

Effects of Maternal Lead Acetate Exposure during Lactation on Postnatal Development of Testis in Offspring Wistar Rats

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Abstract

Objective(s)

During recent years, there has been an increasing interest in contribution of environmental pollutants as heavy metals to human male infertility. Present study was aimed to investigate the effects of maternal lead acetate exposure during lactation on postnatal development of testis in offspring rats.

Materials and Methods

A total of 60 female rats randomly divided into four equal groups; control and three treatment groups received 20, 100 and 300 mg/kg/day lead acetate via drinking water from day 2 to day 21 of lactation. At 7, 14, 21, 28, 60, 90 and 120 days after birth, the testis weight and volume of offspring were measured and their epididymal semen analyzed. Following tissue processing, 5 μ m sections were stained with haematoxylin-eosin and evaluated with quantitative techniques. Testicular parameters in different groups were compared by one-way ANOVA.

Results

Testis weight and volume of offspring decreased significantly in a dose-related manner in moderate ($P < 0.05$) and high ($P < 0.01$) doses groups. Dose-dependent significant reductions were seen in seminiferous tubules diameter and germinal epithelium height during neonatal, prepubertal and postpubertal periods in moderate ($P < 0.05$) and high ($P < 0.01$) doses groups until 90 and 120 days after birth, respectively. Significant decreases were observed in mean sperm density of offspring at puberty in moderate and high doses groups until 90 and 120 days after birth, respectively. Testosterone levels decreased significantly in a dose-related manner at puberty in moderate and high doses groups.

Conclusion

Present study showed maternal lead acetate exposure during lactation caused dose-related and long-term alterations of testicular parameters in offspring rats.

Keywords: Lead acetate, Male infertility, Postnatal development, Testis

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Introduction

In recent years, there has been an increasing interest in the contribution of occupational and environmental exposures to toxic pollutants to declining sperm concentration and human male infertility (1-4). There are epidemiological evidences that exposure to industrial metal aerosols may be detrimental to male reproduction system (5, 6). One of the first materials to be demonstrated as detrimental to fertility was lead (7). Lead appears in homes in many forms as lead piping, lead-containing solders, paints, ceramic glazes, pewter and base metal utensils and fixtures. Also, cream powder, lipstick and hair color have lead. Agricultural soil contamination may be responsible for lead found in many herbal medicines and cigarettes. There is lead in soils near human dwellings. Children that playing in and eating such soil may exhibit significantly elevated lead levels (8).

The role of lead in male subfertility factor is of particular current interest (9). In men, especially in professional workers, lead exposure results in infertility and sterility (10), testicular atrophy, cellular degeneration, and reductions in seminiferous tubule diameter (STD) and sperm count, depending on the dose and time of exposure (11, 12). In animal models, lead exposure consistently decreases male reproductive function at the level of the hypothalamic-pituitary-testicular axis (13). The effects of lead on adult rat testis have been widely studied and observations demonstrate that Pb in particular alters those organs, as evidenced by testicular necrosis and atrophy in rodents (14). However, the effects of chemical agents on fertility and development may consider the fact that exposure to some materials during critical period of development may affect the adult life and even generations to come. In humans and experimental animals the gastrointestinal absorption of lead after chronic exposure also exerts a wide number of adverse biological effects during lactation, more so than in adult life (15), since neonates absorb 40-50 times more lead than adults (16). It appears from the evidences that the neonatal period is a critical stage in the process of sexual development and maturation in primates. Interferences with normal brain-

pituitary-gonadal function during this period appear to impact adversely on subsequent reproductive function (17). These data further emphasize the importance of fully understanding the regulatory mechanisms that govern neonatal gonadal function in the primate, if we are to eliminate, control or minimize the potential risk resulting from its disruption in humans. Given the recent evidence that the reproductive potential of the human male has declined rather dramatically over the last 50 years, and that clinical conditions associated with abnormal testicular function are on the rise, continued investigation in this area would appear to be imperative (17). Therefore, present study was undertaken to investigate the effects of maternal lead exposure during lactation on postnatal development of testis during neonatal, prepubertal and postpubertal periods in offspring Wistar rats.

Materials and Methods

Animals

A total of 60 pregnant female Wistar rats were obtained from animal house of Jondishapour University of Medical Sciences, Ahwaz. The animals were housed in stainless steel cages under standard animal house conditions with a 12 hr light/dark cycle and a temperature of 25 ± 2 °C. They received standard pellet food and distilled water was available *ad libitum*. After childbirth, mothers and their pups divided into four equal groups (N= 15 in each group); the control and three treatment groups that received 20, 100 and 300 mg/kg/day lead acetate in drinking water from day 2 to day 21 of lactation. The doses were established from related studies of reproductive toxicity (18- 20).

Quantitative analysis

At 7, 14, 21, 28, 60, 90 and 120 days of age five pups were randomly selected and body weight measured. At 7 and 14 days of age testes were removed, separated from the epididymis, weighed and fixed by immersion in Bouin's solution for 24 hr. At 21, 28, 60, 90 and 120 days of age right testes were removed and weighed and left testes fixed by whole body perfusion (21), then removed and post-fixed by

immersion in Bouin's solution for 24 hr. In every sampling stage the volumes of left testes were measured by Cavellieri method (22). For this purpose, each testis embedded in paraffin and serial 5 μm sections, with a random start, were prepared along the long axis of the organ, stained with haematoxylin-eosin and used for quantitative analysis.

Tissue samples from right testes were excised and processed for paraffin embedding sections. Tissue sections with 5 μm thickness were stained with heamatoxylin-eosin and used for quantitative analysis at light microscopic level. For measuring of seminiferous tubule diameter and germinal epithelium height, 90 round or nearly round cross-sections of seminiferous tubules were randomly chosen in each rat. Then, using an ocular micrometer of light microscopy (Olympus BH), at a magnification of $\times 40$, two perpendicular diameters of each cross-section of seminiferous tubules were measured and the mean of these was calculated. Also, germinal epithelium height in 4 equidistance of each cross-section of seminiferous tubules measured and the mean of these was calculated.

Semen analysis

At 60, 90 and 120 days of age the epididymis was separated carefully from the right testis. The epididymis was divided into 3 segments; head, body and tail. The epididymal tail was trimmed with scissors and placed in 1.0 ml of 0.1 M phosphate buffer of pH 7.4. It was then vigorously shaken for homogeneity and dispersal of sperm cells. Semen samples were assessed for number and gross morphology without the investigator knowing which samples were from which group. A 10 μL aliquot of the epididymal sperm suspension was transferred to each counting chamber of the hemocytometer (Paulmarine, Germany) and allowed to stand for 5 min. The cells which settled during this time were counted by a light microscope at $200\times$ magnification. The sperm heads were counted and expressed as million/ml of suspension (23). The sperm morphology was also determined using eosin-nigrosin staining method. For this purpose 10 μl of 1% eosin Y and nigrosin was added to a

test tube containing 40 μl of sperm suspension and were mixed by gentle agitation. Then, sperm were incubated at room temperature for 45-60 min for staining and then re-suspended with a Pasteur pipette (24). Two hundred sperm per animal were examined microscopically at $40\text{-}100\times$ magnifications, and the number of morphologically abnormal sperm was recorded to give the percent age of abnormal sperm.

Hormone assay

At 60, 90 and 120 days of age serum concentrations of testosterone in offspring were measured by using a diagnostic radioimmunoassay kit from Immunotech, France. For determination of testosterone concentration, blood samples were collected from heart and serum was removed after centrifugation and kept at $-20\text{ }^{\circ}\text{C}$ until analysis. A total of fifty μl of standard, control and serum samples were pipetted into coated tubes labeled with 1 ml of I_{125} . Total testosterone reagent was immediately added to each tube. The tubes were vortexed and placed in water bath at $37\text{ }^{\circ}\text{C}$ for 3 hr. All tubes, except total count tubes, were decanted, aspirated and counted for 1 min in a gamma counter. Double measurements were carried out and the average results were reported in ng/dl.

Statistical analysis

All data were analyzed using SPSS version 10.0 for windows. Testicular parameters in different groups were compared by one-way ANOVA and Tukey's test was used as a *post hoc* test. Differences were considered to be significant when $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Results

Atrophy and degeneration of seminiferous tubules and loss of spermatogenesis in testis of offspring Wistar rats were observed at puberty in all lead-treated, particularly in the highest dose, groups (Figure 1).

The means of testis weight increased significantly in 100 ($P < 0.05$) and 300 ($P < 0.01$) mg/kg/day doses groups from 14 to 28 days of age and decreased significantly from 60 to 90 days of age in 100 mg/kg/day

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($P < 0.05$) and from 60 to 120 days of age in 300 mg/kg/day ($P < 0.01$) doses groups. No statistically significant differences were seen between 20 mg/kg/day dose and the control groups (Figure 2). Significant decreases were seen in the means of testis volume from 7 to 90 days of age in 100 mg/kg/day ($P < 0.05$) and from 7 to 120 days of age in 300 mg/kg/day ($P < 0.01$) doses groups in comparison with the control group, so that these differences were more significant in the highest dose groups (Figure 3).

Statistically significant decreases were found in the means of seminiferous tubule diameter from 14 to 90 days of age in 100 mg/kg/day ($P < 0.05$) and from 7 to 120 days of age in 300 mg/kg/day ($P < 0.01$) doses groups in comparison with the control group, so that these changes were more significant in the highest dose groups (Figure 4). The means of germinal epithelium height decreased

significantly from 28 to 90 days of age in 100 mg/kg/day ($P < 0.05$) and from 28 to 120 days of age in 300 mg/kg/day ($P < 0.01$) doses groups, so that these differences were more significant in the highest dose groups (Figure 5).

Significant decrease was observed in the mean sperm density at 60 and 90 days of age in 100 mg/kg/day ($P < 0.05$) and at 60, 90 and 120 days of age in 300 mg/kg/day ($P < 0.05$) doses groups (Table 1). Also, the mean percentage of morphologically normal sperm decreased significantly ($P < 0.05$) in 300 mg/kg/day dose group from 60 to 120 days of age in comparison with the control group (Table 1). Also, testosterone serum levels reduced significantly at 60 days of age in 100 mg/kg/day ($P < 0.05$) and at 60, 90 and 120 days of age in 300 mg/kg/day ($P < 0.01$) doses groups (Figure 6).

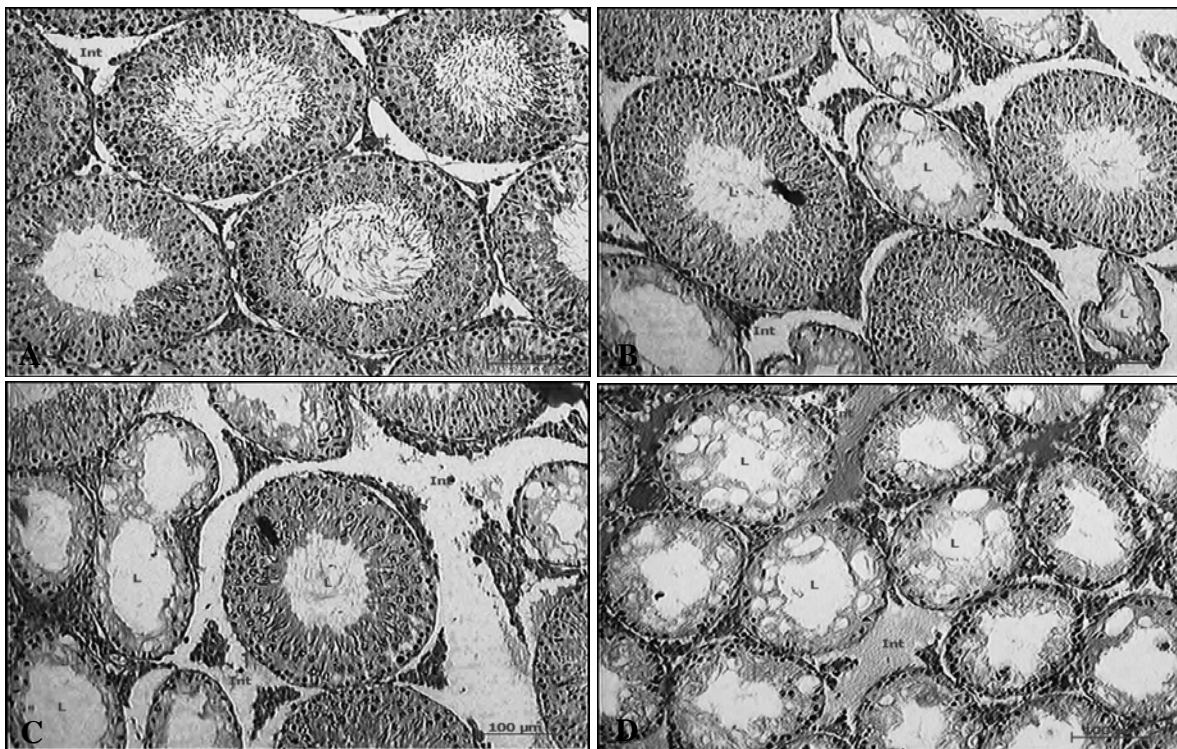


Figure 1. Histological sections of offspring Wistar rats testis at 60 days of age in experiment groups; control (A), 10 (B), 100 (C) and 300 mg/kg/day (D) lead-treated groups (Scale bar: 100 μ m, 350 \times , H&E). Atrophy and degeneration of seminiferous tubules and loss of spermatogenesis, decrease of germinal epithelium height, increase of tubular lumen and presence of vacuoles in germinal epithelium were seen in all treatment, particularly in the highest dose, groups.

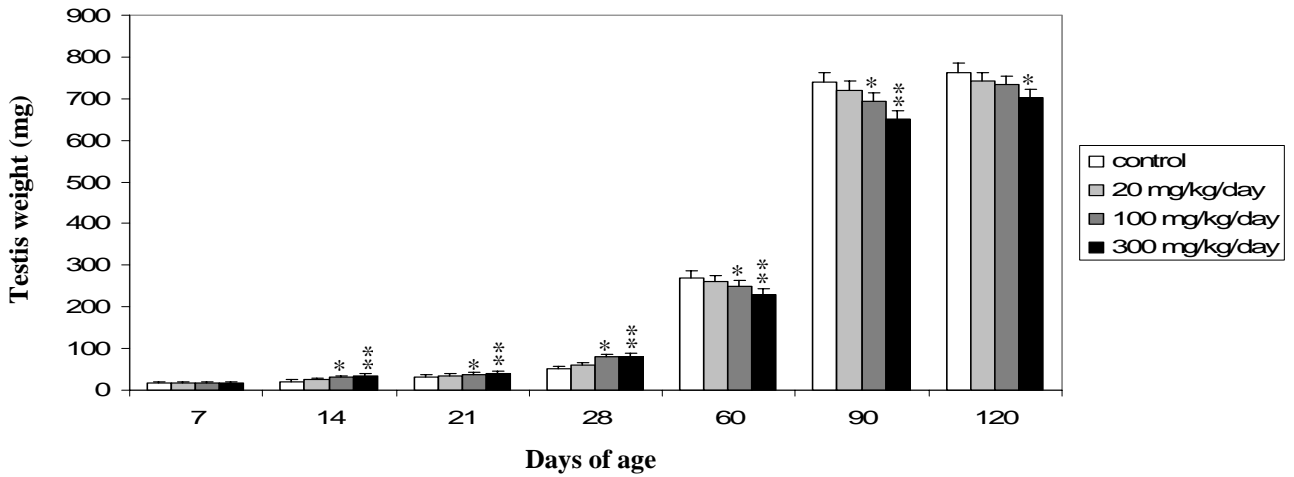


Figure 2. Mean±SEM testis weights (mg) in the control and neonatal lead-treated offspring Wistar rats during different stages of postnatal development.
* $P < 0.05$, ** $P < 0.01$

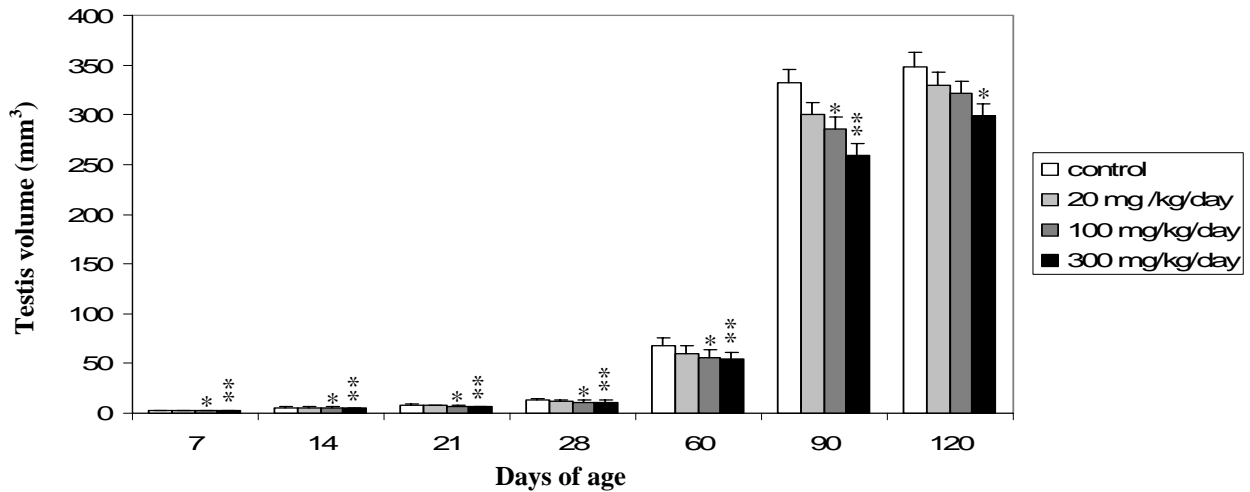


Figure 3. Mean±SEM testis volumes (mm³) in the control and neonatal lead-treated offspring Wistar rats during different stages of postnatal development.
* $P < 0.05$, ** $P < 0.01$

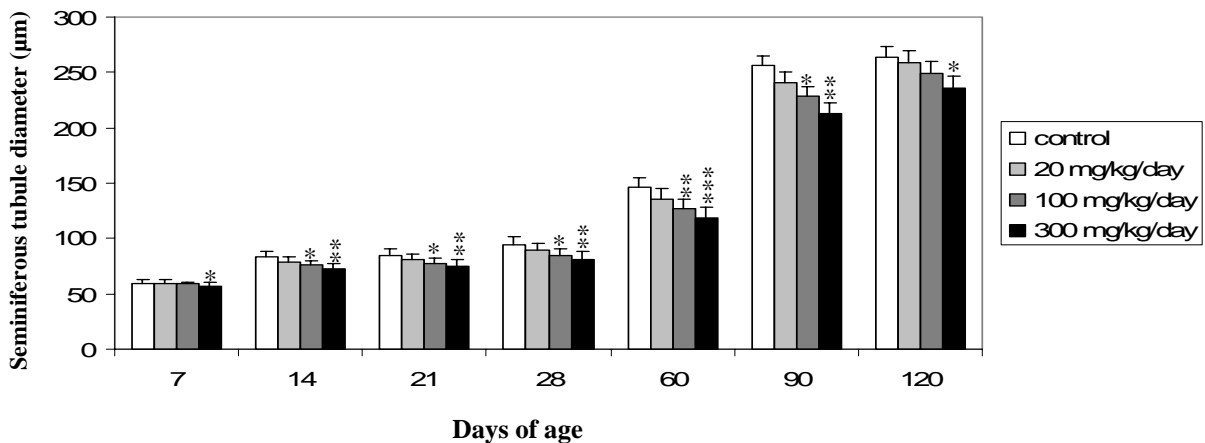


Figure 4. Mean±SEM seminiferous tubules diameter (µm) in the control and neonatal lead-treated offspring Wistar rats during different stages of postnatal development.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

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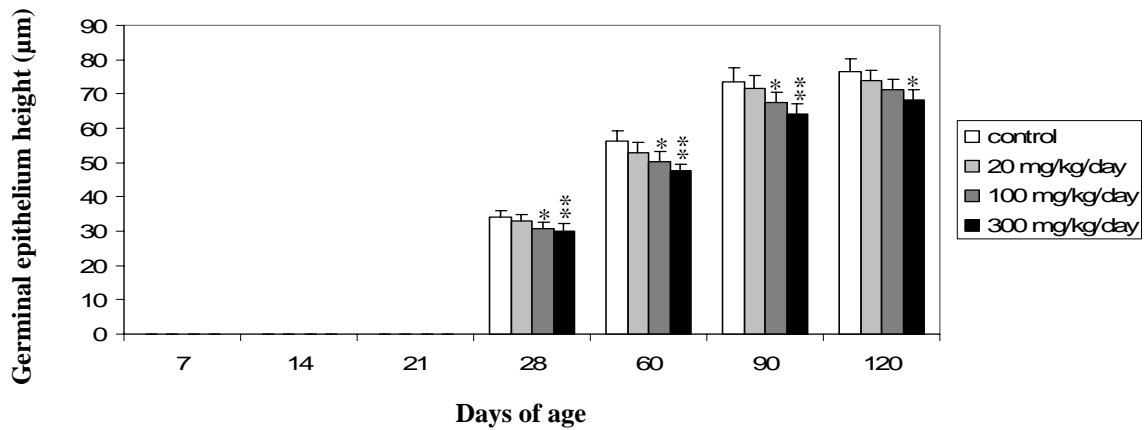


Figure 5. Mean±SEM germinal epithelium height (µm) in the control and neonatal lead-treated offspring Wistar rats during different stages of postnatal development.
* $P < 0.05$, ** $P < 0.01$

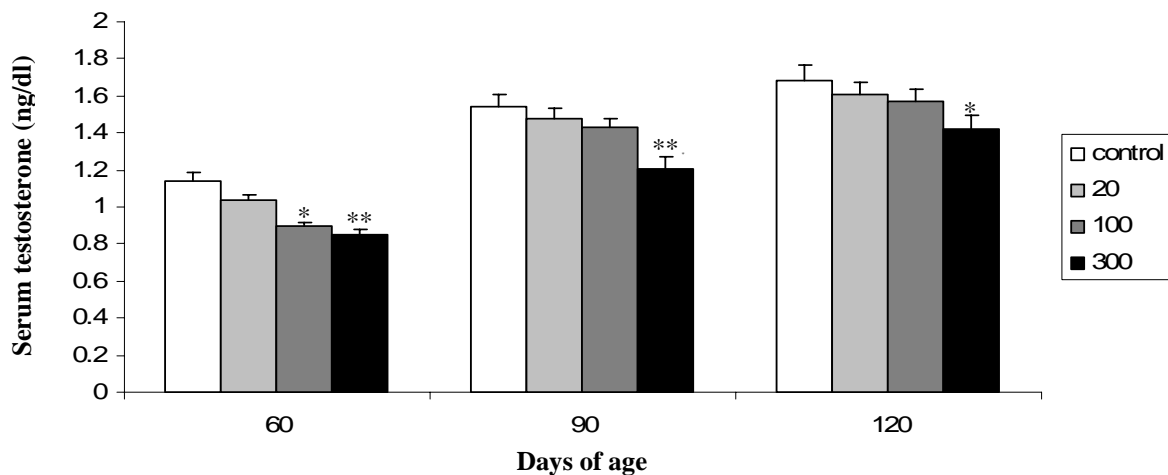


Figure 6. Mean±SEM serum testosterone levels (ng/dl) in the control and neonatal lead-treated offspring Wistar rats during different stages of postnatal development.
* $P < 0.05$, ** $P < 0.01$

Table 1. Mean±SEM sperm density (million/ml) and percentage of sperm with normal morphology (%) in the control and neonatal lead-treated offspring Wistar rats during different stages of postnatal development.

Groups	Days of age	Sperm count (million/ml)	Normal morphology (%)
Control	60	60.10±2.44	79.40±3.17
	90	61.33±2.31	78.09±4.46
	120	63.20±2.28	77.66±4.78
20 mg/kg/day	60	58.31±2.24	75.94±5.05
	90	60.41±2.30	75.71±4.22
	120	60.37±2.25	76.20±4.48
100 mg/kg/day	60	52.52±3.26*	73.31±3.61
	90	54.21±3.10*	72.27±3.50
	120	56.60±3.28	73.61±3.44
300 mg/kg/day	60	48.31±3.15**	62.66±4.45*
	90	50.65±2.31**	64.51±3.11*
	120	52.32±3.21*	67.39±3.40*

*Significant difference between the control and treatment groups.
* $P < 0.05$, ** $P < 0.01$

Discussion

It has long been suggested that at least one half of the cases of the human male infertility of unknown etiology may be attributable to various environmental and occupational exposures (25). The present study was designed to determine short- and long-term developmental effects of maternal exposure to different doses of lead acetate during lactation on testicular structure in offspring Wistar rats. We have found that testicular parameters decrease in offspring Wistar rats following maternal exposures of lead acetate at doses above 100 mg/kg/day. These dose-related changes suggest that the effects of maternal lead exposure are expressed in offspring. The results revealed that weight of testis in 100 and 300 mg/kg/day doses groups increased significantly during early postnatal development from 14 to 28 days of age, that it could be made by lead accumulation in soft tissues such as testis (26), however decreased significantly during late postnatal development from 60 to 120 days of age, that it could be caused by significant atrophy of seminiferous tubules in testis of offspring (27). Testis weight reduced significantly by 8.13% and 14.78% in 100 and 300 mg/kg/day doses groups at 60 days of age, respectively. At 120 days of age, testis weight reduced significantly by 7.89% only in 300 mg/kg/day dose group. Acharya *et al* (2003) showed that testes weights significantly decline in lead-treated mice compared to respective control groups (28). Corpas *et al* (2002) reported that lead ingested by experimental pups, caused decrease in mean testis weight on postnatal days 21 (16). Also, Ahmad *et al* (2003) showed that testis weight decrease in adult albino male in lead-treated group (27). However, Graca, *et al* (2004) reported that lead causes increase in mean testis weight in adult rat (29). Allouche *et al* (2009) found that there were no changes in body weight gain and in absolute or relative weight of testes, epididymidis and seminal vesicles in adult albino Wistar male rats that were given 0.0%, 0.025%, 0.05%, 0.1% and 0.3% lead acetate in distilled drinking water for 24 weeks (30).

Moreover, testis volume decreased significantly in a dose-related manner from 14 to 120 days of age. Testis volume reduced

16.95% and 19.62% in 100 and 300 mg/kg/day doses groups at 60 days of age, respectively. At 120 days of age, mean testis volume reduced significantly (14.09%) only in 300 mg/kg/day dose group. Seminiferous tubule diameter reduced by 12.90% and 18.86% in 100 and 300 mg/kg/day doses groups at 60 days of age, respectively. At 120 days of age, seminiferous tubule diameter reduced significantly by 10.45% only in 300 mg/kg/day dose group. Al-Omar *et al* (2000) reported that lead causes decrease in seminiferous tubules diameter in adult rats (31). Corpas *et al* (2002) showed that lead acetate causes decrease in the diameter and epithelial thickness of rat seminiferous tubules (16). Germinal epithelium height reduced by 10.72% and 15.37% in 100 and 300 mg/kg/day doses groups at 60 days of age, respectively. At 120 days of age, germinal epithelium height reduced significantly by 10.77% only in 300 mg/kg/day dose group. Sperm production reduced by 12.60% and 19.61% in 100 and 300 mg/kg/day doses groups at 60 days of age, respectively. At 120 days of age, sperm production reduced significantly by 12.94% only in 300 mg/kg/day dose group. Percentage of morphologically normal sperm reduced significantly during puberty only in 300 mg/kg/day dose group. Allouche *et al* (2009) found that sperm velocity decreases in all adult albino Wistar male rats given 0.025%, 0.05%, 0.1% and 0.3% lead acetate in distilled drinking water for 24 weeks, while reduction of sperm motility was observed in rats exposed to 0.05%, 0.1% and 0.3% lead acetate without statistically significant differences compared to the control group. However, there was a significant increase greater than 100% in the total percentage of abnormal sperm in groups treated with 0.1% ($P < 0.01$) and 0.3% lead acetate ($P < 0.05$) (27).

Our quantitative results indicate that the statistically significant reductions in testis volume, seminiferous tubule diameter and germinal epithelium height observed in lead-treated groups are dose-related and highest at 60 days of age. It has been shown that lead acetate intoxication during spermatogenesis can delay spermiation as well as release of immature spermatogenic cells in the tubules of testes (16). Furthermore, lead could be

concentrated in the nucleus and perturbs cell proliferation and DNA synthesis *in vivo*, so treatment could affect germinal cells, during pre- and early postnatal development, when prospermatogonia undergo mitosis or when Sertoli and Leydig cells appear (32). Sokol *et al* (1985) reported that dietary exposure to lead results in a dose-related suppression of spermatogenesis and serum testosterone levels in the young adult male rats (33). Moorman *et al* (1998) demonstrated a dose-dependent inhibition of spermiation in mature male rabbits that were given lead acetate by subcutaneous injection in the dose range of 0 to 3.85 mg/kg on a Monday-Wednesday-Friday basis (34).

Moreover, our findings show that degrees of reductions in testis volume, seminiferous tubules diameter and germinal epithelium height increase from early weeks to 60 days of age, nearly by the onset of puberty, but decrease afterward, so it seems that lead has a transient effects and testicular parameters become better gradually until 120 days of age. However, the present study shows that early neonatal life comprises a sensitive period for induction of permanent adverse effects by lead acetate on testicular parameters of the male offspring rats, so that their reproductive efficiency reduces at puberty. The reversibility of the toxic effects of lead was demonstrated on the hypothalamic-pituitary-testicular axis in prepubertal and pubertal male Wistar rats (35). Sokol and Berman (1991) indicated that prepubertal rats may be less sensitive to the toxic effects of lead than rats whose exposure begins after puberty has been initiated (36).

Serum testosterone levels were suppressed significantly at puberty in 100 and 300 mg/kg/day doses groups. Continuous

intrauterine lead exposure of male rodents also showed harmful effects on male sexual maturity and reduced neonatal sex steroid levels (37). Sokol (1989) reported that serum testosterone, intratesticular sperm counts, and sperm production rates in male Wistar rats, 27 days and 52 days old, that were given *ad libitum* access to 0.0% or 0.6% lead acetate containing water for 30 days, were suppressed significantly ($P < 0.001$) in the lead-treated groups (35). However, in addition to the effects of lead on hormone levels, Assennato *et al* (1987) describe a reduction in sperm concentration by a direct, non-hormonal effect, in sperm production or transport (38). Also, Murthy (1991) has been shown a direct effect of lead on the testes in rodents (39). Moreover, significant ($P < 0.01$) increase in serum testosterone level of adult albino Wistar male rats, that were given lead acetate for 24 weeks, was noted in animals administered 0.05% lead acetate in drinking water. Allouche *et al* (2009) reported that the increase of testosterone level observed in adult albino Wistar male rats suggests that lead may target testicular function (30).

Conclusion

Consequently, the present study indicates that maternal lead acetate exposure during neonatal period has dose-related and long-term effects on postnatal development of testis in offspring Wistar rats and reduces their reproductive efficiency at puberty.

Acknowledgment

The authors wish to thank the Vice Chancellor for Research of Shahid Chamran University, Ahwaz, Iran for the research grant.

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