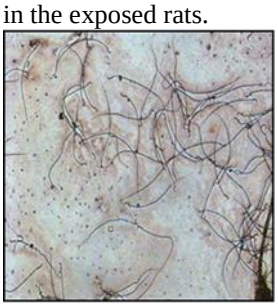


Figure 4A

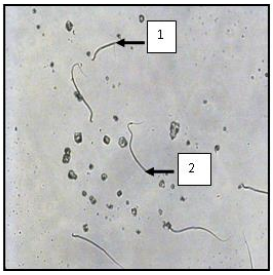


Figure 4 B

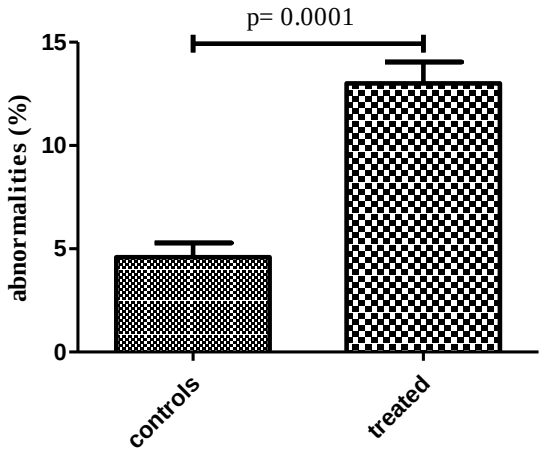


Figure 4C

Quantitation of lead and zinc in the testicular homogenate

After 90 days of exposure to lead acetate, the concentration of lead and zinc were determined in the testicular homogenate by flame Atomic Absorption Spectrophotometer (Varian, spectra AA20). The results are consigned in table 2. These results indicate a highly significant increase in the concentration of lead (p=0.0003) and a significant reduction of zinc (p=0.023) in the rats exposed compared to the control rats (Figure 5A and 5B).

Table 2. concentration of lead and zinc of controls and treated animals.

Animals	Lead concentration	Zinc concentration
Controls	0.0546±0.054 µg/mL	4.867±0.378 µg/mL
Treated	0.6257±0.078 µg/mL ***	3.407±0.307µg/mL *

Figure 5. Effect of lead and zinc in the testicular homogenate. The heavy metals assay revealed a significant increase in lead (p = 0.0003) in the testes of treated rats compared with controls (Figure 5A) and a significant reduction in zinc concentration (p = 0.023) was observed in the treated rats as compared to the untreated control rats (Figure 5B).

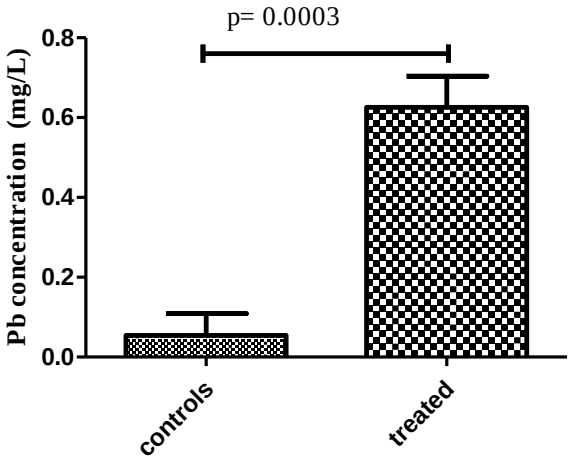


Figure 5A

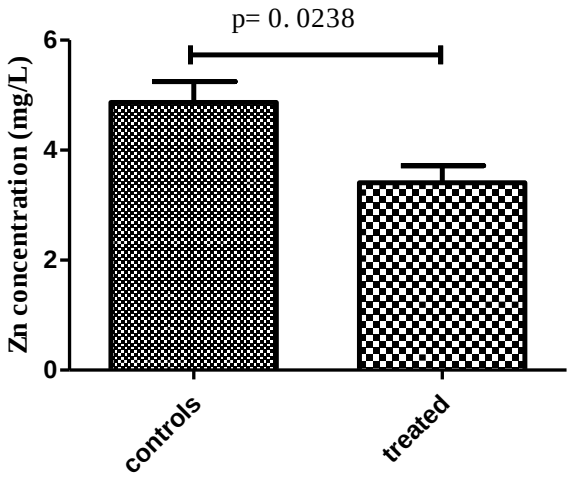


Figure 5B

Discussion

The routine method to evaluate the potential fertility of a male is the realization of the spermogram which consists of the analysis of the quantitative and qualitative seminal parameters. In the present study, chronic exposure of lead acetate to rats induced a significant change in sperm parameters (vitality, motility, number and morphology). These changes could be attributed to the deleterious effects of lead-induced oxidative stress. Several authors agree on the induction of lead oxidative stress [20-23] and its adverse effects on spermatozoa [24, 25]. Moreover, spermatozoa were the first cells on which susceptibility to oxidative stress was studied [26].

The imbalance between antioxidant and pro-oxidant in this study was measured by the zinc content in the testicular homogenate of the rats. According to Ben Ali et al. [27], zinc is a trace element that fights sperm alteration by free radicals. The results corroborate with this hypothesis since we found that the average of zinc concentration in the testicular homogenate of the control rats was significantly higher than that of the exposed rats. In addition, the results revealed a highly significant positive correlation between zinc concentration and sperm vitality. Lewis-Jones et al. [28], noted that in addition to its stimulatory effect on sperm production, zinc protects the latter from degradation. This zinc action on spermatozoa appears to be confirmed in the present study as the zinc concentration in the testicular homogenate of the exposed rats is inversely correlated with sperm abnormalities. Also, Zago and Oteiza [29], showed that zinc deficiency would alter the antioxidant defenses, compromising the mechanism of DNA repair, exposing sperm to oxidative damage. In fact, exposure of lead to animals has led to an imbalance between sperm defense systems against oxidative stress, characterized by an increased production of free radicals. Under physiological conditions small amounts of reactive oxygen species (ROS) are produced by spermatozoa and several trappers act to reduce the concentration of these ROS in seminal plasma [30]. These ROS are necessary for capacitation, acrosomal reaction and finally fertilization [31-33]. However, excessive ROS production and/or decreased sequestration results in excess oxidative stress in sperm, resulting in alterations in sperm nuclear DNA, decreased mobility and impairment of their membrane integrity [34-36]. The oxidative stress that is deleterious is therefore characterized by an imbalance between the production of ROS and the antioxidant capacity of the organism [37]. These free radicals by attacking the membranes of the spermatozoa damage them and make them permeable. The observation concerning the damage of the plasma membrane of the spermatozoa and their permeability seem to be confirmed in this work by the highly significant increase of the abnormal spermatozoa on one hand and on the other hand, the decrease of the vitality in the treated rats. This membrane permeability of the spermatozoa following their alteration by the free radicals has, as corollary, the entry of the vital dyes, particularly the eosin inside the spermatic cells, signaling their death. The results also showed a significant decrease in the total number of spermatozoa and in the motility of these germ cells in the epididymis of the treated rats. This significant decrease in these two parameters is probably due to the actions of lead on the different levels of spermatogenesis control and to the deleterious effects of the oxidative stress induced by this heavy metal on the spermatozoa [38]. Moreover, the concentration of lead in the testicular homogenate is negatively correlated with the number of spermatozoa. In other words, when the concentration of lead increases in the testes, the number of spermatozoa in the epididymis decreases. The results obtained are in agreement with those of Lamina et al**.** [14]. These authors observed a very significant decrease in the number of epidydimal spermatozoa in rats after two months of lead exposure, however no differences were reported in rats after one month of exposure. According to Chinoy**,** [39] the decrease in the number of epidydimal spermatozoa is due to the cessation of spermatogenesis in the testes. Moreover, the concentration of this heavy metal was significantly higher in the exposed rats compared to the control rats (unexposed). Thus, we found a highly significant positive correlation between motility and sperm vitality in exposed rats. The results obtained corroborate the recent studies carried out by Bansal and Bilaspuriand Gharagozloo and Aitken [40,41]**.** These authors have shown that the oxidation and lipid peroxidation of the spermatozoa membranes increase the anomalies of the intermediate piece, resulting in a decrease in motility.

This study shows that in adult rats, chronic exposure to lead induces changes in spermatic parameters, leading to a decrease in male fertility. In addition, measurement of testicular lead is a good marker of lead exposure.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgements

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