Article **ECORFAN Journal**
December 2016 Vol.2 No.3 6-18

Effect of lead on reproductive physiology: The model study rat

HANDAL-SILVA, Anabella*†, MORÁN-PERALES, José L. and GARCÍA-SUÁSTEGUI, Wendy A.

Departamento de Biology and Toxicology of Reproduction Instituto de Cienciasde la Benemérita Universidad Autónoma de Puebla. 14 Sur 6301 Col. San Manuel C.P. 72570 Puebla, México.

Received July 21, 2016; Accepted September 20, 2016

Abstract

AIM: to determine the lead (Pb)-induced reproductive disturbances in CII-ZV rats. MATERIALS AND METHODS: 40 female CII-ZV rats were divided into 4 groups of 10 rats each one, a control group and three treatment groups that received graded doses of lead acetate 0.003, 0.03 and 0.6 g/L via oral route for 30 days. We determined blood lead (Pb) concentrations by anodic stripping voltammetry (ASV), progesterone and 17 estradiol by radioimmunoassay. RESULTS: We found a direct relation between the concentrations of Pb administered and determined in blood. Pb administration induced morphological and physiological alterations in the ovary, changes in development and maturation of follicles as well as on steroid hormone secretion. CONCLUSION: Pb induced reproductive disturbances in CII-ZV rats altering the homeostasis of Hypothalamic-pituitary gonadal axis.

Effect, reproductive physiology, model

Citation: HANDAL-SILVA, Anabella, MORÁN-PERALES, José L. and GARCÍA-SUÁSTEGUI, Wendy A. Effect of lead on reproductive physiology: The model study rat. ECORFAN Journal-Republic of Guatemala 2016, 2-3: 6-18

* Correspondence to Author (email: ahandals@yahoo.com.mx)
† Researcher contributing first author.

Introduction

Lead is an important environmental pollutant present in nature due to natural and anthropogenic sources (ATSDR 2005).

Exposure to lead occurs mainly through feeding, ingestion of water or inhalation and its deposition is evident in several tissues, such as kidney, liver, brain and bones (Bresslery Goldstein, 1991; Russell Moser, 1995; Michael J. Et al., 1999). Studies in both animals (Anwer et al., 1988, Antonio and Leret 2000, Burger J. et al., 2005, Yara M. Müller et al, 2008) and in humans (Al-Saleh 1994; Fraser et al., 2006). It has also been studied the effect of heavy metals on the reproductive system and the toxicity of Cd and Pb in human reproduction (X. Wang et al., 2004; LW Jackson et al., 2008; LW Jackson et al., 2011; Al-Saleh et al., 2008).

It is well documented that lead exerts a wide range of adverse biological effects on the reproductive system (Murakami, K. et al, 1993; Zhigang, D. L. et al, 1997). Lead crosses the placental barrier and accumulates in fetal tissue during the gestational period (Barraclough, C.A, 1982, 1983). The effect of Pb on the female reproductive system causes a reduction in luteinizing hormone (LH) binding and follicle-stimulating hormone (FSH) binding, altered in vitro steroidogenesis in granulosa cells isolated from rats (PN Priya et al. , 2004). Studies conducted by Katalin Paksy et al. 2011, showed that lead levels in ovarian follicular fluid do not represent a danger for the secretion of progesterone in the ovary. There was also a decrease in serum gonadotropin levels (Pillai A. et al, 2003) and serum progesterone (Gupta S. et al, 2002) by simultaneous exposure to Pb and cadmium and it was shown that in vitro exposure of cells From granulosa to Pb and Cd, cause a decrease in the production of gonadotropins and binding steroids. (Priya P. et al., 2004).

Also Laxmipriya P. Nampoothiri and Sarita Gupta (2006) demonstrated that lead and cadmium cause significant reduction in gonadotropin binding, which alters the androgenic enzymatic activity of granulosa cells.

Lead can interfere with steroidogenesis (Wiebe, JP, et al, 1983), and may affect androgen receptors (MacLean, FC et al., 1961) and inhibit Leydig cell testosterone production in vitro (Caffey, J. 1961). Research conducted by Neeta Adhikari et al. (2000) and Derbrand, B.C. et al. (1974) demonstrated that lead induces damage in spermatogenesis, decreases sperm production. The investigations of Sokol R. Z. et al. 1994; Laskey, J.W., and Phelps, P.V. 1991; Masser, 1995; Hoyer, P. et al., 2001) reported that lead in vivo causes suppression of the hypothalamic-pituitary-testicular axis. Studies by Rebeca Z. 1994 and Blazka, M. et al., 1994 showed that the toxic effects of lead on reproductive hormones in the male rat are reversible. The present investigation will allow to obtain approximate information on the health risk of people exposed to lead with doses higher than the determined average of blood lead in the general population, with the objective of applying control measures in environmental health.

Methodology.

Experimental design

The mean blood lead level in the human population of Santiago Xalizintla (Municipality of San Nicolás de los Ranchos in the State of Puebla) was 9 □g / dL value that is within the limits permissible according to NOM-199-SSA1-2000, ENVIRONMENTAL HEALTH. The lead concentration to be evaluated was 9 □g / dL lead in blood, which is approximately 0.03g / L, taking into account the average weight and volume ratio of human and rat.

ISSN-On line: 2414-8849

The rats were provided by Claude Bernard Bioterio and were treated according to the rules of the Mexican Council on Care and Use of Experimental Animals based on the NOM-062-ZOO 1999 standard and the current CICUAL-BUAP parameters. Female rats of the newborn CII-ZV strain were used with a light / dark cycle controlled 12/12 hrs, with free access to the mother until the age of weaning (21 days), food (Labdiet 5008) and water ad Libitum until the day of the sacrifice. Three experimental groups and a control group of 10 rats were formed each group.

After the day of weaning (26 days) the rats were given lead acetate in the drinking water. The doses to be studied were four higher and one lower than the average dose of blood lead: 0.0 (control), 0.003, 0.03 and 0.6 g / L (three experimental doses) administered during 18 consecutive estrus cycles. On each of the four days of the third estrous cycle (estrus, diestrus I, distro II and proestrus) groups of 2 rats were weighed and randomly sacrificed simultaneously with 2 specimens from the control group. The autopsy was dissected and the ovaries were weighed. From each animal blood was obtained from the trunk in two vacutainer tubes with heparin, and the lead concentration was determined by the "ese" lead analyzer. To the second tube the blood was allowed to coagulate for 30 minutes, centrifuged and the serum removed, which was stored at -20 ° C until the quantification of progesterone, 17 / □-estradiol. On each day of the estral cycle, the control group and each experimental concentration, 2 rats.

Histological analysis

After weighing the ovaries; the histological analysis was performed, the sections were stained with hematoxylin - eosin and analyzed in the Axioplan II Confocal motorized microscope.

Radioimmunoassay

Quantification of steroid hormones.

Quantification of progesterone and 17β-estradiol was performed by the solid-phase radioimmunoassay method, with a Coat-A-Count Kit.

Preparation of the standard curve of progesterone and 17β-estradiolProgesterona

The standard curve was performed in duplicate, using calibrators of 0.1, 0.5, 2.0, 10, 20 and 40 ng / mL.

Estradiol

The standard curve was performed in duplicate, 0.0, 20, 50, 150, 500, 1800 and 3600 pg / mL calibrators were used and dilutions were performed to obtain standards of 0.0, 5.0, 10, 20, 50, 75, 150 and 250 pg / ML.

Statistic analysis

The results obtained were analyzed by the Kruskal-Wallis test followed by the Dunn test and ANOVA followed by Tukey.

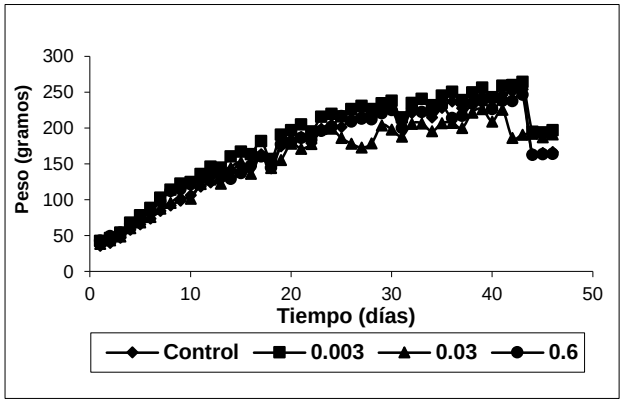
Results

Morphometry

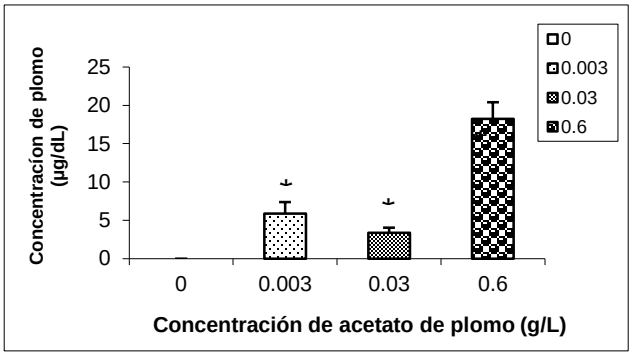
The body weight of the rats was similar in all experimental groups relative to the control group (Graph 1).Results related to lead concentration in blood showed a general tendency to increase and was significant in concentration 0.6 g / L. At the concentration 0.03 g / L, there was a decrease in blood lead concentration compared to 0.003 g / L and control (Graph 2). Comparing the weight of the right and left ovaries between the treated and control groups was similar, and decreased in the concentration 0.6 g / L, in relation to the control group (Graph 3).

ISSN-On line: 2414-8849

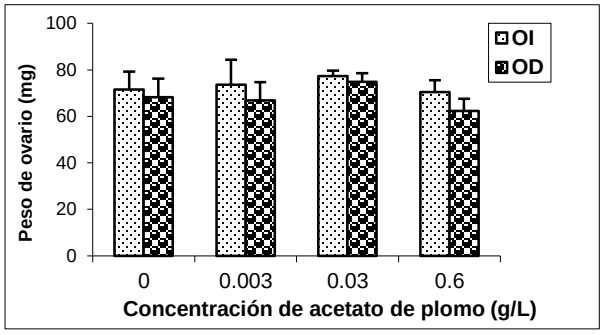
In figure 4 it was observed that as the blood lead concentration increased, the total number of follicles decreased and in the concentration 0.003 g / L significantly decreased.



Graphic 1 Mean ± ha of the ovarian weight of the control group and the groups treated at different concentrations of lead acetate □*P<0.005 vs control (ANOVA followed by Tukey).

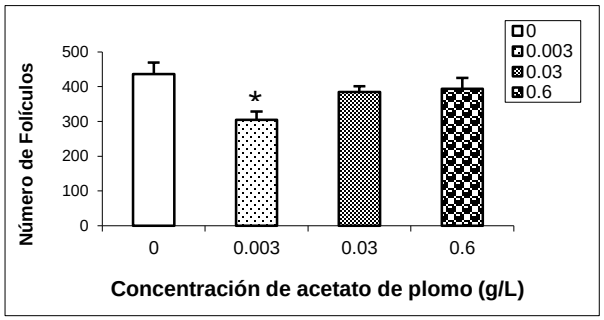


Graphic 2 Mean Â ± SEM of the blood lead concentration of the control group and the groups treated with lead acetate. * P <0.0001 vs control (ANDEVA followed by Tukey).

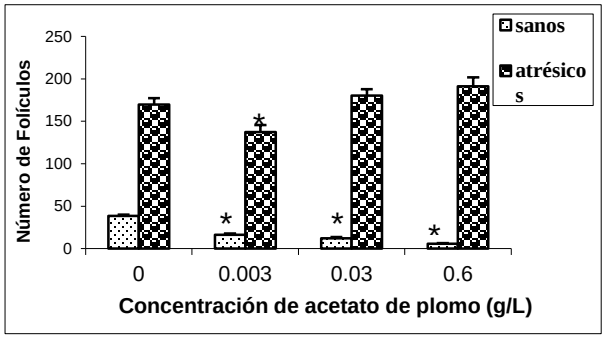


Graphic 3 Media ±e.e.m. Graph 2. Mean ± SEM of ovarian mass (both ovaries) of the control group and groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

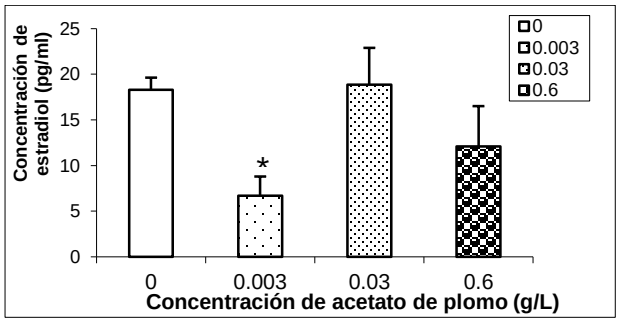
In Figure 5 a significant decrease in the number of healthy follicles was observed compared to the control group. At concentrations of 0.03 and 0.6 g / L the number of atresic follicles is similar to the control group and significantly lower in the 0.003 g / L group.



Graphic 4 Media ±e.e.m. del Graph 2. Mean æem of the total number of follicles in the Control group and the groups treated with lead acetate. * P <0.05 vs control (Kruskall - Wallis followed by the Dunn test).



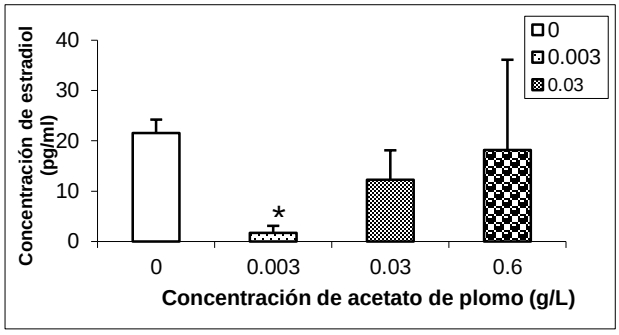
Graphic 5 Media ±e.e.m. del número de folículos Graph 2. Mean ± eem of the total number of healthy and atresic follicles of the control group and those treated with lead acetate. * P <0.0001 vs control (Kruskall - Wallis followed by the Dunn test).



Graphic 6 Media ± eem Graph 2. Mean ± SEM of the total estradiol concentration of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

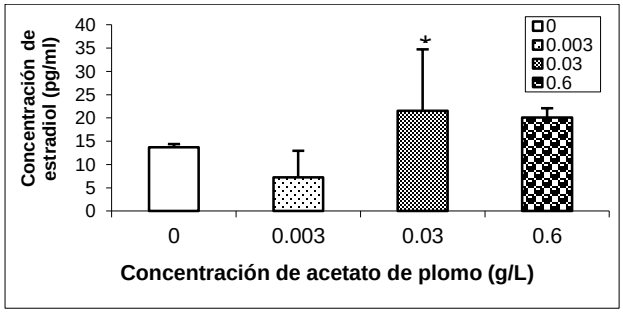
The total estradiol concentration decreased in the concentrations 0.003 and 0.6 g / L and in the concentration 0.03 g / L remained similar to the control (Graph 6).

Plasma levels of oestradiol in proestrus at all concentrations were lower than controls and in the concentration 0.003 g / L the estradiol level was significantly lower (Graph 7).

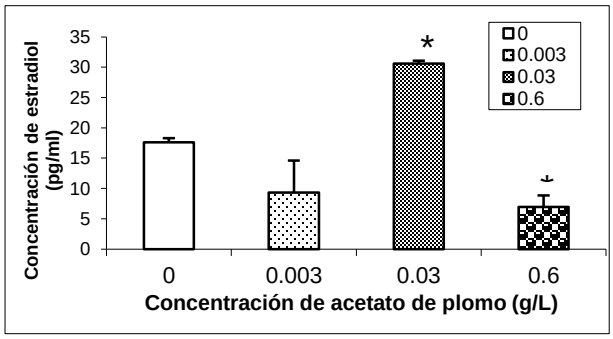


Graphic 7 Media ± eem de la Graph 2. Mean æem of the estradiol concentration in Proest of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

Plasma levels of oestradiol estrus increased in relation to control and were significantly lower in the concentration 0.003 g / L (Graph 8).



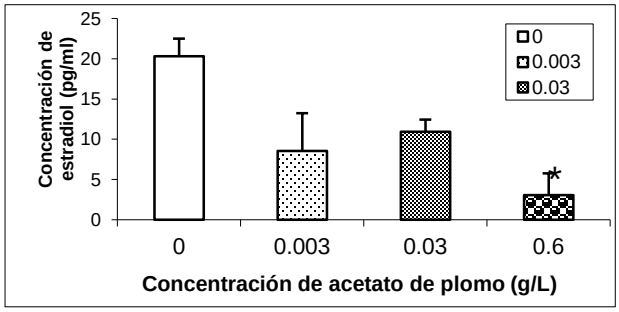
Graphic 8 Mean ± SEM of the estradiol concentration in estrus of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).



Graphic 9 Mean ± SEM of the estradiol concentration in right-handed 1 of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

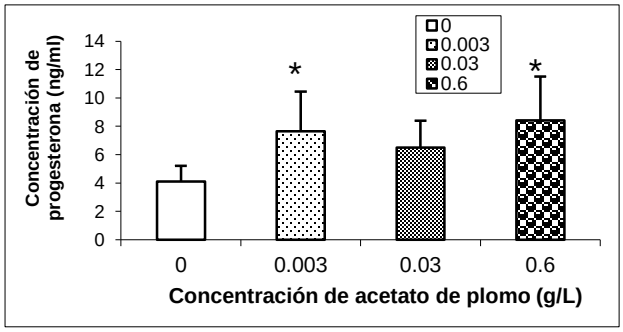
Plasma levels of estradiol in diestrus 1 decreased, being significant in the concentration 0.6 g / L. At the 0.03 g / L concentration the estradiol level increased significantly compared to the control (Graph 9).

Plasma levels of estradiol, at right ventricle 2, decreased and was significant at the concentration 0.6 g / L compared to control and other experimental groups (Graph 10).



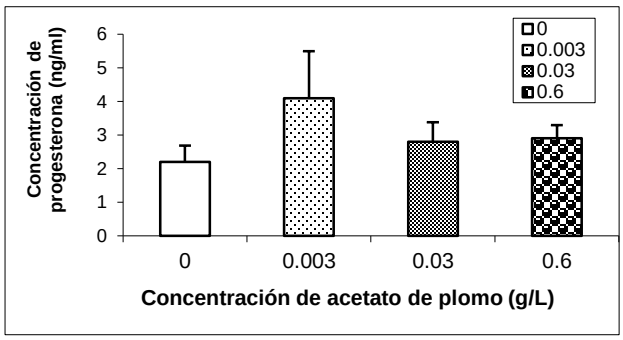
Graphic 10 Mean ± SEM of the estradiol concentration in right-handed 2 of the control group and the groups reated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

Plasma progesterone levels increased in the 0.003 and 0.6 g / L concentrations and were significantly higher in the control group (Graph 11).



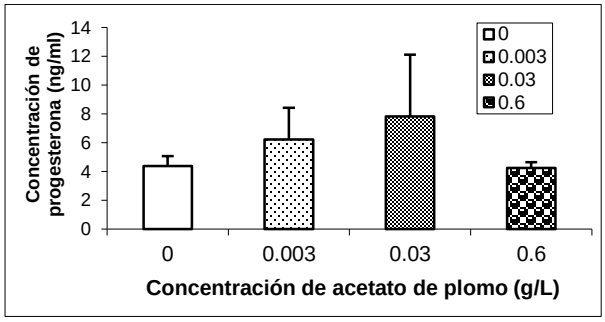
Graphic 11 Mean ± eem of the progesterone concentration of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

Plasma levels of progesterone in proestrus increased and were significant in the concentration 0.03g / L in relation to the control (Graph 12).



Graphic 12 Mean ± eem of the progesterone concentration of the control group and the groups treated with lead acetate. * P <0.05 vs control, (ANOVA, followed by the TUKEY test).

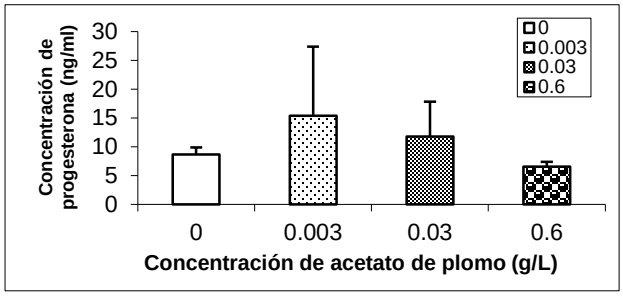
Plasma levels of progesterone in estrus increased at concentrations of 0.003 and 0.03 g / L and in the concentration 0.6 g / L was similar to the control group (Graph 13).



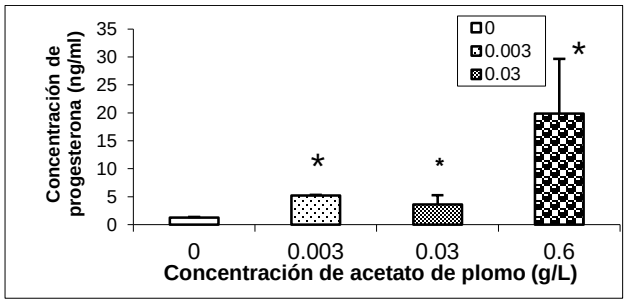
Graphic 13 Mean ± SEM of the estrus progesterone concentration in the control group and the groups treated with lead acetate. * P <0.0007 vs control, (ANOVA, followed by the TUKEY test).

Plasma levels of progesterone in diestrus 1 increased by 0.003 and 0.3 g / L relative to control and decreased significantly in the concentration 0.6 g / L (Graph 14).

ISSN-On line: 2414-8849



Graphic 14 Mean ± ha of the progesterone concentration in right-handed 1 of the control group and the groups treated with lead acetate. * P <0.0007 vs control, (ANOVA, followed by the TUKEY test).



Graphic 15 Mean ± SEM of the progesterone concentration in right-handed 2 of the control group and the groups treated with lead acetate. * P <0.0007 vs control, (ANOVA, followed by the TUKEY test).

Plasma levels of progesterone in diestrus were significantly increased in all treated groups compared to the control group and were very high at 0.6 g / L (Graph 15).

Pathology

Similar morphological alterations were observed in the development of proliferative and cytological phenomena and were more aggressive and invasive in the 0.6 g / L concentration of lead acetate.

Figure 1 shows granulosa cells that do not have a nucleus, these cells are also located in the antrum in the follicular fluid.

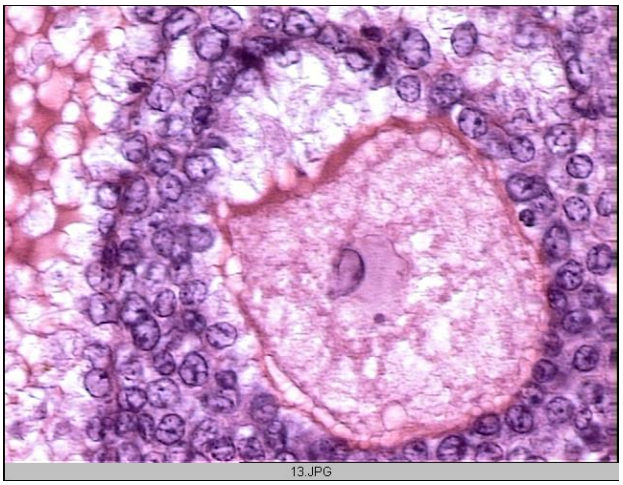


Figure 1 Transverse section of ovary. The oocyte presents nucleus and nucleolus and is atresic, stained with Hematoxillin-eosin 40x.

The follicle oocyte is atretic is surrounded by anucleated cells, has lost its shape and there are some pycnotic cells in the crown, rupture of the pellucid membrane is observed and the follicle begins to luteinize.

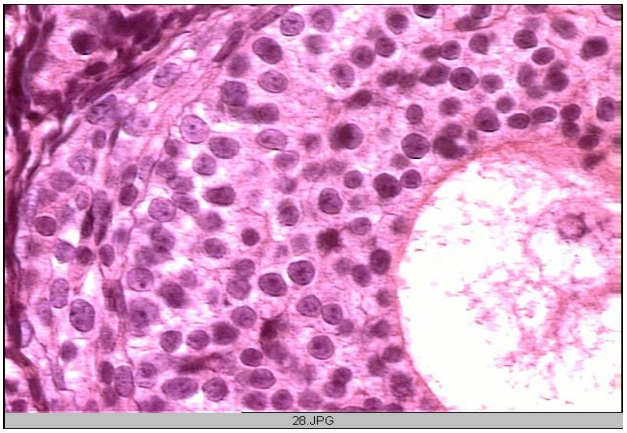


Figure 2 Transverse section of ovary. Primary follicle with atresic oocyte and internal teak thickening. Tinted with Hematoxillin-eosin 40x.

Figure 2 shows an atresic follicle, presenting an oocyte with its nucleus, nucleolus and zona pelucida; there are presence of surrounding anucleated cells, granulosa cells with picnosis, desquamation and large intercellular spaces.

ISSN-On line: 2414-8849

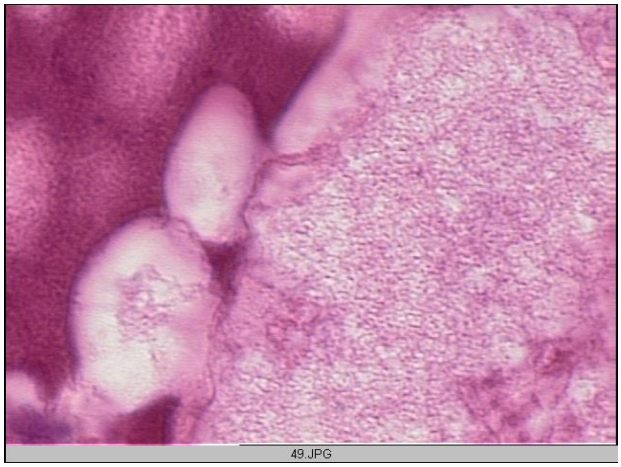


Figure 3 Transverse section of ovary. Infiltration by anucleated cells into the oocyte. Tissue with Hematoxillin-eosin 100x.

Figure 3 shows how channels form between the pelucidal zone and the oocyte membrane through which the contents of the anucleated cells, formed in the radiated corona, can be directed into the oocyte and the degradation of the pellucida and oocyte membrane.

Discussion

Lead acetate has been shown to reduce the weight of some organs of the reproductive system depending on the dose, duration and age of the animal (Sokol, R. Z et al, 1991; Nahan, E et al., 1992, Correa et al., 2004, Yara M. R et al, 2008). Based on the results obtained on body weight and ovarian mass in most of the experimental groups, it was observed that lead does not affect the body weight of the rat, however, in the concentration 0.6g / L the weight of the ovary decreased (Figure 1).

Research by Palminger et al. (1991) showed that most lead in blood is fixed in erythrocytes (Lorentzo AV et al, 1977) and that blood lead levels remain constant despite continued exposure to Lead, in this situation, the body has to maintain the homeostatic balance by accumulating surplus lead in bone and other tissues (Fei Yu et al., 2008).

This criterion coincides with the results, which showed that the administration of 0.6 g / L lead acetate reflected a concentration of 19 □g / dl lead in blood (see graph 2), suggesting that there is a limit of Transport of lead in blood and that circulating lead surplus is redistributed and accumulated in bone and other organs (Fei Yu et al., 2008); In this case we can suggest that one of the affected organs could be the ovary due to the morphological alterations observed during the follicular development. This criterion agrees with the results obtained by Flower et al. 1994; Khan-Dawood et al .; Wilson C.A. Et al. 1992, Fei Yu et al., 2008, A. Pollack et al, 2011; C.M. Gallagher et al, 2010; E.F. Krieg Jr., H.A. Feng 2011; K. Paksy et al, 1997; L.W. Jackson et al, 2011.

It is known that granulosa and teak cells are sensitive or vulnerable to heavy metals, by research conducted by Krinitz et al (1978); Petrusz et al (1979); Vermande-Van Eck et al. (1960), showed that lead salts cause follicular atresia, inhibit follicular development, ovulation does not occur and puberty is delayed. This suggests that lead crosses the granulosa layer and interferes with the process of steroidogenesis (P.N. Priya, A. Pillai, S. Gupta, 2004). This information coincides with the results obtained in the concentration of 0.6g / L lead acetate, a reduction in the number of secondary and tertiary follicles was observed and follicular atresia increased (Graph 5). These results coincide with the work of (Petrusz, P et al, 1979; Maxim Khotimchenko et al 2006). We can infer that as the concentration of lead in blood increases, the number of healthy follicles decreases and the atresics increase (Graphs 4 and 5).

There is evidence that lead exerts its toxic effects on the hypothalamic-pituitary-gonad axis produced by inhibition in the synthesis and release of gonadotropins. In this sense the works carried out by Ronis and col (1998).

ISSN-On line: 2414-8849

They showed that in prepubertal females exposed to lead they had delayed vaginal opening and the estrous cycle was interrupted. By in vitro studies, lead blocks the secretion of GnRH in the middle eminence, an event associated with low PGE2 secretion. We also found low levels of IGF-1 in the hypothalamus required to activate the GnRH / LH release systems (P.S. Christensen et al, 2016). Therefore it can not be ruled out that lead exposure disrupts the circulatory development of GnRH within the hypothalamus and the mean eminence.

The results showed that, by administering minimal concentrations of lead acetate, estradiol concentrations decreased and progesterone increased proportionally as the administered lead acetate concentration increased (L. W. Jackson et al., 2011) (see graphs 6-15).

These results demonstrate that exposure to lead is associated with increased DNA and RNA and protein synthesis; so we can infer that there is a relationship between the concentration of lead in the nucleus and the subsequent alterations in cell division.

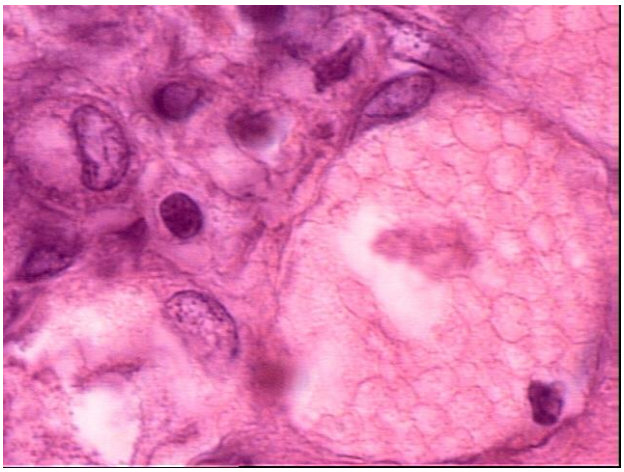


Figure 4 Transverse section of ovary. Formation of irregular bodies in the corpus luteum. Dyed with Hematoxillin-eosin 100x.

A characteristic sign of lead poisoning is intracellular inclusion bodies. These were located in granulosa cells in the process of desquamation and in the corpus luteum, these results coincide with those of Terry D. Oberley et al. (1995). (Figure 4).

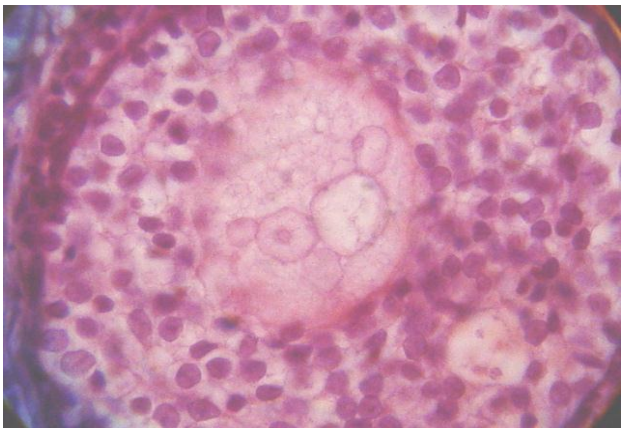


Figure 5 Transverse section of ovary. Presence in some follicles of multiple and multinucleated oocytes. Tissue with Hematoxillin-eosin 100x.

Another characteristic alteration was the presence of multiple oocytes within an ovarian follicle, some oocytes presented several nuclei (Figure 5).

The antecedents and results obtained can be inferred that lead delays the vaginal opening, alters the estrous cycle, and interferes with the secretion of FSH, LH, in the production of progesterone and estradiol in the follicle, producing an increase in the atresia of healthy follicles.

These results support the hypothesis that as the concentration of lead acetate in the bloodstream increased, the greater the morphological alteration in the ovary and the physiology in controlling the secretion of ovarian hormones, growth and Gonadal maturation (A. Pollack et al, 2011, CM Gallagher et al, 2010, EF Krieg Jr., HA Feng 2011, K. Paksy et al, 1997; LW Jackson et al, 2011).

ISSN-On line: 2414-8849

It is concluded that the administration of lead acetate alters the normal functioning of the hypothalamic-pituitary-gonadal axis and directly influences the ovary physiology. There is a direct relationship between the concentration of lead administered and that determined in blood. Alterations in the secretion of ovarian hormones caused morphological alterations and changes in follicular development and maturation characteristic of the concentration 0.6g / L.

References

Antonio MT, Leret ML. 2000. Study of the neurochemical alterations produced in discrete brain areas by perinatal low-level lead exposure. Life Sci 67:635–642.

Anwer J, Ali S, Mehotra NK. 1988. Antagonistic eVect of zinc in lead treated developing chick embryos. Drug Chem Toxicol 11(1):85–95.

ATSDR. 2005. Draft toxicological proWle for lead. Agency for toxic substances and disease registry. US Public Health Service, Atlanta.

Al-Saleh IAS. 1994. The biochemical and clinical consequences of lead poisoning. Med Res Rev 14:415–486.

Barraclough, C. A. 1982. Sex steroid regulation in reproductive neuroendocrine processes. En: "Handbook of fhisiology". Sección 7. Vol. II. Cap. 2. End. R. O. Greep & E. B. Astwood. Am. Physiol. Soc. Washington, D.C. pp. 29-52.

Barraclough, C. A. 1983. The role of catecholamines in the regulation of gonadotropin secretion. Acta Morflologica Hungarica. 31: 101-116.

Burger J, Gochfeld M. 2005. EVects of lead on learning in herring gulls: an avian wildlife model for neurobehavioral de Wcits. Neurotoxicology 26:615–624.

Blazka, M. E., Harry G. J. Luster, M. I. 1994. Effect of lead acetate on nitrite production by murine brain endothelial cell cultures. Toxicology and Applied Pharmacology. 126: 191 – 194.

Bressler JP, Goldstein GW. 1991. Mechanisms of lead neurotoxicity. Biochem Pharmacol 41:479–484.

Caffey, J. 1961. Pediatric. X-ray. Diagnosis. 4th ed. Year Book. Medical Publisher Chicago. pp. 852.

Correa M, Roig-Navarro AF, Aragon CMG. 2004. Motor behavior and brain enzymatic changes after acute lead intoxication on diVerent strains of mice. Life Sci 74(16):2009–2021.

C.M. Gallagher, B.S. Moonga, J.S. Kovach. 2010. Cadmium, follicle-stimulatinghormone, and effects on bone in women age 42–60 years, NHANES III, Environ. Res. 110 (1): 105–111.

Derbrand, B. C., Der, R., Griffin, W. T. and Fahim, M. S. 1974. Effects of lead acetate on reproduction. Am. J. Obstet. Gynecol. 115:1058-1065.

E.F. Krieg Jr., H.A. Feng. 2011. The relationships between blood lead levels andserum follicle stimulating hormone and luteinizing hormone in the National Health and Nutrition Examination Survey 1999–2002, Reprod. Toxicol. 32 (3): 277–285.

Fei Yu, Yingjun Liao, Yaping Jin, Yue Zhao, Yahao Ren, Chunwei Lu, Gexin Li, Yanxi Li, Jun Yang. 2008. Effects of in utero meso-2,3-dimercapto succinic acid with calciumand ascorbic acid on lead-induced fetal development. Arch Toxicol. 82:453–459. DOI 10.1007/s00204-007-0267-5.

ISSN-On line: 2414-8849

Fraser S, Muckle G, Després C. 2006. The relationship between lead exposure, motor function and behaviour in Inuit preschool children. Neurotoxicol Teratol 28:18–27.

Gupta S, Laxmipriya, Gohil V. 2002. Simultaneous exposure of lead and cadmium on granulosa cells, progesterone and luteinising hormone in proestrous rats. Adv Pharmacol Toxicol. 3: 23–30.

Hoyer, P. E., Terkalsen, O. B. F., Byskov G. A., Nielsen, H. 2001. Fetuin and fetuin messenger RNA in granulose cells of theratovary. Biology of Reproducction. 1655 – 1662.

Al-Saleh, S. Coskun, A. Mashhour, N. Shinwari, I. El-Doush, G. Billedo. 2008. Exposure to heavy metals (lead, cadmium and mercury) and its effect on the out come of in-vitro fertilization treatment, Int. J. Hyg. Environ. Health 211(5–6): 560–579.

Katalin Paksy, István Gáti, Miklós Náray, Klárarajczy. 2011. Lead accumulation in human ovarian follicular fluid, and in vitro effect of lead on progesterone production by cultured human ovarian granulosa cells. Doi: 10.1080/152873901300018093. Pages 359-366.

K. Paksy, K. Rajczy, Z. Forgacs, P. Lazar, A. Bernard, I. Gati. 1997. Effect ofcadmium on morphology and steroidogenesis of cultured human ovarian granulosa cells, J. Appl. Toxicol. 17 (5): 321–327.

Krinitz, B. 1978. Rapid scrining field test for detecting cadmiun and lead extracted fron glazed ceramic dinnerware: collaborative study; J. Assoc. Off Anal. Chem. 61: 1124-1129.

Lasley S. M. and Gilbert M. E. 1999. Lead inhibits the rat N-methyl-D-aspartate receptor channel by binding to a site distinct from the zinc allosteric site. Toxicology and Applied Pharmacology. 159: 224 – 233.

Laskey, J. W., and Phelps, P.V.; Effect of cadmiun and other metal cations on in vitro Leidig cells testosterone production; Toxicol. Appl. Pharmacol.; 1991; 108:296-306.

Laxmipriya P. Nampoothiri, Sarita Gupta. 2006. Simultaneous effect of lead and cadmium on granulosa cells: A celular model for ovarían toxicity. ReproductiveToxicology 21 (2006) 179–185.

Lorentzo, A. V., Gerwirtz, M, Maher, C., and Davidowski, L. I. 1997.The equilibration of lead between blood and milk of lactating rabbits; Life Sci. 21:1679-1984.

L.P. Nampoothiri, S. Gupta. 2006. Simultaneous effect of lead and cadmium ongranulosa cells: a cellular model for ovarian toxicity, Reprod. Toxicol. 21 (2): 179–185.

L.W. Jackson, M.D. Zullo, J.M. Goldberg. 2008. The association between heavy metals, endometriosis and uterine myomas among premenopausal women: national Health and Nutrition Examination Survey 1999–2002, Hum. Reprod. 23 (3): 679–687.

L.W. Jackson, P.P. Howards, J. Wactawski-Wende, E.F. Schisterman. 2011. The association between cadmium, lead and mercury blood levels and reproductive hormones among healthy, premenopausal women, Hum.Reprod. 26 (10): 2887–2895.

MacLean, F. C., and Urist, M. R. 1961. Bone: Introduction to physiology of Skeletal Tissup. Ed. ". Chicago. The University of Chicago Press. pp. 149.

ISSN-On line: 2414-8849

Maxim Khotimchenko and Irina Serguschenko. 2006. La absorción y excreción de plomo en las ratas tratadas con sales insolubles de pectina y alginato. International Journal of Toxicology, 25:195–203, 2006. ISSN: 1091-5818 print. DOI: 10.1080/10915810600683291.

Masse, R., and pinin-Ltaillade, G.; Impairment of testicular endocrine function after lead intoxication in the adult rat; Toxicology; 1995; 1-3:101-109.

Michael J. McCabe Jr., Kameshwar P. Singh, John J. Reiners Jr. 1999. Lead intoxication impairs the generation of a delayed type hypersencibility response; Toxicology. 139:255-264.

Murakami, K., Feng, G., AND Chen, S. G. 1993. Inhibition of brain protein kinase C subtypes by lead. J. Pharmacol. Exp. Ther. 264: 757 – 761.

Nahan, E., Huang, H. F., Pogach, L. 1992. Lead acetate does not impair of Sertoli cell function marker proteins in the adult Sprague Dawley rat. Arch. Environ. Health; 47:370-375.

Neeta A., Neelima S., and D. K., Saxena. 2000. Effect of lead on Sertoli-germ cell coculture of rat; Toxicology Letters. 116:45-49.

Palminger, I., and Oskarsson, A.; Transfer of lead via rat milk and tissue uptake in the suckling off spring. In trace elemts in health and disease; Royal Chem. Society, London; 1991; pp. 172-212.

Petrusz, P., Weaver, C. M., and Grant, L.D. 1979. Lead poisoning and reproduction: Effects on pituitary and serum gonadotropíns in neonatal rats. Environ. Res. 19: 383-396.

Pillai A, Priya L, Gupta S. 2003. Effects of combined exposure to lead and cadmium on the hypothalamic-pituitary axis function in proestrous. Food Chem Toxicol; 41: 379–84.

Pollack, E. Schisterman, L. Goldman, S. Mumford, P. Albert, R. Jones. 2011. Cadmium, lead, and mercury in relation to reproductive hormones and an ovulation in premenopausal women, Environ. Health Perspect. 119 (8): 1156–1161.

P.S. Christensen, J.P. Bonde, L. Bungum, A. Giwercman, G. Toft, B.A.G. Jönsson, I.O. Specht. 2016. Environmental cadmium and lead exposure and anti-Müllerian hormone in pregnant women. Reproductive Toxicology. Volume 61, June 2016, Pages 114–119. doi:10.1016/j.reprotox.2016.03.047.

P.N. Priya, A. Pillai, S. Gupta. 2004. Effect of simultaneous exposure to lead and cadmium on gonadotropin binding and steroidogenesis on granulosa cells: anin vitro study, Indian J. Exp. Biol. 42 (2) (2004) 143–148.

Priya PN, Pillai A, Gupta S. 2004. Effect of simultaneous exposure to lead and cadmium on gonadotropin binding and steroidogenesis on granulosa cells: an in vitro study. Indian J Exp Biol. 42: 143–8.

Rebecca, Z., Socol, Helen Ocuda, Harris M., Nagler, and Nancy Berman; Lead exposure in Vivo alters the fertility potential of sperm in Vitro; Toxicology and pharmacology; 1994; 124:310-316.

Ronis, M. J. J., Gand y J., Badger, T. 1998. Endocrine mechanisms underlyng reproductive toxicity in the developing rat chronically exposed to dietary lead; J. of toxicology and environmental health part A. 54: 77-99.

Russell Moser, Terry D. Oberley; Effects of lead administration on developing rat kidney; Toxicology and applied pharmacology; 1995; 131:85-93.

Sokol, R. Z., and Berman, N.; The effect of age of exposure on lead-induced testicular toxicity; Toxicol; 1991; 69:269:278.

ISSN-On line: 2414-8849

Sokol R. Z., Okuda, H., Nagler, H. M., and Berman, N. 1994. Lead exposure in –Vivo alters the fertility potential of sperm in-Vitro; Toxicology and Applied Pharmacology. 124:310-316.

Terry D. Oberley, Aaron L. Frieman. 1995. Effects of lead administration on developing rat kidney; Toxicology and applied pharmacology. 131: 94-107.

Vermande-Van Eck, Gertrude J., and Wister Meigs, J. 1960. Changes in the ovary of the rhesus monkey after chronic lead intoxication. Fert Steril. 11: 223-234.

Verity, M. A. 1990. Comparative observations on inorganic and organic lead neurotoxicity. Environ. Health Perspect. ; 89: 43-48.

Wilson, C. A., and Leigh, A. J. 1992. Endocrine toxicology of the female reproductive system. In Endocrine Toxicology; Cambridge Univ. Press, Oxford. pp. 313-395.

Wiebe, J. P., Salhanick, A. I., and Myers, K. I. 1983. On the Mechanism of action of lead in the testis: In Vitro, suppression of fish receptor, cyclic AMP and steroidogenesis. Life Sci. 32:1997-2005.

X. Wang, J. Tian. 2004. Health risks related to residential exposure to cadmium in Zhenhe County, China, Arch. Environ. Health 59 (6) (2004) 324–330.

Yara M. R. Müller, Lilianna B. D. Rivero, Márcia C. Carvalho, KarolineKobus, Marcel Farina, Evelise M. Nazari. 2008. Behavior alimpairments related to lead induced developmental neurotoxicity in chicks. Arch Toxicol. 82:445–451. DOI 10.1007/s00204-007-0266-6.

Zhigang, D. L. and Harvey, A. B. 1997. The influence of Pb on expression of acetyl cholinesterase and the acetylcholine recepor. Toxicology and Applied Pharmacology. 145: 237 – 245.

ISSN-On line: 2414-8849