ITALIAN JOURNAL OF PUBLIC HEALTH

Effects of oral intoxication by lead acetate on pituitary-testicular axis in the pubertal rat

Hachfi Lamia[1], Trimèche Mounir[2], Chekir Adel[3], Kerkeni Abdelhamid[3], Najjar Mohamed Fadhel[4], Sakly Rachid[1]

[1]Ecole Supérieure des Sciences et Techniques de la Santé, Monastir, Tunisia; [2]Hopital Farhat Hached, Service d'Anatomie Pathologique, Sousse, Tunisia; [3]Laboratoire de Biophysique,Faculté de Médecine, Tunisia; [4]Hopital Fattouma Bourguiba, Laboratoire de Biochimie, Monastir, Tunisia.

Correspondence to: Hachfi Lamia, Ecole Supérieure des Sciences et Techniques de la Santé, Av Avicenne, 5019 Monastir, Tunisia.

E-mail : hachfilamia@yahoo.com

Abstract

Background: The aim of this work is to study the effects of lead on pituitary-testicular axis. It is about a direct toxicity on testicular cells or an indirect toxicity via abnormalities of the hypothalamic-pituitary axis?

Methods: Forty male rats were divided equitably into four groups. One of these groups received tap water containing 0.3% lead acetate for one month. A second group was assigned to the same protocol but the intoxication was conducted for two months. The third and the fourth groups were used as reference. At the end of the experiment, the rats were anaesthetized and blood was collected for the proportioning of the FSH, the LH, testosterone and lead. The testes and epididymides were removed quickly and weighed. For the determination of testosterone in testicular fluids, a cruciate incision was made in the tunica albuginea of each right testis and centrifuged at 54xg for the extraction of interstitial fluid. Thereafter, the seminiferous tubules were extruded and centrifuged at 6000xg to collect seminiferous tubule fluid. The left testis of each animal was cut in two halves, one half was used for the histological examination and the other was suitably treated for the determination of the lead concentration. The epididymides were homogenized in solution containing Triton X-100 and spermatozoa were counted using Malessez cells. The plasma FSH and LH were determined by RIA using reagents from a commercial kit (BioInternational, France), while testosterone was determined by electrochemiluminescence (Elecsys, rochediagnostics). The lead concentration in blood and Tissues was measured by flameless atomic absorption spectrophotometry with the Zeeman effect background (Hitachi Z-9000).

Results: In the rats poisoned for one month, no notable effect was noted in spite of a blood lead concentration of 0.721 µg/ml. On the contrary, in animals exposed to lead acetate for two months, the blood level passed to 2.721 µg/ml and plasma testosterone, FSH and LH dropped by about 45%. In parallel, the secretion of testosterone by the Leydig cells fell by 35% and epididymal sperm count dropped by 14%. The histological examination showed lead deposits in the walls of the seminiferous tubules.

Conclusions: Although we do not exclude a directly toxic action of lead on the interstitial cells and on the Sertoli cells, it seems that the hypothalamic-pituitary axis is the major target of lead since the decrease in the plasmatic LH and FSH was associated with a reduction in testosterone secretion and with a reduction in the number of the epididymal spermatozoa.

Key words: FSH; LH, testosterone, lead, rat, spermatozoa

Introduction

Lead is considered as one of the major environmental pollutants. It may have different origins such as contaminated food, unsanitary preservation of food, lead hydrous piping, industrial pollution, road traffic [1] and drinking water [2]. The main sources of exposure for humans are food (ranging from 0.4 µg/kg bw/week to 10.1 µg/kg bw/week) and drinking water (ranged from 0.23 µg/kg bw/week to 0.35 µg/kg bw/week) [2]. On the basis of the recent studies, the provisional tolerable weekly intake (PTWI) was fixed at 25µg/kg BW [3] and the blood lead level should not exceed 0.4 mg/L [4]. Exposure to lead could damage nervous systems by provoking neuropsychological disturbances

[5], motor and sensory conduction velocities [6], and alter the heme synthesis [7]. It can also induce acute nephrotoxicity [8], cause disturbances of the reproductive system [9-12] and increase blood pressure [13, 14]. Some other studies have shown that it can increase the risks of lung cancer [15]. In fact, there has been 1.4-fold increase in the overall cancer incidence and a 1.8-fold increase in the incidence of lung cancer among those who have had a blood level higher or equal to mumol.L-1. Likewise, it has been reported that brain cancer risk has increased among caucasian men and women due to their lead high-level exposure, with a significant twofold excess among Caucasian men [16]. In animals, several experimental studies have reported impairment of the spermatogenesis [17- 20] but the mechanisms implied in the pathogenesis are not yet completely understood. Indeed, some works suggested a direct toxic action on the hypothalamic-pituitary axis, such as the study of Stump and co-workers [21] which showed that lead accumulates preferentially in the median eminence of the hypothalamus and that of Sokol and co-workers [22] which mentioned a fall in the plasma FSH associated with a reduction in the number of spermatozoa in the testis. In addition, other studies showed that the toxicity of lead appears primarily on the interstitial cells by the inhibition of enzymes synthesis implied in the steroidogenesis and of the hormonal receivers [23, 24]. In this study we propose to investigate the action of lead on the pituitary-testicular axis in the rat. It is about a direct toxicity on testicular cells or an indirect toxicity via abnormalities of the hypothalamic-pituitary axis?

Materials and methods

Animals

One-month old male Wistar rats were purchased from SIPHAT farm (Radès, Tunisia). They were housed in plastic cages in a separate room having a 12-h light-dark cycle and controlled temperature (22 + 2°C). Animals were given food (Sico, Sfax, Tunisia) and water ad libitum and acclimated to their new environment for 7 days prior to initiation of the lead treatment. The rats were divided randomly into four groups consisting of 10 rats in each one (2 served as control groups, 2 as intoxicated). Animals were maintained during the experimental period in accordance with the Canadian standards for animal care (CCAC Guide vol. 1 (2nd ED) 1993) upon obtaining the authorization of the ethical committee of the Faculty of Medicine (Monastir ,Tunisia).

Diet

Control groups received tap water containing no lead acetate whereas the treated groups received tap water containing 0.3% lead acetate. The 0.3% lead acetate solution was prepared by dissolving 7.5 g of lead acetate in 2.5 litres of tap water. One milliliter of 5 N HCl was added to all bottles including those of controls to preclude the precipitation of insoluble lead acetate (pH of aqueous solution 7.6). One of the control groups and one of the treated groups were sacrificed after 30 days of the trial and the remainder of the groups after 60 days.

Study design

After being weighed, the rats were anaesthetized with ether and blood was collected into heparinized Pasteur pipettes from the brachial artery. 100 µl of collected blood was transferred into a polystyrene tube and treated as described below for the determination of the blood lead concentration and the remainder of blood was centrifuged for 15 min at 4000 x g and the serum stored at -80°C for the determination of the levels of LH, FSH and testosterone. The testes were removed and once separated from their epididymides, they were washed with the physiological salt solution and weighed. One of the two testes was cut in two halves, the first was treated for lead extraction, and the second was used for histological examination (HE). The second testis was used for the extraction of the intertial and seminiferous tubule fluids according to the method of Turner and co-workers [25]. Briefly, a cruciate incision was made in the tunica albuginea of the distal pole and the whole testis was centrifuged at 54 x g at 0°C for 15 min to collect interstitial fluid. Thereafter, the testis was decapsulated and the seminiferous tubule was extruded through the hub of a syringe and then centrifuged at 6000 x g at 0°C for 15 min to collect the supernatant above the collapsed seminiferous tubules. The Interstitial and the Seminiferous tubule fluids were then diluted 25 to 1 using 50 nM tris Ca (pH 7.5) with 5% human serum albumin (HAS) and were snap-frozen in liquid nitrogen and finally stocked at – 80°C.

Lead determination in blood and tissues

100 µL of whole blood was diluted 10 times with ultrapurified water. Diluted blood was mixed with 100 µL of 2M HNO3 (Merck, Darmstadt, Germany) and then incubated for deproteinisation in a hot water bath of 70°C for five minutes. After centrifugation (4000 t/min), lead concentration was measured in the diluted

supernatant by flameless atomic absorption spectrophotometry with the Zeeman effect background (Hitachi Z-9000).

Lead concentration was extracted from the testes according to the method of Niemi et al [26]. Briefly, samples were homogenized and evaporated to dryness in an oven at 100°C (16h). Dried samples were ashed in a muffle furnace at 500°C for 12 h. Ashed samples were cooled to room temperature and 1.0 mL of 2M HNO3 was added and the volume was adjusted to 25 mL with deionized water. The metal was then measured as described above. The detection limits were 40 µg/L for blood and 0.05 µg/g for tissue lead.

Testicular testosterone and plasmatic hormonal parameters

The plasma FSH and LH were determined by RIA using reagents from a commercial kit (SB-Testo, CIS BioInternational, Gif-sur-Yvette, France), while testosterone was determined by electrochemiluminescence (Elecsys, rochediagnostics). The electrochemiluminescence is a form of chemiluminescence, which permits a high amplification of the signal. The reaction of chemiluminescence which entails light emission was preceded by an electrochemical reaction. The actors of this reaction were magnetic microparticles papered of streptavidine, antibodies marked with the biotin, antibodies marked with ruthenium and tripropylamine. The detection limits were 0.02, 2.00 and 025 ng/mL respectively for testosterone, FSH and LH. The intra-assay coefficients of variation were respectively 10, 7 and 8%.

Spermatozoa count

The left and the right epididymides (caput, corpus and cauda) of each rat were minced with scalpels into small pieces and homogenized in 4 ml of 0.9% NaCl solution containing 0.01% Triton X-100 [27]. Then, 10 µl of the epididymis homogenates were still diluted in 990 µl of 0.9% NaCl solution. After agitation of samples, an aliquot was dropped into a Malessez cells and spermatozoa were counted using the light microscope. The total number of spermatozoa was then calculated taking into account the dilution and the volume of physiological salt added during homogenisation. For each rat, an average was established from both epididymes and results were finally expressed per gram of epididymis weight.

Histological study

One half of testis issued from each rat was fixed in bouin, cut into 7 µm thick sections and stained with hematoxylin and eosin to examine any pathological changes and lead crystals.

Statistics

The Shapiro-Wilk test was applied first (using XLSTAT 2009.2.01) to assess the normality of the distribution of the data. If $W > W1-\alpha,n$ a normal distribution may be concluded. In this case we applied the Senedecor-Fisher test in order to assess the homogeneity of variances. If the F ratio (Variance A/Variance B; A>B) is lower than the critical limit provided by a pre-established table (table F), we proceeded to the Student test. If this was not the case we concluded that there were not any differences between the means. The significance of the student test was set using Statistica (Statistica Kernel version 6, StatSoft, France). Differences at $p< 0.05$ were considered statistically significant.

Results

Body and organ weights

At the beginning of the experiment, the rats weighed 80 + 5 g. After one month and two months the body weight passed respectively to approximately 191 g and 230 g in treated and control animals.

At all steps of the trial, the testis and the epididymis weights in the animals poisoned with lead were found to be similar to those of the control animals. Likewise, no differences were observed when reporting the testis and the epididymis weights to body weight (Table 1).

Blood and tissue lead values

After 30 days of exposure to lead, the blood lead concentration was of 0.731 + 0.07 µg/mL, whereas the concentration in the testis was of 671 + 18 ng/g (Table 2) . After 60 days of intoxication, the blood lead has tripled compared to that of 30-days poisoned animals (2.19 + 0.08 µg/mL; $p<0.001$) and testicular accumulation doubled (1496 + 39 ng/g; $p< 0.001$). In the control animals lead was found both in the blood and in the testis at the two stages of the experiment (respectively: 0.18 + 0.04 and 0.30 + 0.06 µg/ml; 67 + 18 and 75 + 29 ng/g) because of the presence of lead in the drinking water at the rate of 40 µg/L.

Hormonal parameters

At the end of the 30-day treatment period, neither the plasma FSH, nor LH and testosterone were found to be different from those of the control animals (Table 3). However, after 60 days of intoxication, the three hormones underwent a

Table 1. Body and organ weights and relative organ weights after 30 and 60 days of exposure to lead acetate.

Body and organ weights and relative organ weights	Groups	Period of treatment	
		30 days	60 days
Body Weight (g)	Control	191.50 ± 10.96	230.00 ± 26.83
	Contaminated	191.00 ± 15.13 **NS** *p=0.9335*	228.00 ± 20.49 **NS** *p=0.8535*
Epididymis weight (g)	Control	0.292 ± 0.049	0.442 ± 0.021
	Contaminated	0.306 ± 0.061 **NS** *p=0.5789*	0.441 ± 0.019 **NS** *p=0.9123*
Ratio of epididymides to BW ($\times 10^{-3}$g/g body weight)	Control	1.52 ± 0.23	1.95 ± 0.23
	Contaminated	1.60 ± 0.30 **NS** *p=0.5118*	1.96 ± 0.20 **NS** *p=0.9185*
Testis weight (g)	Control	1.068 ± 0.086	1.178 ± 0.058
	Contaminated	1.045 ± 0.069 **NS** *p=0.5178*	1.223 ± 0.085 **NS** *p=0.1836*
Relative testis weight ($\times 10^{-3}$g/g body weight)	Control	5.59 ± 0.41	5.19 ± 0.65
	Contaminated	5.49 ± 0.40 **NS** *p=0.5877*	5.41 ±0.53 **NS** *p=0.4177*

Data are means ± SD; NS = différence not significant

Table 2. Blood and testicular lead in rats after 30 and 60 days of exposure to lead acetate.

Blood and testicular lead	Groups	Period of treatment	
		30 days	60 days
Blood lead (µg/ml)	Control	0.18 ± 0.04	0.30 ± 0.06
	Contaminated	0.73 ± 0.07 ***	2.19 ± 0.08 ***
Lead in testis (ng/g)	Control	67 ± 18	75 ± 29
	Contaminated	671 ± 18 ***	1496 ± 39 ***

Data are means ± SD, *** $p < 0.001$

Table 3. Plasma testosterone, FSH and LH after 30 and 60 days of exposure to lead acetate.

Hormonal parameters	Groups	Period of treatment	
		30 days	60 days
FSH (ng/ml)	Control	2.39 ± 0.20	4.92 ± 0.62
	Contaminated	2.24 ± 0.17 **NS** *p=0.0875*	2.69 ± 0.49 ***
LH (ng/ml)	Control	6.41 ± 1.45	2.28 ± 0.45
	Contaminated	5.38.20 ± 1.40 **NS** *p=0.1240*	1.25 ± 0.45***
Testosterone (ng/ml)	Control	5.50 ± 1.32	5.82 ± 0.73
	Contaminated	4.62 ± 1.38 **NS** *p=0.1620*	3.18 ± 0.58***

Data are means ± SD
NS : difference not significant, *** $p < 0.001$

Table 4. Concentration of testostérone in testicular fluids after 30 and 60 days of exposure to lead acetate.

Parameters	Groups	Period of treatment	
		30 days	60 days
Testosterone (STF) (ng/ml)	Control	320.93 ± 110.42	458.48 ± 73.10
	Contaminated	325.48 ± 91.80 **NS** *p=0.9210*	350.14 ± 37.32 ***
Testosterone (IF) (ng/ml)	Control	479.15 ± 162.77	682.60 ± 104.85
	Contaminated	412.59 ± 158.49 **NS** *p=0.3670*	442.40 ± 75.57 ***

STF: seminiferous tubular fluid; IF: intertitial fluid
Data are means ± SD; NS : non significatif; *** $p < 0.001$

Table 5. Epididymal sperm count in rats after 30 and 60 days of exposure of lead acetate.

Parameter	Groups	Period of treatment	
		30 days	60 days
Sperm count ($\times 10^6$/g epididymis weight)	Control	45.60 ± 6.70	222.85 ± 21.51
	Contaminated	49.29 ± 4.33 **NS** *P=0.1608*	191.17 ± 25.52 ** *p=0.0077*

Data are means ± SD; ** $p < 0.02$

significant fall of about 45% compared with the control animals (respectively 2.69 + 0.49 vs 4.92 + 0.62 ng/mL, p< 0.001; 1.25 + 0.45 vs 2.28 + 0.45 ng/ml, p< 0.001; 3.18 + 0.58 vs 5.82 + 0.73 ng/mL, p<0.001).

Testosterone in testicular fluids

After one month of intoxication, the concentration of testosterone in the interstitial fluid and seminiferous tubule fluid was found to be identical to that observed in the control animals (Table 4). However, after two months of exposure to lead, a fall of about 34% was observed in the interstitial fluid (442.40 + 75.57 vs 682.60 + 104.85 ng/mL, p< 0.001) and about 24% in the seminiferous tubule fluid (350.14 + 37.32 vs 458.48 + 73.10 ng/mL, p< 0.001).

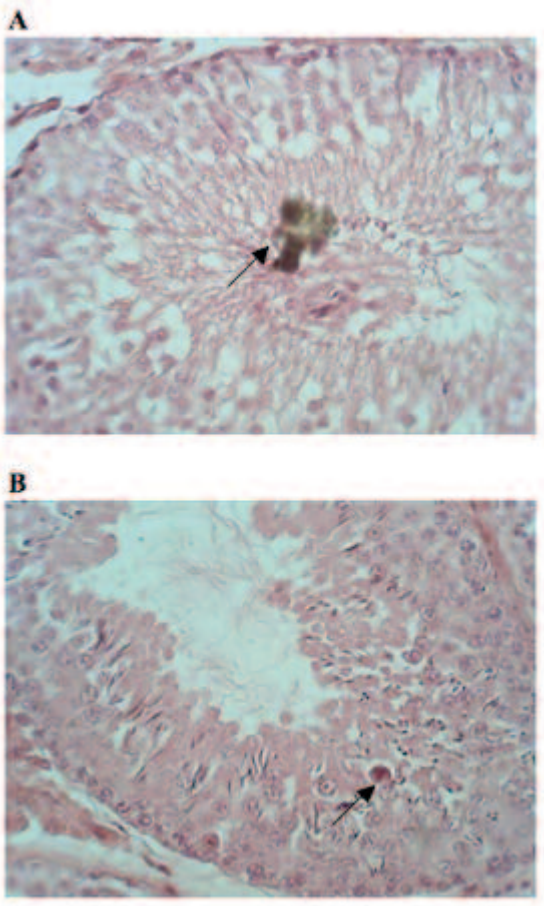
Epididymal sperm count

No effect was observed on the epididymal sperm count after 30 days of exposure to lead (45.60 X 10 6 / g in the control vs 49.29 X 10 6 / g of epididymis in the treated). On the other hand, after 60 days of intoxication a significant reduction of about 14% was observed in the animals poisoned with lead (222.85 X 10 6 + 21.51 X 10 6 vs 191.174 X 10 6 + 25.52 X 10 6 / g of epididymis, p<0.02) (Table 5).

Testicular histology

The histological examination did not show any deterioration of the Sertoli junctions and a normal aspect of the interstitial cells was observed in all poisoned animals. However, lead deposits in the walls of the seminiferous tubules and some images of abnormal mitosis of germinal cells were observed only in the animals intoxicated with lead for two months. (Figure 1).

Figure 1. Lead crystals in seminiferous tubule (A) and abnormal mitosis (B) after 60 days of exposure to lead acetate (magnification x 400).



Discussion

Our results showed that the chronic administration of lead acetate for one month led to a rise in the blood lead concentration up to 0.73 µg/mL. This level is slightly higher than that authorized by the National Institute for Safety and Health in the United States (0.5 mg/L) [28] and the European Council (0.7 mg/L) [29]. In the animals exposed to lead for two months the blood lead reached a concentration of 2.19 µg/mL, assuming that the animals sacrificed after two months of exposure to lead had toxic levels for at least a month. In the sacrificed control animals, although the concentrations of blood lead were found below 0.4 µg/mL in the two reference groups, blood lead was significantly higher in the 60-day controlled group than in 30-day controlled group (p<0.001). This could be explained by the fact that the lead concentration in the tap water to which the rats were subjected contains about 40 µg / L.

Contrary to the study of Thoreux-Manlay and co-workers [30] which reported an increase in the relative weights of the testis and epididymides in rats poisoned with 8mg/Kg/day by I.P route for five weeks, no variation was observed in our study. On the other hand, our results were consistent with those reported by Gorbel and co-workers [20] in spite of their use of an amount of lead three times greater than ours.

Concerning the hormonal levels, our results showed of about 45% in both the plasma LH and plasma testosterone in the animals exposed to lead for two months. If a decrease in testosterone was reported for blood lead lower than ours [20, 22] no study has reported a variation of the LH for lower blood lead [22, 31, 32]. On the contrary, Thoreux-Manlay and co-workers [24] observed a reduction in LH of about 32% at blood lead concentration approximately eight times higher than ours.

The fall in plasmatic testosterone seems to be due to the fall in the secretion of the testosterone (35%) by the Leydig cells confirmed by the analysis of testosterone in the interstitial fluid.The disorder of the synthesis of testosterone could result in a decrease in the secretion of the LH and a reduction in the number of receivers to the LH [33] and/or in a reduction in the expression of enzymes cytochromes, P450scc, P450c17 and the 3β-hydroxysteroid dehydrogenase implied in the biosynthesis of the testosterone and which was highlighted on a culture of interstitial cells issued from rats poisoned for 5 weeks with lead acetate at a rate of 8 mg/Kg/day [24].Although our results do not confirm a direct toxic action of lead on the level of the Leydig cells, it seems certain that lead has had a strong toxic effect on the hypothalamic-pituitary axis. Indeed, in spite of the spectacular fall in plasma testosterone observed after two months of exposure to lead, the LH did not undergo any increase. This confirms a disruption of the feedback control exerted by testosterone on the synthesis of the LH in pituitary gland or on the secretion of LHRH in hypothalamus which appeared dependent especially on the testosterone [34]. The study of Petrusz and co-workers [35] showing that the pituitary contents in LH remained unchanged whereas that of the FSH was increased in young rats poisoned with lead was in contradiction with our results and highlights the need for further studies in this field.

Also, our results showed a fall of about 45% in the plasma FSH after two months of exposure to lead. This was in agreement with the study of Sokol and co-workers [22] in which lead was administered at the same amount as in our study and only for one month, but was inconsistent with the results of other works [30, 31, 36]. The heterogeneity of the results concerning the FSH could be explained mainly by the use of different amounts of toxicant, the varied period of exposure and the multiple routes of administration of the toxicant.

It seems that the fall in the FSH is the principal cause of the reduction in the number of the spermatozoa observed after 60 days of exposure to lead. Indeed, the disturbances of the number, the motility and the morphology of the spermatozoa were observed in rodents, monkeys or in man [12, 37, 38] for blood lead higher than 0.4 µg/ml. The FSH plays, in fact, a key role in the reproductive function in males. It stimulates the proliferation of the Sertoli cells [39], induces the synthesis of the ABP [40], which is implied in the transport of testosterone and the dehydotestosterone from the testis towards the epididymis and increases the expression of the transferrine in the Sertoli cells [41].

The deterioration of the spermatogenesis observed in our study seems to be due to a drop in the secretion of FSH, but we should not rule out that this effect is amplified by a certain dysfunction of the Sertoli cells caused directly by lead. This hypothesis can be confirmed by the presence of lead deposits preferentially in the wall of seminiferous tubules. On the contrary, the data reported by Wadi and Ahmad [42] supported that lead targets testicular spermatogenesis and sperm rather than hypothalamic-pituitary axis. Using lead at 0.25%, these authors reported a reduced number of sperm within epididymis, while plasma FSH was not affected in adult mice. Considering the constancy of FSH reported in poisoned animals [30, 31, 36] and its reduction in other [22], it is yet difficult to understand the role of this hormone in the spermatogenesis disorders in the intoxicated animals.

Although our study showed a fall in the concentration of testosterone in the seminiferous tubule fluid in the animals poisoned with lead for two months, concentration still 20 times higher than that essential for the maintenance of normal spermatogenesis [43-45], excluded the possibility of a disorder of the spermatogenesis related to a deficit of testosterone in the seminiferous tubule fluid. If we referred to the data of Turner and co-workers [25] which reported testosterone concentrations of about 50 ng/mL in the seminiferous tubule fluid and 73 ng/mL in the interstitial fluid in the normal rat, the concentrations obtained in our study in the 60 day old rats (30 days after exposure to lead) were at least 5 times higher both in interstitial fluid and seminiferous tubular fluid. The high sensitivity of the chemiluminescence used for the proportioning of testosterone and the method employed for the extraction of the testicular fluids could explain largely the values obtained in our study in comparison with previous works.

In conclusion, the results of this study demonstrate that the chronic administration of lead acetate in rats induced, after two months of exposure, a deterioration of the spermatogenesis and a very significant fall in the secretion of testosterone by the Leydig cells. Although we cannot exclude a certain direct toxicity of lead on the interstitial cells and the Sertoli cells, it seems that the major target of the toxicity of lead occurs on the hypothalamic-pituitary axis via a reduction, at the same time, in the secretion of LH and FSH. Further studies are still required to explain the mechanism of toxicity of lead and especially to

clarify the role of the hypothalamic-pituitary axis in testicular dysfunction.

References

1) Carrilo LL, Sauchez LT, Carrido F, et al. prevalence and determinants of lead intoxication in Mexican children of low socio-economic status. Environ Health Perspect 1996; 104: 1208-1211.
2) WHO. Lead.WHO Food Additives Series 44. Geneva: WHO; 2000.
3) JECFA. 53rd report of the Joint FAO/OMS Expert Committee on Food Additives. Rome: FAO/OMS; 1999.
4) Occupational Safety and Health Administration (OSHA). Final standard for occupational exposure to lead: supplementary information, health effects. 43 Federal Register. No. 220, pp. 52954-5; 1978
5) Hogstedt C, Hane M, Agrell A, et al. Neuropsychological test results and symptoms among workers with well-defined long-term exposure to lead. Br J Ind Med 1983; 40: 99-105.
6) Seppäläinen AM, Hernberg S, Vesanto R, et al. Early neurotoxic effects of occupational lead exposure: A prospective study. Neurotoxicology 1983; 4: 181-192.
7) Piomelli S. Chemical toxicity of red cells. Environ Health Perspect 1981; 39:65-70.
8) Goyer RA. Mechanisms of lead and cadmium nephrotoxicity. Toxicology Letters 1989; 46:153-162.
9) Goyer RA. Transplacental transport of lead. Environ Health Prospect 1990; 91: 89101-105.
10) Mushak P, Davis JM, Crocetti AF, et al. Prenatal and postnatal effects of low-level lead exposure: Integrated summary of a report to the U.S. Congress on childhood lead poisoning. Environ Res 1989; 50:11-36.
11) Biava PM, Audisio R, Centonze A, et al. An epidemiological study of the health conditions of Milan traffic police with respect to pollution from vehicular traffic. Med Lav 1992; 83: 249–258.
12) Apostoli P, Kiss P, Porru S, et al. Male reproductive toxicity of lead in animal and humans, Asclepios Study Group. Occup Environ M 1998; 55 : 364–374.
13) Schwartz J. Lead, blood pressure, and cardiovascular disease in men. Environ Health Pespect 1995; 91: 71-75.
14) Hertz-Picciotto I, Croft J. Review of the relation between blood lead and blood pressure. Epidemiologic Reviews 1993;15: 352-73.
15) Anttila A, Heikkilä P, Nykyri E, et al. Excess lung cancer among workers exposed to lead. Scan J Work Environ Health 1995; 21: 460-469. 1995.
16) Cocco P, Dosemeci M, Heineman EF. Brain cancer and occupational exposure to lead. J Occup and Environ Med 1998; 40: 937-42.
17) Der R, Fahim Z, Yousef M, et al. Environmental interaction of lead and cadmium on reproduction and metabolism of male rats. Res Commun Chem Pathol Pharmacol 1976; 14: 689.
18) Eyden BP, Maisin JR, Mattelin, G. Long-term dietary lead acetatate on survival, body weight and seminal cytology in mice. Bull Environ Contam Toxicol 1981; 20: 226.
19) Wyrobek AJ, Bruce WR. The induction of sperm-shape abnormalities in mice and humans. Chem Multagens 1978; 5: 257.
20) Gorbel F, Boujelbene M, Makni-Ayadi F, et al. Impact of lead given in drinking water on the endocrine and exocrine sexuel activity in pubescent rats. Determination of an apoptic process. Comptes Rendus Biologies 2002; 325: 927-940.
21) Stumpf WE, Sar M, Grant LD. Autoradiographic locolalization of (210)Pb and its decay products in rat forebrain. Neurotoxicology 1980; 1: 593.
22) Sokol RZ, Madding CE, Swerdloff RS. Lead toxicity and the hypothalamic-pituitary-testicular axis. Biology of reproduction 1985; 33: 722-728.
23) Wiebe JP, Salhanick AI, Myers KI. The mechanism of action of lead in the testes: in vitro suppression of LH receptors, cyclic AMP and steroidogenesis, Life Sci 1983; 32: 1997.
24) Thoreux-Manlay A, Goascogne CL, Segretain D, et al. Lead affects steroidogenesis in rat Leydig cells in vivo and in vitro. Toxicology 1995; 103: 53-62.
25) Turner TT., Jones CE, Howards SS, et al. On the androgen microenvironment of maturing spermatozoa. Endocrinology 1984; 115: 1925-1932.
26) Niemi A, Venalainen ER, Hirvi T, et al. The lead, cadmium and mercury concentrations in muscle, liver and kidney from Finnish pigs and cattle during 1987-1988. Zeitschrift fûr Lebensmittel Untersuchung and Forschung 1991;192: 427-429.
27) Blazak WF, Treinen KN, Juniewicz PE. Application of testicular sperm head counts in the assessment of male reproductive toxicity. In: Chapin RE, Heindel JJ, editors. Methods in Toxicology, vol 3A. San Diego: Academic Press, 1993: 86-105.
28) NIOSH Recommandations for Occupational Health Standards. MMWR Supplement, October 7, Vol.32, Center for Disease Control, Atlanta, Georgia. 1983.
29) Lauwerys R, Haufroid V, Hoet P, Lison D. Toxicologie Industrielle et Intoxications Professionnelles. 5ème edition. Paris: Masson, 2007.
30) Thoreux-Manlay A, Vélez de la Calle JF, Olivier MF, et al. Impairment of testicular endocrine function after lead intoxication in the adult rat. Toxicology 1995; 100: 101-109.
31) Nathan E, Huang HFS, Pogach L, et al. Lead acetate does not impair secretion of Sertoli cell function marker proteins in the adult Sprague-Dawley rat. Arch Environ Health 1992; 47: 370-375.
32) Pinon-Lataillade G, Thoreux-Manlay A, Coffigny H, et al. Effect of ingestion and inhalation of lead on the reproductive system and fertility of adult male rats and their progeny. Hum Exp Toxicol 1993; 12: 165-172.
33) Kempinas WG, Melo VR, Oliveira-Filho RM, et al. Saturnism in the male rat: endocrine effects. Braz J Med Biol Res 1990; 23 : 1171-1175.
34) Rotten D. Régulation de la synthèse et de la sécrétion de FSH. In : Thibault C , Levasseur MC, editors. La reproduction chez les mammifères et l'homme. Paris: Ellipses, 1991: 89-111.
35) Petrusz P, Weaver CM., Grant LD, et al. Lead poisoning and reproduction: effects on pituitary and serum gonadotropins in neonatal rats. Enviro Res 1979; 19: 383.
36) Sokol RZ, Berman N. The effect of age of exposure on lead-induced testicylar toxicity. Toxicology 1991; 69: 269-278.
37) Moorman WJ, Skaggs SR, Clark JC, et al. Male reproductive effects of lead, including species extrapolation for the rabbit model. Reprod Toxicol 1998; 12: 333-346.
38) Foster WC, McMahon A, Rice DC. Sperm Chromatin structure is altered in cynomolgus monkeys with environmentally relevant blood levels. Toxicol Ind Health 1996; 12: 723-735.
39) Plant T.M, Marshall GR. The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. Endocr Rev 2001; 22: 764-786.
40) Louis BG, Fritz IB. Stimulation by androgens of the production of androgen binding protein by cultured sertoli cells. Mol Cell Endocrinol 1977; 7: 9-16.
41) Sylvester SR. Secretion of transport and binding proteins. In: Russell LD, Griswold MD, editors. The Sertoli cell. St. Louis: Cache River Press, 1993: 201-216.
42) Wadi SA, Ahmad G. Effects of lead on the male reproductive system in mice. Toxicol Environ Health 1999; 56: 513-521.
43) Rommerts FFG. How much androgen is required for maintenance of spermatogenesis? J Endocrinol 1988; 116 : 7-9.
44) Sharpe RM, Donachie K, Cooper I. Re-evaluation of the intratesticular level of testosterone required for quantitative maintenance of spermatogenesis in the rat. J Endocrinol 1988; 117: 19-26.
45) Zirkin BR, Santulli R, Awoniyi CA, et al. Maintenance of advanced spermatogenic cells in the adult rat testis : quantitative relationship to testosterone concentration within the testis. Endocrinology 1989;124: 3043-3049.