The Effect of lead exposure of mice during pregnancy on the morphology of epididymal and testicular spermatozoa of their offspring

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ABSTRACT

Introduction: *The aims of this study were to assess the differences in the percentages of abnormal morphology between the epididymal and testicular spermatozoa of mature male offspring mice whose mothers were injected with various doses of lead acetate during gestation.* ***Materials and Methods:*** *Seventy two healthy female mice were divided into three major groups according to the number of injections involving 1, 2 or 3 injections at 8th day; 8th and 13th days; and 8th, 13th and 18th days of gestation period, respectively. Each major group was subdivided into four minor groups according to the dosage of lead administered (0, 25, 50 and 100) mg/Kg.* ***Results:*** *The percentages of abnormal morphology of epididymal and testicular spermatozoa were studied and the data were statistically analyzed. The results of this study proved that an increased number of injections and/or dose of lead acetate injected to the mothers during gestation cause an elevation in the percentage of abnormal morphology of both epididymal and testicular spermatozoa of the male mice offspring.* ***Conclusion:*** *In conclusion this study demonstrated that lead acetate when exposed prenatally have toxic effects on the sperm in the offspring male mice resulting in abnormal morphology of spermatozoa. The most likely causative factor is disturbances in the phase(s) of spermatogenesis and/or spermiogenesis.*

KEYWORDS: Mice, Lead, Abnormal sperm morphology, Epididymis, Testes.

INTRODUCTION

Sperm morphology is the most important seminal parameter, which correlates with the rate of human in vitro fertilization and sperm fertilizing ability in the hamster egg-sperm penetration assay.[1,2] Francavilla et al pointed out that abnormal sperm morphology as an expression of a serious disturbance in the process of spermatogenesis.[3] There are factors that could negatively affect the spermatogenesis including pollutants.[4] Lead is considered as one of the hazardous pollutants and toxins that is found in the environment.[5] Lead is also a very toxic substance; physicians Hippocrates and Nikander recognized occupational lead exposure more than two thousand years ago.[6] Most of the lead in our environment comes from gasoline used in our cars and from exposure to it when lead painted homes and other structures need to be repaired.[7] Previously, Lancranjan et al reported a higher sperm-shape abnormality and reduced sperm count and motility in workers exposed to lead in storage batteries manufacturing plants.[8] Johansson et al discovered a relationship between a high concentration of lead found in the epididymis and a possible disturbance of the maturational changes of the spermatozoa leading to a reduction in the fertility.[9]

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Lead has serious clinical effects on a number of body functions including the physiology of reproduction.[10] Zaki et al reported that lead toxicity is one of the most common syndromes of environmental origin which cause a challenge to our health care.[11] Lead causes both acute and chronic poisoning depending on the length and level of exposure; the toxicity is manifested in various forms of gastrointestinal and neurological symptoms.[12] Therefore, lead toxicity to human organs and systems has been extensively documented for over two millennia.[13] Lead poisoning for more than a century has been the most prevalent and serious disease of environmental origin for young children in the United States.[14]

Air borne lead, particularly lead which has settled in the dust and dirt is gradually being recognized as an important hazardous source of exposure in children.[15] The major sources of lead contamination in addition to gasoline fumes, are industrial emissions, drinking water, lead-based paint and soil.[16] Danielsson et al showed that lead is capable of reaching the embryonic and fetal tissues at all stages of gestation in the mouse.[17] In rats, lead was found in the fetus within minutes of maternal intravenous injection which indicates that lead can easily pass to the fetus.[18] The teratogenic effects of lead intoxication

have been demonstrated frequently in many animal experiments.[19] Therefore, the aims of this study were to firstly assess the harmful effects of lead acetate on sperm morphology between the treated and the control groups, and secondly to show the differences in the percentages of abnormal morphology between epididymal and testicular spermatozoa of mature male offspring mice where the mothers were injected with various doses of lead acetate during gestation.

MATERIALS AND METHODS

Animals

Seventy two healthy mature Swiss albino strain female mice (age: 8 weeks) were obtained from an animal house of Saddam College of Medicine and were kept under suitable environmental conditions such as room temperature that was maintained at about (24+2)oC and exposed to 12 hours per day to light (light program). Vaginal smears were obtained to examine the regularity of at least three consecutive oestrus cycles. When a female was in oestrus stage, it was mated with a male. After the formation of vaginal plug, examination was performed to observe the spermatozoa. The gestation period has commenced and considered the first day of pregnancy (2).

Experimental Design

The female mice were divided into three major groups according to the number of injections (1, 2 & 3). Each major group was further subdivided into four minor groups according to the dosage of lead acetated administered. Table I shows the experimental design of the present study.

Table I. The experimental design of the present study

Quantity of dose	Number of treated females		
	One injection (at 8th day of gestation period)	Two injections (at 8th and 13th days of gestation period)	Three injections (at 8th, 13th and 18th days of gestation period)
0 (Control)	6	6	6
25 mg/Kg	6	6	6
50 mg/Kg	6	6	6
100 mg/Kg	6	6	6

When males offspring reached the period of sexual maturation (two months), they were sacrificed. The testicular and epididymal tissues were collected and placed into two small Petri dishes containing 1 ml of Earl's medium (ICN Biomedical Inc., Costa Mesa, CA, USA) until the solutions were homogenized

Microscopic examination for the percentage of abnormal sperm morphology

All slides were prepared for microscopical examinations from 40, 38, 34 and 34 offspring males where their mothers were injected with 0, 25, 50 and 100 mg/Kg of lead acetate during gestation, respectively. A drop of spermatozoal suspension was mounted between the slide and the cover slide. Each sample was examined at 40X magnification. At least 200 spermatozoa were observed for the calculation of percentage of the total numbers of spermatozoa.2 The percentage of abnormal sperm morphology was calculated from the following formula:

Abnormal sperm morphology

(%) = No. of abnormal sperm X 100
Total sperm count

Statistics

In addition to the standard methods to determine the mean and standard error of the mean (SEM), one way analysis of variance (ANOVA) was used to test the significant difference between treatments (0, 25, 50 and 100 mg/Kg) of lead acetate. Also, when the analysis of variance was significant, the multiple comparisons by improved Tukey's test were used to determine which group of treatment showed a significant difference. Student t-test was used to determine the significant difference between the testicular and epididymal spermatozoa for the percentage of abnormal sperm morphology of the control and the treated groups.20

RESULTS

Percentage of abnormal morphology of epididymal sperm

The results of the present study showed that the injection of lead acetate with various concentrations (25, 50 and 100) mg/Kg to pregnant mice mothers caused a significant ($P<0.05$) increased in the percentages of abnormal sperm morphology within the epididymis of mature male mice offspring as compared to its control groups (Table II).

Table II. Percentages of abnormal morphology of mice epididymal sperm born to mothers treated with different lead acetate concentrations and doses

Quantity of dose	Number of injections		
	One injection (at 8th day of gestation period)	Two injections (at 8th and 13th days of gestation period)	Three injections (at 8th, 13th and 18th days of gestation period)
0 (Control)	32.4	31.5	33.3
	1.48	1.29	0.8
25 mg/Kg	35.31 # c	39.53 *	40.77 * # a
	0.96	0.96	2.85
50 mg/Kg	38.36 * c	43.50 *	46.23 * a
	1.9	2.07	1.36
100 mg/Kg	41.50 * ** c	43.64 *	49.05 * ** a
	1.78	1.89	1.39

Values are presented as mean + SEM.

*: Significantly (P<0.05) different from its control.
**: Significantly (P<0.05) different from 25 mg/Kg group.
: Significantly (P<0.05) different from 100 mg/Kg group.
a : Significantly (P<0.05) different from one injection group.
c : Significantly (P<0.05) different from three injections group.

The percentages of abnormal sperm morphology were significantly (P<0.05) increased in the groups whose mothers were injected with a dose 100 mg/Kg of lead acetate as compared to a dose 25 mg/Kg of lead acetate. Also, three injections of various concentrations (25, 50 and 100) mg/Kg of lead acetate significantly (P<0.05) increased the percentages of abnormal sperm morphology as compared to one injection of the same concentrations (Table II).

Percentage of abnormal morphology of testicular sperm

Table (3) showed that the various concentrations (25, 50 and 100) mg/Kg of lead acetate causes an increased in the percentages of abnormal morphology of testicular sperm when compared to its control groups (Table III). The highest percentage of abnormal sperm morphology was obtained by an injection dose of 100 mg/Kg of lead acetate as compared to the groups which were injected by a dose of (25 and 50) mg/Kg of lead acetate. Similarly, the percentage of abnormal sperm morphology was significantly (P<0.01) increased by one injection of 50 mg/Kg of lead acetate as compared to one injection of 25 mg/Kg of lead acetate (Table III). As compared to one injection, two and three injections of 25 mg/Kg of lead acetate caused an increased in the percentage of abnormal morphology of testicular sperm (Table III).

Quantity of dose	Number of injections		
	One injection (at 8th day of gestation period)	Two injections (at 8th and 13th days of gestation period)	Three injections (at 8th, 13th and 18th days of gestation period)
0 (Control)	26.7	31.1	25.1
	1.29	1.82	1.46
25 mg/Kg	39.75 * $ # b c	47.23 * a	47.88 * # a
	1.58	0.96	1.91
50 mg/Kg	49.18 * **	49.80 *	51.07 *
	1.6	1.14	0.95
100 mg/Kg	49.20 * **	50.28 *	54.60 * **
	1.61	2.04	1.55

Table III. Percentages of abnormal morphology of mice testicular sperm born to mothers treated with different lead acetate concentrations and doses

Values are presented as mean + SEM.

*: Significantly (P<0.01) different from its control.
**: Significantly (P<0.05) different from 25 mg/Kg group.
$: Significantly (P<0.01) different from 50 mg/Kg group.
: Significantly (P<0.05) different from 100 mg/Kg group.
a : Significantly (P<0.01) different from one injection group.
b : Significantly (P<0.01) different from two injections group.
c : Significantly (P<0.05) different from three injections group.

After one injection of a dose (25, 50 and 100) mg/Kg of lead acetate, the percentages of abnormal morphology of testicular sperm were significantly increased (P<0.01) as compared to the epididymal sperm (Figure 1). Similar results were reported for two and three injections of the same doses (Figures 2 and 3). Within the control groups, the percentages of abnormal morphology of testicular sperm were reduced either significantly (P<0.01) or none significantly (P>0.05) than the epididymal sperm (Figures 1 - 3).

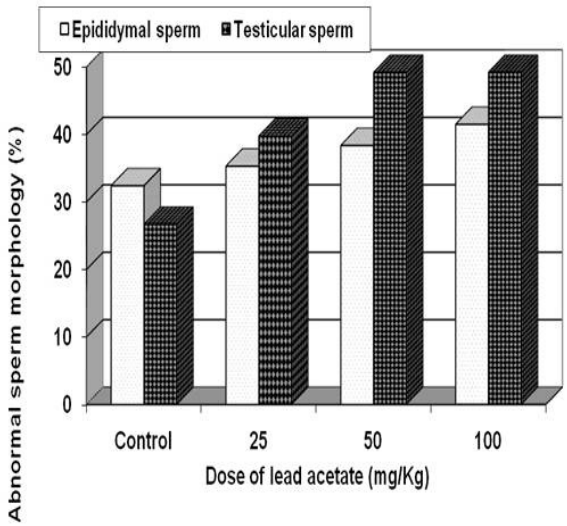

Figure 1. Comparison between percentages of abnormal morphology for epididymal and testicular spermatozoa of mice born to mothers treated at day 8th of gestation period with different doses of lead acetate
a : Highly significantly (P<0.01) different from other groups.

DISCUSSION

Exposure to lead can cause behavioral effects such as irritability and physical effects like fatigue, headache and reduced sex drive.6 In adult, lead usually enters the body through inhalation and digestion; Gordon et al found that lead usually enters through respiratory tract in adults and they also stated that 30-70% of inhaled lead get into the circulation.[21]

Normal morphology of the sperm was considered as one of the most important sperm criteria to achieve normal fertilization and embryonic development.[2] Therefore, abnormal sperm morphology was investigated as a true expression of serious disturbances in the spermatogenesis.[22] It's known that the phases of spermatogenesis are negatively affected by several factors including pollutants such as lead.[23]

In this study, administration of lead acetate with different doses and number of injections to pregnant mothers were associated with an increased in the percentages of abnormal morphology for both epididymal and testicular spermatozoa in the offspring male (Tables II and III). According to these results, lead was observed as being able to pass through the placenta of pregnant mice and reached and accumulated within the tissues of fetus. Similar results were found by Danielesson et al who proved that the lead is capable of reaching embryonic and fetal tissues at different periods of gestation in the mouse.[17] Also, McMichael et al also demonstrated that the lead was able to transfer across the human placenta around the 12th to 14th week of gestation.[23] These results were in agreement with the results obtained by Conffigny et al in which the lead was able to cross the placenta easily as shown by the similar levels in both the mothers and their fetuses.[24] Additionally, lead accumulated in many organs and body fluids especially the gonads and seminal fluid in addition to the testicular tissue and caused harmful effects on the performance of reproduction.[25]

In the present study, there were significant increase, in the percentages of abnormal sperm morphology within both the epididymis and testis of adult offspring as compared to the control group (Tables II and III). Certainly, this abnormal sperm morphology is due to lead compounds ability to induce chromosomal aberrations which could lead to the induction of dominant lethal mutations in the structure of the sperm.[26] Corpas et al found that the lead could disturb mitosis in vivo and alter Sertoli cells proliferation, which produce significant decrease in the sperm functions within the testis of adult offspring.[27] It is reported that lead accumulates in all the male reproductive organs especially in high concentrations in the epididymis where it causes alteration in the functions of epididymis which lead to higher percentage of abnormal sperm morphology. Dawson et al showed that the presence of lead in the seminal fluid exerts toxic effects on sperm parameters such as morphology, motility and viability.28 However, Apostoli et al showed that exposure of rats to concentration of inorganic lead > 40 ¤g/dL in the blood was the reason for the impairment of male reproductive performance as a result of reduction of sperm count and changes in sperm morphology.[29]

In general, the percentage for abnormal sperm morphology within the testis was higher than the epididymis for all doses and number of injections of lead acetate (Figures 1-3). This may be due to either a direct effect of lead on sperm formation within the seminiferous tubules as the spermatozoa within the epididymis are more mature and stable than within the testis.[30] Similar results were reported by Wyrobeck and Bruce, where they noticed increased abnormal sperm morphology in the mice seminal fluid which was collected after acute exposure to lead acetate by peritoneal injections.[31] When the testicular tissue is damaged by lead, the process of spermatogenesis and most of the functions of sperm would be impaired. Hilderbrand et al reported that the exposure of male rats orally to lead at a concentration of 50¤g/dL led to inhibition of normal spermatogenesis.[32] Acharya et al reported that intraperitonial injection of lead acetate to swiss mice stimulated testicular weight loss with constant increase in the incidence of abnormal sperm population and decrease in the total sperm count.[33] They observed damage in the germ cells leading to heavy loss of germ cells and significant decline in the sperm count. Johansson and Wide demonstrated a relationship between a high concentration of lead found in the epididymis and a possible disturbance of the maturational changes of the spermatozoa leading to a reduction in the fertility.9 Lead acetate may inhibit spermatogenesis by a disturbance of metabolic activity of the Sertoli cells.[34] McGivern et al proved that administration of lead during gestation period causes higher risk for reduced reproductive efficiency in adulthood, and this might be attributed to either the dysfunction of the Sertoli cells which are responsible

for the environment of the germ cell proliferation and maturation, or the hypothalamus-pituitary-gonadal (HPG) axis which control and/or regulate germ cell proliferation and maturation.[35]

CONCLUSION

It is our conclusion that lead acetate induced negative effects on the epididymal and testicular tissues by causing a number of disturbances to the process of spermatogenesis. Further studies of the concentration, viability and motility of spermatozoa of the offspring of lead treated mothers during pregnancy are in progress.

RECOMMENDATIONS

Pregnant women should not be exposed to lead and must be prevented from working in factories using lead salts. As children and fetuses are more susceptible to lead poisoning because of a greater absorption rate of their growing bodies, educational and awareness programs should be undertaken to prevent lead exposure in children.36 Certain regulatory measures are also needed to ensure employee protection thereby preventing contamination of homes and automobiles from occupational exposure to lead.

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