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HISTOARHITECTONICS OF OVARY AND UTERUS AFTER LONG-TERM EXPOSURE TO LOW LEAD LEVELS IN FEMALE RATS

Eugenia Dumitrescu[1] | Viorica Chiurciu*[2] | Roxana Popescu[3] | Florin Muselin[1] | Diana Brezovan[1] | Marius C. Pentea[1] | Romeo T Cristina[1]

1) USAMVB, Faculty of Veterinary Medicine Timisoara, Romania; **2)*** Drugs Production Dept., Romvac Company, Voluntari, Romania, chiurciu@romvac.ro;
3) University of Medicine and Pharmacy `Victor Babes` Timisoara, Romania

KEY WORDS

histoarhitecture, genital, lead, rat, reproductive toxicology, AAS

Introduction

Lead is a heavy metal widely dispersed in the environment, which remains in biotopes for large time periods. High levels of exposure can occur especially in industrial areas, more present in the developing countries. In contrast to other metals, lead does not have any physiological role in the body and there isn't known a minimum level that would be considered as non-toxic. Lead can affect all great systems from the cardiovascular, gastrointestinal, urinary and nervous to reproductive systems. Usually, exposure to lead is via dermal, oral, or by inhalation, lead being also involved in the transplacental congenital intoxication, with the following consequences. Generally, the mammalian females' reproductive lead toxicology information available is sparser than those for male, because of differences in gametes genesis and of cyclic nature of female's reproduction function.

Aim of present study appeared following our anterior researches in rat females, in this topic: three months study (lead levels in organs and tissues, hormone levels and sexual cycle duration); *in utero* exposure; suckling and pre-pubertal exposure and respectively two generation study in female rats, to continue to follow lead's accumulation levels in the ovaries, Fallopian tubes and uterus in a 12 months chronic exposure study [7-13].

Materials and Methods

Animals

Animals were purchased from the authorized biobase of the "Victor Babes" University of Medicine and Pharmacy from Timisoara. It was in our concern to choose, as possible, all females with the same body weight, the average weight for experimental groups being of: E1 = 220.0 ±0,72 g; E2 = 222.5 ±1,16 g; E3 = 220.5 ±0,82 g and respectively C = 221.0±0,82 g. Before starting the experiment, rat females were subjected to a seven-day period of acclimatization and them health was confirmed clinically. The animals were kept in the same experimental room for all experimental period. Rat females were housed in four polycarbonate cages with 750 x 720 x 360 mm (L x w x h) dimensions, eight females each / cage / group. As bedding wood shaving was used. The environmental temperature was maintained at 20 ± 2 °C and relative humidity of 55 ± 10%. During experimentation period, the light cycle was 12 hours light and 12 hours dark. Non-sterile pelleted diet (Diet, Biovetimix, code 140-501, Romania) and tap water were offered *ad libitum*.

The evaluation on reproductive system integrity of leads toxic effect biomarkers was carried out on 28 white Wistar female rats at sexual maturity (120 life days), divided in four groups: three experimental (E1, E2, E3) and one control (C). The individuals from E groups were exposed to lead, as soluble lead acetate (Merck, Germany) consumption, in drinking water, twelve months, as follows: E1: 0.050 mgL^{-1} (maximum admitted level in drinking water, after Romanian drinking water quality Law) [17], E2: 0.100 mgL^{-1} and respectively E3: 0.150 mgL^{-1}. The control group received, in the same housing conditions, only tap water.

In about 24 hours after the last administration, rats were euthanized. Euthanasia method used was that by overdosing anaesthetic agents using association: ketamine (Ketamine 10%, CP Pharma), dose 300 $mgkg.bw.^{-1}$ + xylazine (Narcoxyl, Intervet International), dose 30 $mgkg.bw.^{-1}$, in the respect of Directive 2010/63/EU and SVH AEC SOP.26, Euthanasia of mice and rats document.

Organs sampling and citohistological exam

Ovaries, Fallopian tubes and uterus were freshly collected and fixed in alcohol (80 vol.) to be prepared histologicaly. After sampling, citohistological examination was accomplished. Fragments of tissue were fixed in alcohol 80°. Paraffin blocks containing tissue fragments were sectioned on microtome, resulting in 5 µm thick sections. Sections were stained by hematoxylin & eosin method using the hematoxylin-eosin (H&E) method. Histological images were captured by using Olympus CX 41 software program, at the magnification of X 40-100.

Samples digestion and Atomic Absorption Spectrometry

Digestion was accomplished using CEM Mars X microwave digestion oven (CEM Microwave Technology Ltd. UK). Samples of sexual organs of 1.0 g were putted into digestion flasks with 10 ml nitric acid (Merck, Germany) and 5 ml of perhydrols, at 600 W, for 20 minutes at 120°C. Evaluation of lead's levels in sexual organs was accomplished after digestion by Atomic Absorption spectrometry (AAS), using an AA240 Zeeman, with graphite furnace (Varian instruments Inc. USA), programmable sample dispenser (PSD 120) (with detection limit of: 0.06 μgL^{-1}). Absorbance was determined by peak measuring and the concentration was determined based on New Rational calibration algorithm, in μgL^{-1} units. Calibration curve was made to a 283.3 nm wavelength, on five standard lead element levels, in HNO_3, 0.1%, with Slit Width of 0.5 nm. To every 10 determinations, recalibration was made, (RSD < 10%).

Statistical Analysis

All data were analyzed using *Graph Pad Prism* 5.0 (San Diego, USA). The data in different groups were compared by one way ANOVA with *Bonferroni* correction, to counteract the problem of multiple comparisons. Statistical differences were considered to be significant when $p < 0.05$, or lower.

Results

Results of lead levels found in rat female organs: ovary, uterus and Fallopian tubes, after 12 months chronic exposure to the lead acetate are presented in table and figure 1.

Table 1. Lead concentration of (**µg/g**) in tissues and organs / groups

Organ	Lot	X ±Sx	D.S.	Confidence level 95%	
				Lower	Upper
Ovary	M	10.68±0.63	1.79	9.19	12.17
	E1	15.18±0.76^{ns}	2.15	13.38	16.97
	E2	83.29±2.79***	7.90	76.69	89.90
	E3	111.40±3.77***	10.68	102.4	120.3
Uterus + Fallopian tubes	M	6.18±0.81	2.29	4.26	8.10
	E1	28.70±1.10***	3.13	26.08	31.32
	E2	54.37±1.42***	4.03	51.00	57.75
	E3	92.73±1.73***	4.91	88.62	96.84

Legend: Comparison with C group: *ns* = not significant; *** = ***p < 0.001***

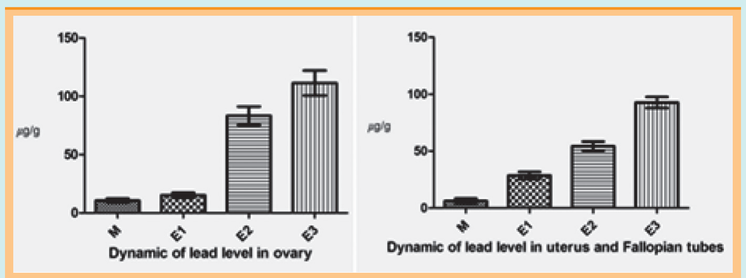

Fig. 1 *Lead levels in ovary and in uterus and Fallopian tube.*

Ovaries

Compared to the group C, the exposure to lead acetate determined a statistical significant increase of lead concentration ($p < 0.001$), in almost all experimental groups as follows: E1/C: +42.13% (ns); E2/C: 6.7 fold higher; E3/C: 9.4 fold higher), obtained results increasing significantly ($p < 0.001$) with the exposure level: E2/E1: 4.4 fold; E3/E2: 0.3 fold; E3/E1: 6.3 fold greater).

Uterus and *Fallopian tubes*

The lead level was also statistically significantly higher ($p < 0.01$) in the experimental groups E versus group C (E1/C: 3.6 fold; E_2/C: 7.7 fold; E3/C: 13.9 fold greater) and significantly ($p < 0.001$) to the level exposure (E2/E1: 0.8 fold; E3/E2: 0.7 fold and respectively E3/E1: 2.2 fold greater). The histologic modifications of studied organs are presented in figures 2 to 9. A large number of ovarian follicles in different stages of evolution were identified by microscopic examination in Control group (figure 2 and 3).

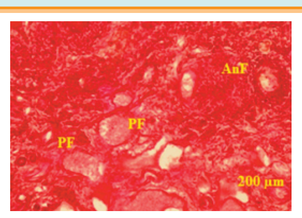

Fig. 2 *Histological investigation of rat's ovary from Control group revealed the presence of ovarian follicles in different stages of evolution (A), with PF (primordial follicles) and AnF (antral follicles). H&E stain, X100.*

The microscopic examination of rat females uterus, showed the presence of a normal structure of mucosa and uterine glands (figure 3).

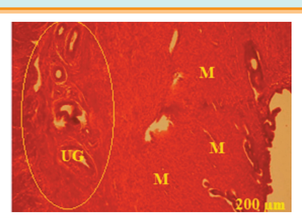

Fig. 3 Histological investigation of rats' uterus from Control group, revealed the presence a normal structure of mucosa (M) and uterine glands (UG); H&E stain, X100.

Experimental group

After the exposure to 0.050 mg L^{-1} lead, areas with optical empty spaces were present in the ovary tissue followed by diffuse oedemas and ovarian follicles denudation (figure 4).

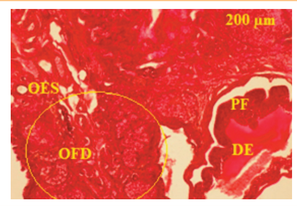

Fig. 4 Histological section in rats' ovary after exposure to 0.050 mgL^{-1} lead; H&E stain, X 100 (OES - optical empty spaces; DE - diffuse edema; PF – primordial ovarian follicles; OFD - ovarian follicles' denudation).

In Figure 5, there can be seen necrosis zones in uterus consequently to 0.050 mgL^{-1} lead exposures. After the exposure to 0.100 mgL-1 lead the rat females' ovary presented large zones of necrosis and follicular edema (figure 6).

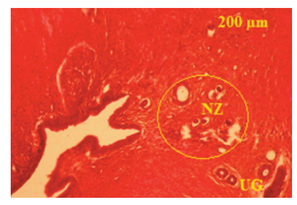

Fig. 5 Histological section in female rats' uterus after exposure to 0.050 mgL^{-1} lead; H&E stains X 100 (NZ- necrosis zone; UG-uterine glands).

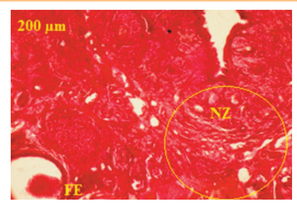

Fig. 6 Histological section in rats' ovary after exposure to 0.100 mgL^{-1} lead; H&E stain, X 100 (NZ- necrosis zone; FE- follicular edema).

The uterus examination after 0.100 mgL^{-1} lead exposure revealed necrosis of uterine glands

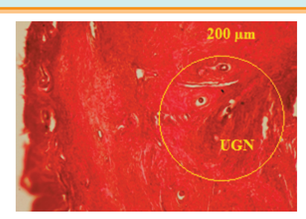

Fig. 7 Histological section in rats' uterus after exposure to 0.100 mgL^{-1} lead; H&E stain X 100 (UGN- necrosis of uterine glands).

The histologic sections made on rat females' ovary after exposure to 0.150 mgL^{-1} lead revealed the heaviest changes of this organ, especially oedemas and necrosis of the ovarian follicles (figure 8). Finally, the microscopic examination of the uterus after exposure to respectively 150 ppb Pb revealed also the necrosis of uterine glands (figure 9).

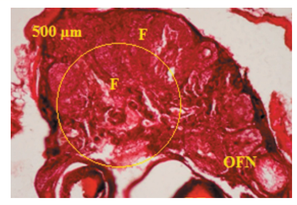

Fig. 8 Histological section in rats' ovary after exposure to 0.150 mg L^{-1}, lead; H&E stain, X 40 (DE - diffuse edemas; OFN - necrosis of ovarian follicles; F-follicles in different stages of evolution).

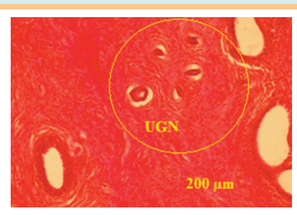

Fig. 9 Histological section in rats' uterus after exposure to 0.150 mgL^{-1}, lead; H&E stain X 100 (UGN - necrosis of uterine glands).

Conclusions

The results of our research on chronic exposure of the rat females to lead acetate in drinking water identified significant increase of lead levels' in the ovaries, Fallopian tubes and uterus, comparatively to the control group linked to the exposure level and followed by the modification of reproductive system integrity biomarkers.

Main structural changes found in ovary were: diffuse edema, necrosis in the ovarian follicles, optical empty spaces, denudation of the ovarian follicles and different stages of follicles' evolution and respectively in uterus and Fallopian tubes: necrosis areas and necrosis of the uterine glands. Based on lead obtained values, the found structural changes can be considered as lead's toxicity biomarkers and can be used by researchers as morphological lead toxicity early detecting parameters the in lab animals.

References: 34 titles

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