JJBS

Volume 16, Number 2,June 2023
ISSN 1995-6673
Pages 223 – 231
https://doi.org/10.54319/jjbs/160206

Jordan Journal of Biological Sciences

Impact of Maternal Exposure to Lead Acetate before Pregnancy Through Lactation Period on the Testicular histomorphologic Indices of Male Offspring of Wistar Rats, A Stereological Study

Ehsan Roomiani[1] , Hassan Morovvati[2,*], Saeid Keshtkar[3], Sareh Najaf Asaadi[4], Shaker Shayestehnia[5] and Arash alaeddini[6]

[1]PhD, Graduate of Comparative Histology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [2] Professor in Anatomical Sciences, Department of Basic Sciences, Faculty of Veterinary, University of Tehran, Iran; [3] PhD, Graduate of Food industry biotechnology, Kaliningrad state university, Kaliningrad, Russia; [4] PhD, Graduate of Comparative Histology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [5] DVM, Department of Veterinary, Faculty of Veterinary Medicine, Islamic Azad University, Iran, Karaj; [6] PhD student of Veterinary Microbiology, Department of Basic Sciences and Health, Faculty of Veterinary, Islamic Azad University of Tehran, Science and Research Branch, Tehran, Iran.

Received: August 11, 2022; Revised: October 19, 2022; Accepted: November 23, 2022

Abstract

Exposure to heavy metals in the products of various industries and the environment is a permanent threat to humans. Much research had shown the adverse effects of lead acetate even at low doses. The present study was conducted to investigate the effect of lead acetate on testicular histomorphologic indices of male offspring of female Wistar rats that were exposed to lead during pre-pregnancy, pregnancy and lactation periods. After adaption, rats were divided into different cages for mating (1 male to 2 females). After diagnosing pregnancy, rats were divided into three groups: pregnancy, lactation, and pregnancy until the end of lactation (PL). During the mentioned periods, 0.2% lead acetate plus 500 µl of glacial acetic acid were available in drinking water. Female rats belonging to the pre-pregnancy group had access to the mentioned compound for 3 weeks before mating. Also, females belonging to the PrePregnancy to the end of Lactation. had access to the mentioned compound for 3 weeks before pregnancy to the end of lactation. Offspring of control and sham groups also had access to drinking water and 500 µl of glacial acetic acid in drinking water, for survey of deposition of lead acetae, from the beginning to the end of the experimental period (63 days), respectively. At the end of the experiment (postnatal day: 63), adults were sacrificed and their left testicles were fixed in 10% formalin. In order to perform morphometric studies, testicular tissue was randomly divided into equal sections, and for hematoxylin-eosin staining routine tissue preparation steps were performed. Finally, the prepared slides were used for stereological examinations.

The results showed that exposure to 0.2% lead acetate had significant reduction on the volumetric density of the seminiferous tubules, the volumetric density of the epithelium of the tubules, the volumetric density of the interstitial tissue and the height of the germinal epithelium of the tubules of experimental groups compared to the control and sham groups. It should be noted that exposure to 0.2% lead acetate, despite the decreasing effects, did not cause a significant change in the length of the tubules and the epithelial surface area of these tubules. We conclude that lead, in the amount of 0.2%, has different effects on the tissue structure of testes of male offspring. Therefore, due to the increasing development of various technologies and industries that are sources of production of this harmful compound, the need to protect the mother and fetus against its adverse effects are felt more than ever.

Keywords: Stereology, Rat testis, PrePregnancy to lactation periods, Lead acetate

* Corresponding author. e-mail: hmorovvati@ut.ac.ir.

1. Introduction

The testicles are the main and important organ of the male reproductive system and are responsible for the production of sex cells (Treuting *et al*., 2017). The activity and steps of spermatogenesis are easily affected by pollution. Therefore, in toxicological and pathological studies, the toxic effects of heavy metals on the spermatogenesis are very important (Morais *et al*., 2012). Existing reports of reduced male fertility point to the role of exposure to toxic and environmental factors in the etiology of human infertility (Benoff *et al*., 2000). Elgawish and Abdel Razak (2014) have stated that lead acetate is the most abundant toxic metal substance in nature. According to Restanty *et al.* (2018) lead acetate is one of the most important heavy metals clinically, and leads to physiological, biochemical, and behavioral disorders. The reproductive system (male and female) is the target tissue of lead exposure. BaSalamah *et al.* (2018) also reported that lead is an unnecessary element that can cause numerous health problems through environmental pollution.

Toxicological studies have shown that lead has negative effects on the nervous system (CNS & PNS), cardiovascular, endocrine, immune and digestive system, blood tissue, urinary system, and male and female reproductive system. It also causes chromosomal abnormalities (Altunkaynak *et al.*, 2013; Restanty *et al.*, 2018). For example, lead can cross the blood-testicular barrier and affect the testes and other accessory organs (Fair and Ricklefs, 2002; Snoeijs *et al.*, 2004). Accumulation of lead in the testes and epididymis affects sexual germ cell differentiation (Apostoli *et al.*, 1999). Lead is also an estrogenic compound that may affect fetal development by crossing the blood-placental barrier (Goyer, 1990; Baghurst *et al.*, 1991; Taupeau *et al*., 2001).

In the present study, an effort was made to evaluate the morphometric characteristics of testicular tissue in male offspring of wistar rats maternally exposed to lead acetate using stereology. Stereology is a set of mathematical laws that interprets two-dimensional information in three dimensions using mathematical principles and statistics and geometry and provide information about the quantity of structures (Nyengaard, 1999; Dehoff, 2000). Stereology provides a set of efficient, unbiased, or minimally biased tools for quantifying functional aspects of three-dimensional morphology. These values include volume, surface area, length, and number, and can be estimated for different levels of structure, from the entire organ to the cell and molecule (Brown, 2017).

Accordingly, due to the complex mechanisms of sperm production (spermatogenesis) and how the testicle develops, the effects of lead on it are probable. So, in this study, with the help of the stereology technique (Boyce *et al.*, 2010; West, 2012), the effect of lead administration in pre-pregnancy to lactation periods on the morphometric characteristics of the testes of male offspring of Wistar rats were examined. These characteristics were: the volumetric density of seminiferous tubules, the volumetric density of tubular epithelium, the volumetric density Interstitial tissue, the surface area and height of germinal epithelium, and the length of seminiferous tubules of testis.

2. Materials and Methods

2.1. Animals

Fourty-two adult Wistar rats (male and female) with 200-210 g average weight, were purchased from the Pasteur Institute, Iran and kept in the embryology laboratory under conditions of 12 hours of light, a temperature of 20-26 °C and adequate water and food. The study was done in accordance with the standard protocol for the care and use of laboratory animals (Faculty of veterinary medicine, University of Tehran, Tehran, Iran. N. 6067543). After adaptation (one week), male and female rats were considered in each cage (1 male to 2 females) for mating. After 12 hours, vaginal plaque was examined for pregnancy and the pregnant rats were randomly distributed into 5 different groups (6 rats in each group) in addition to the pre-pregnancy and the prepregnancy to the end of lactation groups that received lead acetate plus acetic acid 21 days before mating (overall 7 groups).

2.2. Experimental design:

Control (Cont.): animals had access to drinking water and adequate food during the experimental period.

Sham group: animals had access to acetic acid (glacial, 0.05%) in drinking water, for survey of lead acetate deposition, during the experimental period.

Pre-pregnancy (PrePreg.): animals during the pre-pregnancy period (21 days) had access to a combination of lead acetate (0.2%) and acetic acid (glacial, 0.05%) in drinking water.

Pregnancy (Preg.): animals during the pregnancy period (21 days) had access to a combination of lead acetate (0.2%) and acetic acid (glacial, 0.05%) in drinking water.

Lactation (Lac.): animals had access to lead acetate (0.2%) and acetic acid (glacial, 0.05%) in drinking water during lactation (21 days).

Pregnancy_lactation (Preg.Lac.): animals during pregnancy and lactation (42 days) had access to a combination of 0.2% lead acetate and 0.05% glacial acetic acid in drinking water.

Pre-pregnancy_pregnancy_lactation (PrePreg.Preg.Lac.): animals had access to a combination of lead acetate (0.2%) and acetic acid (glacial, 0.05%) in drinking water during the pre-pregnancy, pregnancy, and lactation period (63 days).

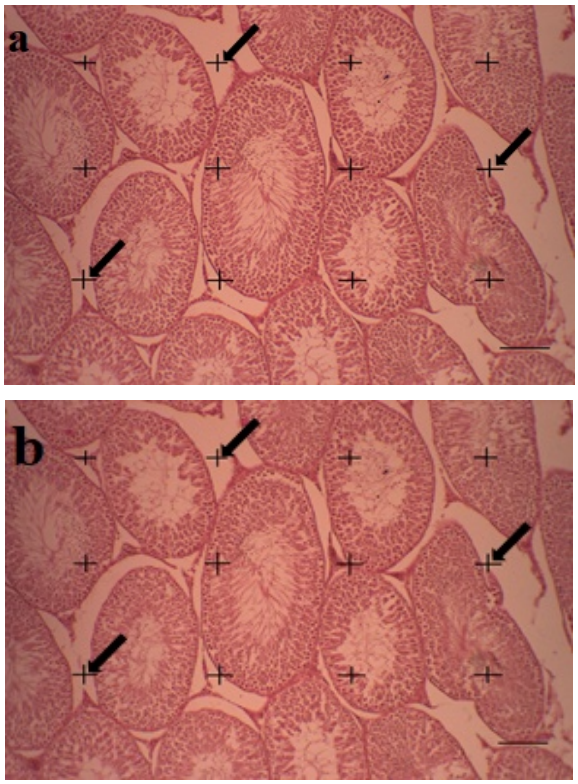
All experimental animals, after the mentioned period, except control group, had access to a combination of 0.05% glacial acetic acid in drinking water to the end of the study. Lead was administered as a 0.2% lead-acetate in drinking water (Barkur and Bairy, 2016). Then, to prevent lead-acetate deposition, 0.05% glacial acetic acid was added to it (Jaco-Movits *et al.*, 2005; Heidmets *et al.*, 2006; Barkur and Bairy, 2016). No deaths were recorded during the experimental period. Also, water c-nsumption was checked daily, and no rejection of water was observed.

2.3. Sampling and Quantitative survey:

To evaluate the stereological features of the testes, samples were taken from the 5 male offspring (left testicle) on day 63 (postnatal day: 63). After fixation of the samples in 10% formalin, sampling was done by the isotropic

random uniform method. Briefly, the testes were first embedded in agar and then placed on the first circle, which consisted of 10 equal parts. In the next step, a random number between 0 and 10 was selected, and the sample in the direction of the selected number was divided into two equal parts. The incision surface of both halves of the testis was placed on the second circle, which also had 10 equal parts. Then, a random number was selected (between 0 - 10) and the samples were cut in the direction of the selected number so that 8 to 10 equal pieces were obtained from each testicle (Sadeghinezhad *et al*., 2021). Samples were prepared for microscopic study. The samples were then embedded in paraffin and 5 µm thickness sections were prepared for Hematoxylin-eosin staining. To perform stereological examinations, images of testicular tissue sections were taken by using of CX40 Jenus (China) light microscope connected to Is1000 Jenus (China) camera. Then, the images were analyzed using Image j software and dedicated plugins for stereology.

The following equation was used to calculate the



absolute volume of the testis (Howard and Reed, 2005):

$$V = \frac{M}{\delta}$$

V: Total structure (testis) volume

M: Testicular weight

d: Testicular density

The following formulas were used to calculate the volumetric parameters of the testis, including the volumetric density of the seminiferous tubules, the volumetric density of the interstitial tissue, and the volumetric density of the germinal layer by point grades (Gundersen *et al*., 1988) (Fig. 1):

$$V_v \text{ (structure)} = \frac{\sum P \text{ structure}}{\sum P \text{ testis}}$$

V (structure)= V_v (structure) × V (testis)

Vv: Volumetric density of the structure

ΣP structure: The sum of the points of collision with structure

ΣP testis: The sum of the points of impact on the testis

Figure 1. A point grid for calculating the volumetric density of the seminiferous tubules - the volumetric density of the epithelium of the tubules and the volumetric density of the interstitial tissue in the testes of 63-day-old male offspring rats. The arrow signs (at the top right of each point) indicate the count of that point (hematoxylin-eosin, scale; 50 µm).

The following equation was used to calculate the length of testicular seminiferous tubules (Howard and Reed, 2005) (Fig. 2).

$$Lv = 2 \times \frac{\Sigma Q}{\Sigma(\text{frame}) \times a(\text{frame})}$$

Lv: Density of length of seminiferous tubules

ΣQ: Number of profiles counted

Σ (frame): The number of frames counted

a: The area of the frame surface

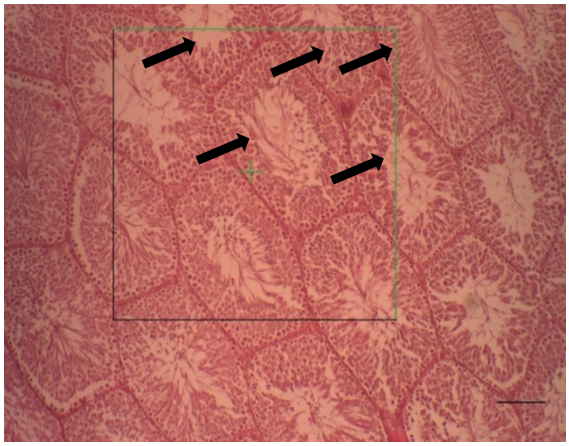


Figure 2. Counting the number of seminiferous tubules (arrow sign) to calculate the length of tubules in the testes of 63-day-old male offspring rats, hematoxylin-eosin, scale; 50µm)

The surface area of the germinal epithelium of tubules was also estimated using a linear probe by the following equation (Howard and Reed, 2005) (Fig. 3).

$$S_v = \frac{2 \times \Sigma l}{l/p \times \Sigma P}$$

$S = S_v \times V_{Germinal\ layer}$

Sv: The surface density of the germinal epithelium of the seminiferous tubules

Σl: The sum of the points of collision of the probe with the edge of the germinal epithelium

l / p: linear probe length per grid point

ΣP: Number of points of contact with the germinal epithelium

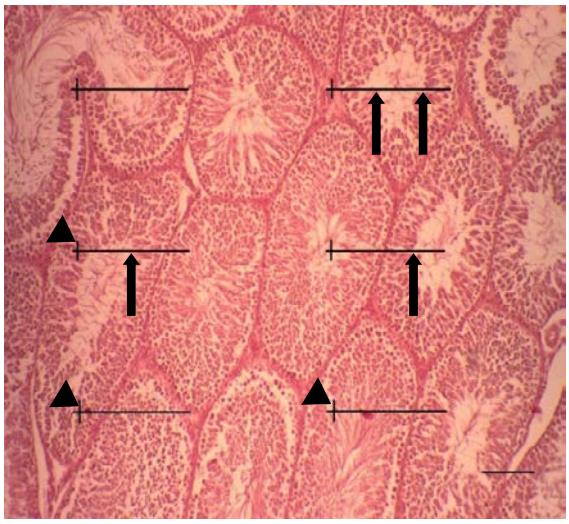


Figure 3. A linear probe to calculate the surface area of the germinal epithelium of tubules of a 63-day-old male offspring rats. The arrow mark indicates countable points for calculating the points of collision at the edge of the germinal epithelium (Σl) and the arrowhead indicates countable points for calculating the point of impact on the germinal epithelium (ΣP) (hematoxylin-eosin, scale; 50 µm).

The height of the germinal epithelium was calculated from the following formula (Nyengaard, 1999).

$$H = \frac{Vv\ (Germinal\ layer)}{Sv\ (Germinal\ layer)}$$

Vv: The density of the germinal epithelium

Sv: surface density of germinal epithelium

2.4. Statistical analysis

Data were analyzed using SPSS software version 22 and expressed as mean ± standard deviation. The normal distribution of data was evaluated using the Kolmogorov-Smirnov test, and comparison between groups was performed by one-way ANOVA and P<0.05 was regarded as a significant level.

3. Results

3.1. Absolute weight and volume of the testis

Statistically, for testicular weight index, a significant increase was observed in control and sham groups compared with pregnancy, lactation, and pregnancy-lactation groups (P <0.05). Among the experimental groups, the means obtained from the pre-pregnancy group was significantly increased compared with the means obtained from the lactation and pregnancy-lactation groups (P <0.05). Finally, the mean of the samples of the pregnancy-lactation group was significantly increased from the mean obtained for the pre-pregnancy-pregnancy-lactation group (P <0.05) (fig. 4). Regarding the absolute volume of the testis, the obtained results were similar to the mentioned results for the testicular weight (fig. 5)

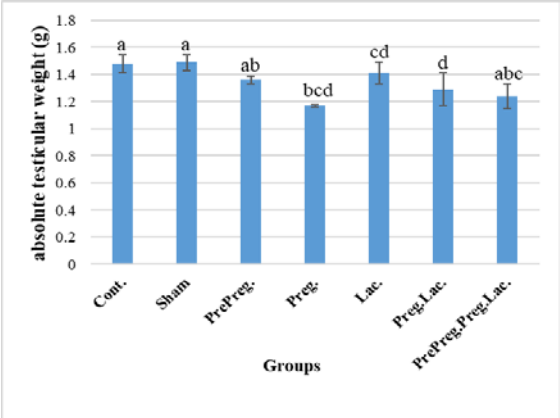


Figure 4.Indicates the results of data on the absolute weight of the testis of 63-day-old male offspring rats exposed to 0.2% lead acetate orally. The results are expressed as Mean ± Std. Heterogeneous letters indicate a significant difference.

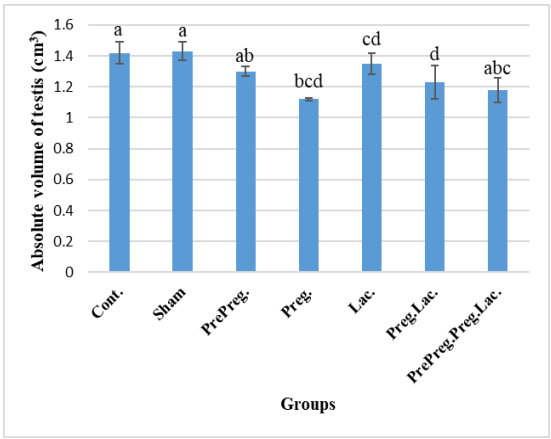


Figure 5, Indicates the results of data on the absolute volume of the testis of 63-day-old male offspring rats exposed to 0.2% lead acetate orally. The results are expressed as Mean ± Std. Heterogeneous letters indicate a significant difference.

3.2. The volume of seminiferous tubules, epithelium of tubules, interstitial tissue, and length of seminiferous tubules

A comparison of the means related to the volume of seminiferous tubules and the volume of epithelium showed that there was a significant difference, increase, between control and sham groups with all experimental groups except the lactation group. Between experimental groups, a significant decrease was recorded in pregnancy group than lactating group (P <0.05) (fig. 6 & 7). The interstitial tissue volume in the testis of the experimental male rat offspring showed only a significant increase in pre-pregnancy group obtained means compared with the control and sham groups (P <0.05) (fig. 8). Comparison of data related to the length of seminiferous tubules in the

testes of 63-day-old offspring of the studied rats did not show a significant difference (P> 0.05) (fig. 9).

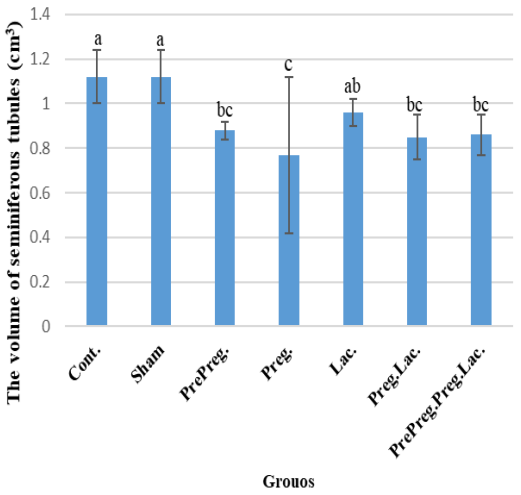


Figure 6. Indicates the results of data related to the volume of seminiferous tubules in the testes of 63-day-old male offspring rats exposed to 0.2% lead acetate orally. The results are expressed as Mean ± Std. Heterogeneous letters indicate a significant difference.

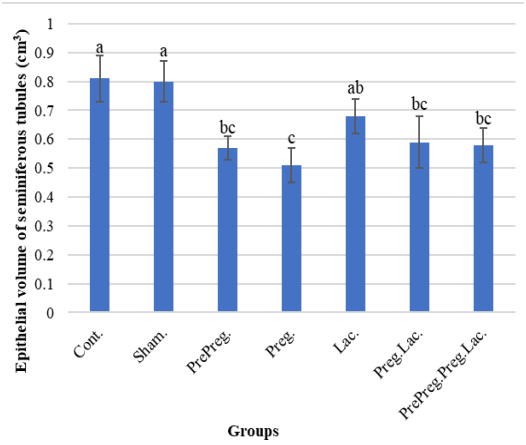
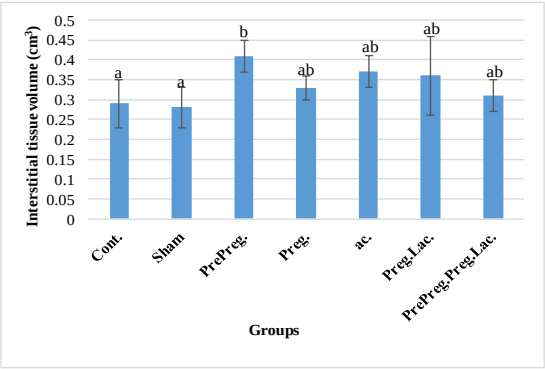


Figure 7. Indicates the results of data on the epithelial volume of the seminiferous tubules in the testis of 63-day-old male offspring rats exposed to 0.2% lead acetate orally. The results are expressed as Mean ± Std. Heterogeneous letters indicate a significant difference.

Figure 8. Indicates the results of data on the interstitial tissue



volume of the testis of 63-day-old male offspring rats exposed to 0.2% lead acetate orally. The results are expressed as Mean ± Std. Heterogeneous letters indicate a significant difference.

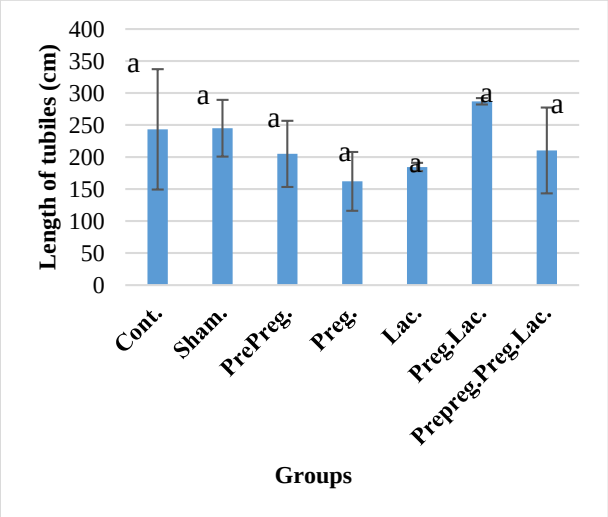


Figure 9. Indicates the results of data related to the length of seminiferous tubules in the testes of 63-day-old male offspring rats exposed to 0.2% lead acetate orally. The results are expressed as Mean ± Std. There was no significant different.

3.3. The surface area of the epithelium of the seminiferous tubules and the height of the germinal epithelium

There was no significant difference in comparison of the means from different groups in the surface area of the seminiferous tubules epithelium of the testis of the experimental animals (P> 0.05) (fig. 10). Statistically, the height of the seminiferous tubules germinal epithelium of the testes showed a significant decrease in the pregnancy, lactation, pregnancy-lactation and pre-pregnancy-pregnancy-lactation groups compared with the control group (P <0.05) (fig. 11).

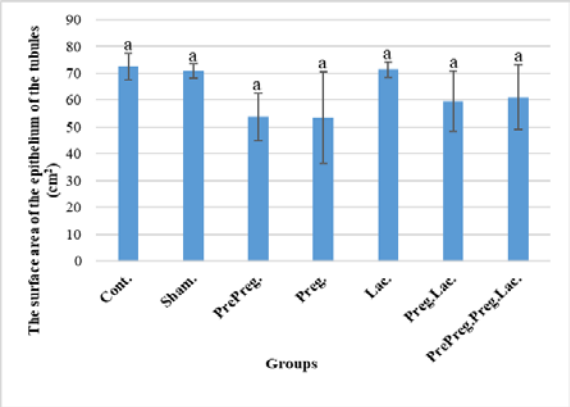


Figure 10. Indicates the results related to the surface area of the germinal epithelium of the seminiferous tubules of 63-day-old male offspring rats exposed to 0.2% lead acetate orally. The results are expressed as Mean ± Std. There was no significant different.

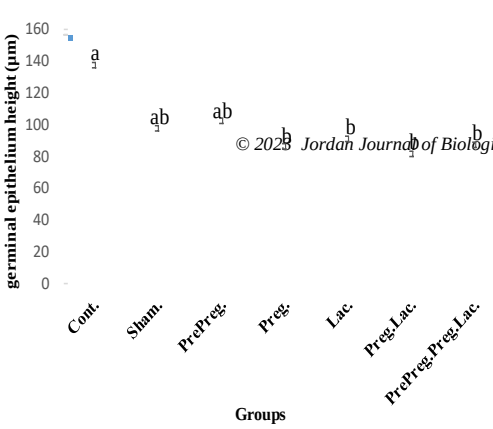
Figure 11. Indicates the results related to the height of the germinal epithelhium of the seminiferous tubules of 63-day-old male offspring rats exposed to 0.2% lead acetate orally. The results are expressed as Mean ± Std. Heterogeneous letters indicate a significant difference.

4. Discussion

Exposure to lead, causes several organs dysfunction including reproductive disorders. If not controlled, lead as an important environmental pollutant can cause serious damage. Recently, lead exposure has been highlighted as an important cause of testicular dysfunction and male infertility. Excessive exposure to lead derivatives reduces sperm quality and reproductive capacity and ultimately causes male infertility (El-Khadragy *et al*., 2020).

In the present study, statistically, for the absolute weight and volume of testes in 63-day-old offspring, we saw a significant decrease between the means of the pregnancy, lactation, and pregnancy-lactation groups compared to control and sham groups (P <0.05). Among the experimental groups, the mean obtained from the pre-pregnancy group showed a significant decrease compared to the means obtained from the lactation and pregnancy-lactation groups (P <0.05). Also, the mean from the pregnancy-lactation group showed a significant decrease compared to the mean obtained for the pre-pregnancy-pregnancy-lactation group (P <0.05). In this regard, Assi *et al*, (2016) in their study stated that lead acetate (10 mg/kg/oral and daily) caused a significant reduction in testicular weight in the Sprague Dawley rats. Other researchers such as Allouche *et al*, (2009) and Elgawish and Abdelrazek (2014) have also shown that different doses of lead acetate induce testicular weight and degenerative testicular changes. Dadkhah *et al.* (2017), in their study, stated that lead acetate caused a decrease in testicular weight in the studied rats, although this reduction was not significant. They mentioned the reason for this decrease as the decrease in germ cells and sperm cells.

Hassan *et al.*, (2019) and Offor *et al.* (2019) reported significant reductions in testicular weight and relative testicular weight during exposure of albino rats to lead acetate, respectively. In another study, Udefa *et al.* (2020) reported a significant reduction in absolute testicular weight of rats exposed to lead acetate. Dorostghoal *et al.* (2011) considered significant testicular weight loss due to high atrophy of the seminiferous tubules. It has been shown that the weight of reproductive organs is used as a basic criterion in toxicologicalstudies (Crissman *et al.*, 2004; Yavasoglu *et al.*, 2008). Mruk and Cheng (2004) have stated that testicular weight is highly dependent on spermatogenic cell mass and that testicular weight loss may be due to impaired spermatogenesis.



Dorostghoal *et al.* (2011) reported a significant decrease in testicular volume in the offspring of Wistar rats following maternal exposure to lead acetate. The results of the present study on the change in absolute testicular volume in 63-day-old rat offspring following maternal exposure to lead acetate are consistent with the results of their study. It seems that anatomical changes in the testis, such as reduction in size and volume, depending on the amount of compound and the duration of exposure, occur due to the production of free radicals in spermatogenic cells and the consequent destruction of these cells. According to Sadeghinezhad *et al.* (2021), there is a positive correlation between testicular volume and the number of spermatogenic cells.

In the present study, comparing the means of the volume of seminiferous tubules and epithelial volume of these tubules showed that there was a significant difference only between control and sham groups with all experimental groups except lactation group, and pregnancy group with lactation group ($P < 0.05$). The volume of interstitial testicular tissue was significantly different ($P < 0.05$) in the control and sham groups compared with the pre-pregnancy group. These significant differences were recorded, while for the volume of seminiferous tubules the volume of the epithelium of these tubules, the volume of interstitial testicular tissue, and the means obtained from all experimental groups showed a decrease compared to the means of the control and sham groups.

In the study of Khodabandeh *et al.* (2021), no significant decrease in the volume of seminiferous tubules following exposure to lead acetate was reported. However, Asadpour *et al.* (2013) reported a decrease in the volume of seminiferous tubules in rats exposed to lead acetate. The results of the present study are in line with the results of their study that this volume reduction may be due to the shrinkage phenomenon in the seminiferous tubules. Sadeghinezhad *et al.* (2021) also considered the decrease in the volume of seminiferous tubules as the reason for the decrease in the number of germ cells due to apoptosis in these cells during the inhibition of calcium pumps in the endoplasmic reticulum.

Khodabandeh *et al.* (2021) stated that lead acetate did not have a significant effect on reducing the epithelial volume of seminiferous tubules. Asadpour *et al.* (2013) also reported a decrease in germ epithelial volume in rats exposed to lead acetate. The results of the present study are in line with the results of the above studies. In another study, Bouazza *et al.* (2018) stated that lead acetate created empty spaces between germ cells. Aftab and Abdul Rauf, (2014) attributed the vacancy between the germ cells to the Sertoli cell damage, which is caused by genotoxic and antiproliferative effects of compounds such as lead acetate. Kumar and Devi, (2018) in a review study stated that exposure to lead acetate destroys the structure of the epithelium and disrupts the process of spermatogenesis. Hassan *et al.* (2019) also reported the loss of reproductive epithelial cells during exposure of albino rats to lead acetate. According to Oboma *et al.* (2018), lead has a cytotoxic effect on sertoli cells, secreting the sex hormone testosterone, and also inhibiting the expression of specific enzymes involved in the production of steroid hormones. They also stated that lead acetate causes hypertrophy of the seminiferous tubules and dilation of interstitial tissue, which in turn impairs the growth of leydig cells. According to their report, lead poisoning during spermatogenesis can decline sperm count as well as the release of immature spermatogenic cells into the seminiferous tubules. Also, lead acetate can accumulate in the cell nucleus and disrupt the process of cell proliferation and DNA synthesis in vivo. Thus, lead treatment can affect germ cells during the prenatal and postnatal periods, when pro-spermatogonia cells undergo mitosis or Leydig cells develop.

Akinola *et al.* (2015) reported that the interstitial connective tissue of the seminiferous tubules of rats did not change when exposed to an aqueous solution of 2.5% lead-acetate. BaSalamah *et al.* (2018) also reported interstitial tissue change (interstitial necrosis) in their study, using 1000 mg of lead acetate per liter of drinking water for four weeks in rats. In another study, Khodabandeh *et al.* (2021) stated that the volume of interstitial tissue in lead-treated animals increased compared to the control group, but the number of Leydig cells did not change, so it appears that this increase does not depend on the number of Leydig cells. Massanyi *et al.* (2007) have stated that lead causes dilation of capillaries in interstitial testicular tissue. Therefore, the increase in interstitial tissue volume in the present study may be due to changes in interstitial tissue components, especially dilation of blood vessels.

In the present study, it was found that maternal exposure to lead acetate during prenatal and lactation periods reduced the length of seminiferous tubules compared with the control and sham groups. Of course, this decrease was not statistically significant. The results of the present study on changes in the length of seminiferous tubules of rat testes were consistent with the results of Khodabandeh *et al.* (2021) who reported that the length of seminiferous tubules in the lead-acetate group decreased compared to the control group, but this decrease was not significant. In justifying the changes in the length of the tubules, it can be said that according to Mustafa (2015), shrinkage of the seminiferous tubules in rats treated with lead acetate is due to contractions of myoid cells. Also, according to Franca and Russell, (1998), three factors, testicular size, the diameter of seminiferous tubules, and the volumetric density of these tubules affect the length of seminiferous tubules. In the present study, it was found that testicular size and volumetric density of seminiferous tubules in experimental groups decreased compared to control and sham groups.

In the present study, despite the decrease in the statistical analysis of data obtained from all experimental groups, except the lactation group, there was no observed significant difference between the results of the studied groups for the surface area of the tubular epithelium. Despite the statistical decrease in the results of the data analysis, the height of the germinal epithelium did not

show a significant difference compared to the control and sham groups. Akinola *et al.* (2015) reported that the thickness of the epithelium of the seminiferous tubules of rats decreased significantly compared to the control group when exposed to an aqueous solution of 2.5% lead acetate. Zhang *et al*. (2014) stated that lead can bind directly to DNA through four binding sites and form the Pb-DNA complex, thereby damaging the DNA double helix structure. Therefore, the loss of germ cells that reduced the diameter of seminiferous tubules and germinal epithelium in adult rats in their study could be due to the genotoxic effect of lead on germ cell DNA and finally the death of these cells. In addition to the ability of lead to directly damage the DNA of germ cells, it can weaken the antioxidant system in the rat testis by increasing the production of free radicals. The results of the present study and related studies support the role of oxidative damage to germ cells due to lead exposure, and may also support thinning of the germinal epithelium of the seminiferous tubules in our study.

Asad *et al.* (2019) stated that lead acetate caused a significant decrease in the height of the reproductive epithelium compared to the control group. BaSalamah *et al.* (2018) in their study reported atrophy of the seminiferous tubules and destruction of germ cells and sertoli cells in the testis while exposed to rat lead acetate. In another study, Bouazza *et al.* (2018) stated that lead acetate reduced the height of the germ epithelium and created empty spaces between the germ cells. Dorostghoal *et al.* (2011) also reported a significant dose-dependent decrease in the height of the germinal epithelium during the neonatal, pre-pubertal, and post-pubertal periods of rats exposed to lead acetate. Udefa *et al.* (2020) attributed the significant decrease in the height of the reproductive epithelium of the seminiferous tubules to rats exposed to lead acetate due to oxidative stress and apoptosis due to lead acetate exposure. According to them, the decrease in the height of the germinal epithelium indicates the cessation of spermatogenesis in the spermatocyte stage while exposed to lead acetate.

5. Conclusion

It has been found that even in low amounts, lead acetate causes problems in different organs of the body, especially in both male and female reproductive system. The results of this study confirmed the results of previous studies about adverse effects of lead acetate on male reproductive system. The adverse effects of maternal exposure to lead acetate on the morphometric parameters of male offspring rats in this study confirm not only a part of wide range spectrum of adverse effects of this compound on the male reproductive system but also make it necessary to take important decisions to protect the mother and the fetus from exposure to heavy metals, especially lead acetate.

References

Aftab A, and Abdul Rauf S. 2014. Anti-proliferative and Genotoxic Effects of Arsenic and Lead on Human Cells in vitro. *Toxicol Environ Health Sci.* **6**: 148-154.

Akinola O B, Oyewopo A O, Aremu Sh A, Afolayan S T, Sanni G O and Biliaminu S A. 2015. Testicular histomorphometry and semen quality of adult Wistar rats following juvenile oral lead intoxication. *Eur J Anat.* **19(1)**: 65-72.

Allouche L, Hamadouche M and Touabti A. 2009. Chronic effects of low lead levels on sperm quality, gonadotropins and testosterone in albino rats. *Exp. Toxicol. Pathol.* **61**:503–510

Altunkaynak M E, Akgul N, Yahyazadeh A, Altunkaynak B Z, Turkmen A P, Anjum M R and Reddy DR P S. 2013. effect of prenatal exposure to lead acetate on testicular lipid peroxidation in adult rats. *Int. j. pharm. biol. sci.* **4(1)**(B): 893-898.

Apostoli P, Porru S and Bisanti L. 1999. Critical aspects of male fertility in the assessment of exposure to lead. *Scand J Work Environ Health.* **25**: 40 – 43.

Asad A, Hamid S and Qamar K. 2018. Effect of Lead Acetate on Basement Membrane of Seminiferous Tubules of Adult Rat Testis and Protective Effects of *Ficus carica*: A Histological Study. *J Coll Physicians Surg Pak.* **28(10)**: 731–734.

Asadpour R, Azari M, Hejazi M, Tayefi H, and Zaboli N. 2013. Protective effects of garlic aquous extract (Allium sativum), vitamin E, and N-acetylcysteine on reproductive quality of male rats exposed to lead. *Vet Res Forum.* **4(4)**: 251–257.

Assi M A, Hezmee M N, Abba Y, Yusof M S, Haron A W, Rajion M A, and Al-Zuhairy M A. 2016. Prophylactic effect of *Nigella sativa* against lead steroidogenesis enzymes, and testicular damage in testes of diabetic rats. *Acta histochemica*, acetate induced changes in spermiogram, reproductive hormones and gonadal histology of rats. *Vet. World*. **9(11)**: 1305–1311.

Baghurst P A, Robertson E F, Oldfield R K, King B M, McMichael A J, Vimpani G V and Wigg N R. 1991. Lead in placenta, membranes, and umbilical cord in relation to pregnancy outcome in a lead-smelter community. *Environ. Health Perspect*. **90**: 315 – 320.

BaSalamah M A, Abdelghany A H, El-Boshi M, Ahmad J, Idris Sh and Refaat B. 2018. Vitamin D alleviates lead induced renal and testicular injuries by immunomodulatory and antioxidant mechanisms in rats. *Sci. Rep*. **8(4853)**: 1-13.

Benoff S, Jacob A, and Hurley I R. 2000. Male infertility and environmental exposure to lead and cadmium. *Hum. Reprod. Update*. **6(2)**: 107–121.

Bouazza S, Demmouche A, Toumi-Benali F, Zouba M, Bahri R, Agher N, Merakchi N, and Ahmar El. 2018. Effect of bee pollen extract on lead-induced toxicity in rat testis. *South Asian J Exp Biol*. **8(3)**: 91-102.

Boyce R W, Dorph-Petersen K A, Lyck L, Gundersen H J. 2010. Design-based stereology: introduction to basic concepts and practical approaches for estimation of cell number. *Toxicol. Pathol*. **38 (7)**: 1011-1025.

Brown D L. 2017. Practical Stereology Applications for the Pathologist. *Vet. Pathol.* **54(3)**: 358–368.

Crissman J W, Goodman D G, Hildebrandt P K, Maronpot R R, Prater D A, Riley J H, Seaman W J and Thake D C. 2004. Best practices guideline: toxicologic histopathology. *Toxicol. Pathol.* ***32*(1)**: 126–131.

Dadkhah N, Shahnazi M, Mesgari Abbasi M and Etemadifar S. 2017. Assessment of the protective effect of vitamin E on the quality of spermatogenesis and sperm parameters in rats exposed to lead. *Adv. Herb. Med.* **3(2)**: 6-14.

Dehoff R T. 2000. Probes, populations, samples, measurements and relations in stereology. *Image Anal. Stereol*. **19**: 1-8.

Dorostghoal M, Dezfoolian A and Sorooshnia F. 2011. Effects of Maternal Lead Acetate Exposure during Lactation on Postnatal Development of Testis in Offspring Wistar Rats. *Iran J Basic Med Sci*. **14(2)**: 122-131.

Elgawish R AR and Abdelrazek H M A. 2014. Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats. *Toxicol. Rep*. **1**: 795-801.

El-Khadragy M, Al-Megrin W A, AlSadhan N A, Metwally D M, El-Hennamy R E, Salem F, Kassab R B and Abdel Moneim A E. 2020. Impact of Coenzyme Q10 Administration on Lead Acetate-Induced Testicular Damage in Rats. *Oxid. Med. Cell*. 12p.

Fair J M and Ricklefs R E. 2002. Physiological growth and immune responses of Japanese quail chicks to the multiple stressors of immunological challenge and lead shot. *Arch. Environ. Contam. Toxicol*. **42**: 77 – 87.

Franca L R and Russell L D. 1998. *The testes of domestic animals. In: Male Reproduction.* A Multidisciplinary Overview, Madrid: Churchill Livingstone.

Goyer R A. 1990. Transplacental transport of lead. *Environ. Health Perspect*. **89**: 101–105.

Gundersen H J, Bagger P, Bendtsen T F, Evans S M, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard J R and Pakkenberg B. 1988. The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS*. **96(10)**: 857–881.

Hassan E, El-Neweshy M, Hassan M, and Noreldin A. 2019. Thymoquinone attenuates testicular and spermotoxicity following subchronic lead exposure in male rats: Possible mechanisms are involved. *Life Sci*. **230**: 132–140.

Heidmets L T, Zharcovasky T, Jurgenson M, Jaako-Movits K and Zharkovasky A. 2006. early post-natal, low level lead exposure increase the number of PSA-NCAM expressing cells in the dentate gyrus of adult rat hippocampus. *Neurotoxicology*. **27**: 39-43.

Howard C V and Reed M G. 2005. *Unbiased Stereology: Three-Dimensional Measurment in microscopy* (2nd ed). Garland Science/BIOS Scientific Publishers, UK.

Jaako-Movits k, Zharkovasky T, Romantchik O, Jurgenson M, Mersalu E, Heidmets L T, and Zharkovasky A. 2005. Developmental lead exposure impairs contextual fear conditioning and reduces adult hippocampal neurogenesis in the rat brain. *Int J Dev Neurosci*. **23**: 350-627.

Khodabandeh Z, Dolati P, Zamiri M J, Mehrabani D, Borsbar H, Alaee S, Jamhiri I and Azarpira N. 2021. Protective Effect of Quercetin on Testis Structure and Apoptosis Against Lead Acetate Toxicity: An Stereological Study. *Biol. Trace Elem. Res*. 199: 3371–3381.

Massanyi P, Massanyi M, Madeddu R, Stawarz R and Lukac N. 2020. Effects of Cadmium, Lead, and Mercury on the Structure and Function of Reproductive Organs. *Toxics*. **8(4)**: 94.

Morais S, Costa F G and Pereira M L. 2012. Heavy Metals and Human Health, Environmental Health - Emerging Issues and Practice, Prof. Jacques Oosthuizen (Ed.), ISBN: 978-953- 307-854-0.

Mruk. D and Cheng C Y. 2004. Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. *Endocr. Rev*. ***25*(5)**: 747–806.

Mustafa H N. 2015. Potential alleviation of *Chlorella vulgaris* and Zingiber officinale on lead-induced testicular toxicity: an ultrastructural study. *Folia Biol.* **63(4)**: 269–278

Nyengaard J R. 1999. Stereologic Methods and Their Application in Kidney Research. *J. Am. Soc. Nephrol.* **10**: 1100-1123.

Oboma Y I, Sylvanus B, Okara P N, Tamuno omie F A and Ibiang O E. 2018. Protective effect of combined aqueous extracts of *Allium sativum* and *Zingiber officinale* against lead acetate induced hepatotoxicity and testicular damage in Rattus norvegicus. *MOJ Anat & Physiol*. **5(5)**:306-313.

Offor S J, Mbagwu H O and Orisakwe O E. 2019. Improvement of Lead Acetate-Induced Testicular Injury and Sperm Quality Deterioration by *Solanum anomalum* Thonn. Ex. Schumach Fruit Extracts in Albino Rats. *J Family Reprod Health.* **13(2)**: 98–108.

Restanty D A, Soeharto S and Indrawan I W A. 2018. The effect of oral lead acetate exposure on bax expression and apoptosis index granulose cells antral follicle in female wistar rat (*Rattus norvegicus*). *Asian Pac. J. Reprod*. **6(2)**: 54-57.

Sadeghinezhad J, Dahmardeh M, Tootian Z, Bojarzadeh H and Yarmahmoudi F. 2021. Study on the effect of maternal administration of oxaliplatin on offspring testes. *J Exp Clin Med.* **38(2)**: 99-106.

Santhosh Kumar R and Asha Devi S. 2018. Lead Toxicity on Male Reproductive System and its Mechanism: A Review. *Res J Pharm Technol*. **11(3)**: 1228-1232.

Snoeijs T, Dauwe T, Pinxten R, Vandesande F and Eens M. 2004. Heavy metal exposure affects the humoral immune response in a free-living small songbird, the great tit (*Parus major*). *Arch. Environ. Contam. Toxicol*. **46**: 399 – 404.

Taupeau C, Poupon J, Nome F and Lefevre B. 2001. Lead accumulation in the mouse ovary after treatment-induced follicular atresia. *Reprod. Toxicol.* **15**: 385–391.

Treuting P M, Dintzis S M and Montine K S. 2017. *Comparative Anatomy and Histology a Mouse and Human Atlas*. Academic press.

Udefa A L, Amama E A, Archibong E A, Nwangwa J N, Adama S, Inyang V U, Inyaka G U, Aju G J, Okpa S and Inah I O. 2020. Antioxidant, anti-inflammatory and anti-apoptotic effects of hydro-ethanolic extract of *Cyperus esculentus* L. (tigernut) on lead acetate-induced testicular dysfunction in Wistar rats. *Biomed. Pharmacother*. **129**: 110491.

West M J. 2012. Introduction to stereology. Cold Spring Harbor Protocols. 843-851. *World Health Organization* (WHO). Lead poisoning and health. 2016; Available from: http://www.who.int/mediacentre/factsheets/ fs379/en/ [Accessed 2017 28/09].

Yavasoglu A, Karaaslan M A, Uyanikgil Y, Sayim F, Ates U and Yavasoglu N U. 2008. Toxic effects of anatoxin- a on testes and sperm counts of male mice. *Exp. Toxicol. Pathol.* **60(4-5)**: 391–396.

Zhang H, Zhang M, Liu R and Chen Y. 2014. Assessing the mechanism of DNA damage induced by lead through direct and indirect interaction. *J. Photochem. Photobiol. B.* **136**: 46-53.