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Modficatons of the Morphological Structure of Ovaries and the Estral Cycles of Albino Rat Females under the Effect of Lead Acetate

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Abstract: With the help of histological, morhpometric and statistical methods, the effect of lead acetate and statistical methods were used to study the lead acetate effect on the morphological structure of white rate female ovaries and the estral cycle exposed to lead acetate. It upsets the folliculogenesis and the rat ovaries, the morphological restructuring of the ovarian cerebral and brain matter. The cerebral matter manifests a pronouced atresion of secondary follicles. The brain matter contains larger vessels proving the intensified blood supply to the ovaries. The obtained data manifest that the lead acetate affect the estral cycle duration, primarily the diastrus stage.

Key words: Ovaries · One-layer epithelium · Protein shell · Brain ovarian matter · Cerebral ovarian matter · Follicular ovarian apparatus · Estral cycle · Estrus · Diestrus and lead acetate

INTRODUCTION

The ecological situation in separate parts of the world assumes a threatening state. The scale of technogenic chemical pollution is not to be assessed precisely, but the available publications evidence a dear price the man is to pay for the civilization progress. A huge contribution to the environment pollution is due to heavy metals, among them the lead and its compounds play a particular role. They represent the polytropic toxins affecting all organs and tissues [1, 2].

Notwithstanding the published data how the lead and its compounds affect the human and animal organisms, many morphological aspects of this interrelation remain little studied [3]. So far the lead effect on the reproductive function has not been fully determined. The earlier our conducted studies show the lead acetate effect on the placenta and fetus development [4, 5], on the morphology of male sexual glands (testicles) [6, 7, 8, 9].

The present study purpose is exploration of alternations of ovarian morphological structure and the estral cycle of albino rat females under the lead acetate effect.

Methods: The biological test object were the multibred reproductive mature male rats aged 18-20 months with the stable estral cycle and body weight 200-250 grams in the summertime. All in all there were 50 animals.

In accordance with the formulated tasks, the animals were split into two groups. The control group comprised 20 females fed on the common pen rations. The control group comprised 30 females fed during 7 days fed on ration of lead acetate $Pb(CH_3COO)_2\ 3H_2O$ in a dose 45 mg/kg/day. The total estral cycle duration, duration of individual phases and their rhythmic alternation, were taken into account. To obtain a fuller characteristic of the estral cycle, the number of cycles per female during the last 22 days of exposure, was studied too.

Due to the short reduction of cycle duration from 4 to 6 days, the experimental and control animal groups at the estrus and diestrus stages were selected according to the follicular and lutein ovarian phases.

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The estral cycle dynamics in the control and experimental animal groups was studied following the rule of one hour. The estral cyclicity was studied was studied by analyzing the uterine smears under the digital microscope Axio Imager.M2 (ZEISS, Japan) at the magnification 100×10:

- The estrus smears consisted virtually of keritinized nucleus-free epithelial cells (≥90%);
- The diestrus smear contained mainly leucocitary cells (≥60%) and rare epithelial cells [10].

The animals were decapitated at the diestrus phase under ether narcosis with chloroform (1:1) observing the European community principles of humanity (86/609/EEC) and Helsinki declaration and following the rules of conducting works when using experimental animals.

The female ovaries from control groups were subjected to histological study. The tissue specimens were fixed in a 10% neutral formalin solution. The fixed specimens were rinsed in running water and then were subjected to dehydration by placing the studied material in alcohol of growing concentration and cast in paraffin following the common technique. Histological ovarian cross sections were prepared 10-15 μ thick and died with hematoxilin eosin to be studied under the digital microscope Axio Imager M2 (ZEISS, Japan) with the software to analyze images AxioVision SE64 Rel. 4.8.3 and ZEN 2011. The preparations were photographed with a digital camera AxioCam MRc5 (ZEISS, Japan) and then the images were processed in the Abode Photoshop Elements 11.

The review miscopy comprised the morphological analysis of the structural tissue ovarian components, after that the morhpometric parameters were studied, namely, the one-layer epithelium covering the ovary, the protein shells; the ovarian cross sectional area; the cerebral and brain matter ovarian area and thickness; the number of various types of follicles and yellow bodies in the ovarian cerebral matter.

The morhpometric measurements were conducted at the magnification 20×10 and 100×10. The resolution of obtained images was 1300×1030 pixelles.

The digital data were processed statistically using the software FStat and Excel. The statistical hypotheses were checked with the Student t-criterion. When assessing the statistical hypotheses, the following significance levels were used: $p \leq 0.05$. The morhpometric studies were processed mathematically using the correlation analysis technique.

Main Part: The accomplished histological and morhpometric studies have revealed the condition of main ovarian structures in albino rats: follicles, white bodies and vessels both in the control and experimental groups.

The accomplished study has revealed in the control group the singe-layer epithelium covering the ovary outside and having the cubic shape. The protein shell has a homogeneous structure and is weakly vascularized (Figure 1). The ovarian cerebral and brain layers are well distinct.

The ovarian critical matter has the follicles at different development stages up to mature graaf bubbles (tertiary follicles). The primordial follicles arrange directly beneath the ovarian protein shell in compact groups. The single primordial follicles are come across just rarely. Some places contain the atrethic follicles. The cells of connective cortical matter base are spindle shaped (Figure 2). The detected yellow bodies are rounded and at the stage of involution or at the forming stage (Figure 3).

The cerebral matter is rather small âcompared with the brain and it is well vascularized. Rather small blood vessels pass from the cerebral matter into the brain. The connective tissue base of the cortical matter is disordered (Figure 4).

After 7 days of lead acetate peroral administration, the accomplished morphological study has revealed that the single layer epithelial cells coving the ovary outside are elongated. The protein shell versus control has a denser structure (Figure 5).

The ovarian cortical matter is increased versus brain. Primordial follicules arrange predominantly as singles. The atrethic follicules have the noteworthy concentration. Atresion most often affects secondary growing follicules; tertiary follicles are less often affected (Figure 6, 7). The detected yellow bodies have irregular shape (Figure 8).

The cerebral matter contains larger vessels versus control. It is evidenced by intensification of ovarian blood supply (Figure 9).

The morphological studies have revealed that the experimental animal group versus control that the single layer epithelium is thinning together with the protein shell; the ovarian cortical matter thickness and area are, respectively, 35.55% ($P \leq 0.05$), 13.88% ($P \leq 0.05$), 12.04% ($P \leq 0.05$) and 6.18% ($P \leq 0.001$). Meanwhile, the ovarian cross section grows in the critical matter area and thickness was, respectively, 2.85% ($P \leq 0.001$), 30.03% ($P \leq 0.001$) and 19.01% larger ($P \leq 0.05$) (Table 1).

The quantitative analysis of the ovarian follicular apparatus has revealed that the experimental animal group versus control shows the reduced number of primordial,

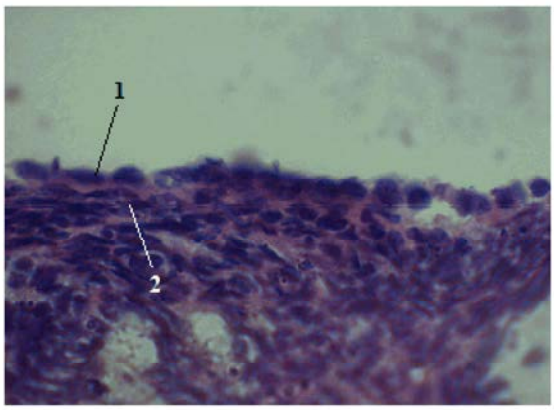
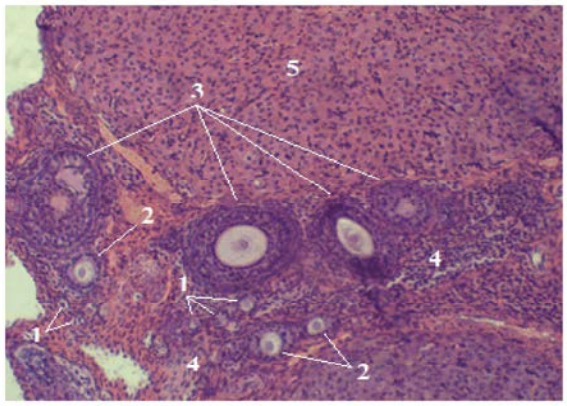
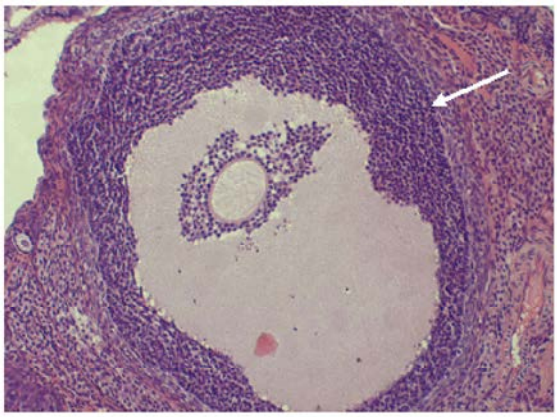


Fig. 1: Ovarian surface (control). Hematoxylin and eosin dye. Magnification 100×10: 1 – single layer epithelium; 2 – protein shell



A



B

Fig. 2: Ovary section (control). Hematoxylin eosin dye. Magnification 20×10: À – cortical ovarian matter; 1 –primordial follicules; 2 – primary follicules; 3 – secondary follicules; 4 – connective tissue base; 5 – yellow body; B – tertiary follicle

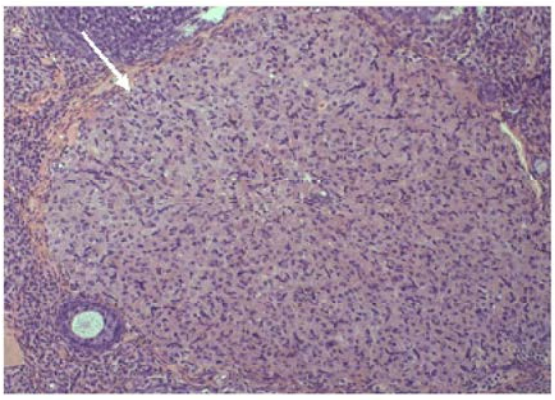


Fig. 3: Ovarian yellow body (control). Hematoxylin eosin dye. Magnification 20×10

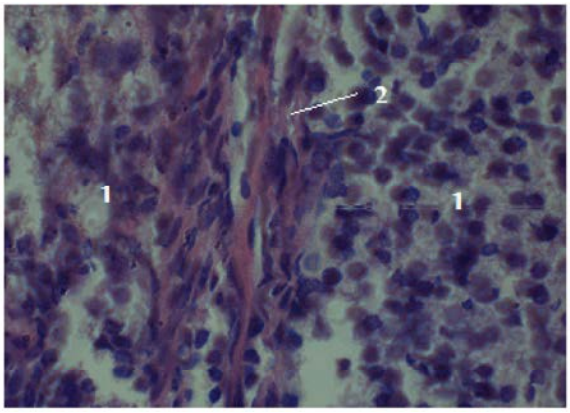


Fig. 4: Ovarian cortical matter (control). Hematoxylin eosin dye. Magnification 100×10: 1 – connective tissue base; 2 – blood vessel

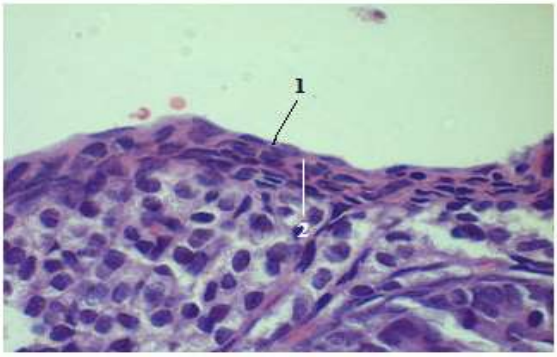


Fig. 5: Ovary surface (experiment). Hematoxylin eosin dye. Magnification 100×10: 1 – single layer epithelium; 2 – protein shell

primary, secondary and tertian follicules and yellow bodies, respectively, by 15.12% (P≤0.05). 18.73% (P≤0.05). 44.32% (P≤0.05). 49.43% (P≤0.05) и 45.30% (P≤0.05). meanwhile, the number of atrethic follicules has grown by 66.23% (P≤0.05) (Table 2, Figure 10).

Table 1: Macroscopic ovarian indicators of albino rats

Indicators	Control	Experiment
Thickness of single layer epithelium, μ	10.07±0.48	6.49±0.24*
Thickness of ovarian protein shell, μ	19.67±0.38	16.94±0.39*
Ovarian cross sectional area, $\times 10^3$, μ^2	9586.76±25.50	9868.51±28.27**
Ovarian cortical matter area, $\times 10^3$, μ^2	6912.15±23.79	6484.89±22.63**
Ovarian root matter area, mcm	1538.98±18.73	1353.73±21.07*
Ovarian cortical matter area, $\times 10^3$, μ^2	2176.05±24.84	3110.23±22.63**
Ovarian cortical matter thickness, μ	799.43±3.79	987.09±5.92*

Note: * – $p \leq 0.05$ versus control animals;
** – $p \leq 0.001$ versus control animals.

Table 2: Quantitative indicators of ovarian cerebral matter structural components in albino rats

Ovarian structural cerebral matter components	Control	Experiment
Primordial follicules	17.72±1.31	15.04±0.78*
Primary follicules	13.88±1.09	11.28±1.07*
Secondary follicules	10.92±0.98	6.08±0.68*
Secondary follicules	7.04±0.77	3.56±0.63*
Atrethic follicules	2.08±0.78	6.16±0.16*
Yellow bodies	4.68±0.68	2.56±0.62*

Note: * – $p \leq 0.05$ versus control animals.

Table 3: Estral stage duration and is separate stages among albino rats and separate stages

	Indicators		
Group	Estral cycle duration, days	Estrus cycle duration, days	Diestrus cycle duration, days
Control	4.13±0.26	1.33±0.11	2.80±0.15
Experiment	4.67±0.28*	1.20±0.12*	3.47±0.19*

Note: * – valid in respect to control $P \leq 0.05$.

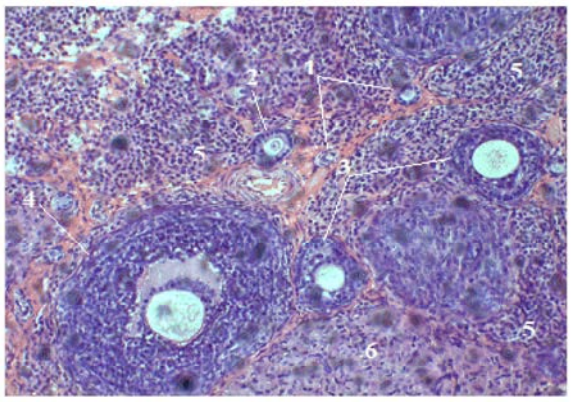
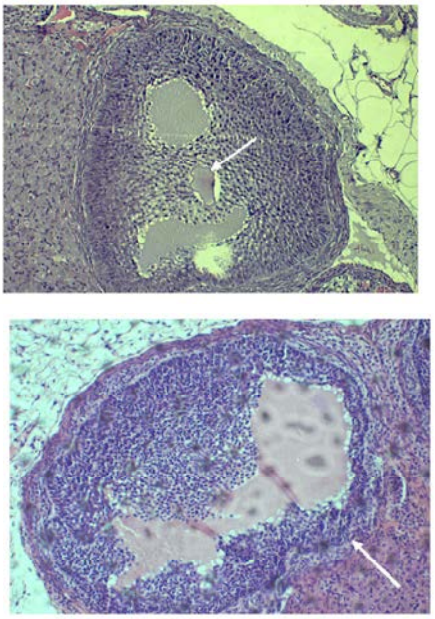


Fig. 6: Ovarian cortical matter (experiment). Hematoxylin eosin dye. Magnification 20×10: 1 –primordial follicules; 2 – primary follicules; 3 – secondary follicules; 4 – tertiary follicle; 5 – connective tissue base; 6 – yellow body.

The obtained data manifest that rat ovaries react to the lead acetate effect by reduction of the reproductive ability. The lead acetate reduces the number of estral cycles during 22 days from 4.27±0.39 to 3.60±0.48, i.e. by 15.69% ($P \leq 0.05$), the duration prolongs by 11.56% ($P \leq 0.05$), the phase sequence gets upset towards diestrus.



A

B

Fig. 7: Follicular atresion (experiment). Hematoxylin eosin dye. Magnification 20×10: A – Follicular atresion onset (the arrow shows wrinkled shining oocyte shell); B – atrethic follicle

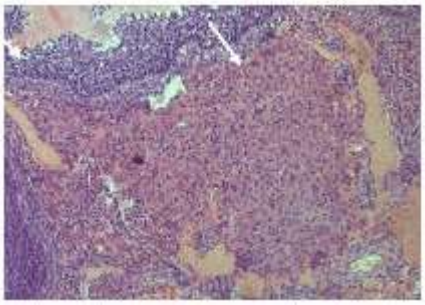


Fig. 8: Ovarian yellow body (experiment). Hematoxylin eosin dye. Magnification 20×10

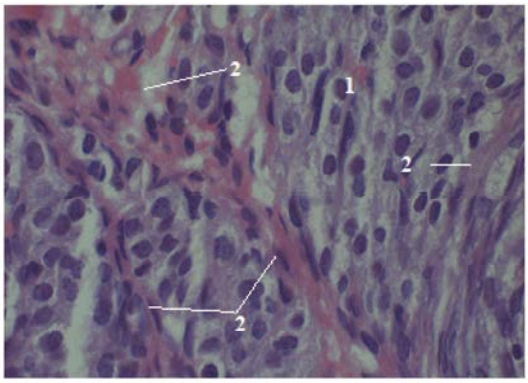


Fig. 9: Ovarian cortical matter (experiment). Hematoxylin eosin dye. Magnification 100×10: 1 – connective tissue base; 2 – blood vessels

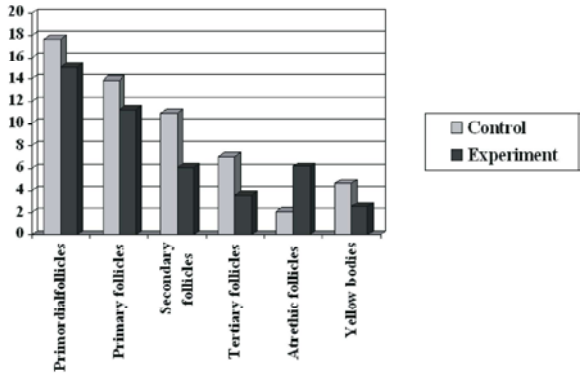


Fig. 10: Quantitative indicators of cerebral matter ovarian structural components of albino rats

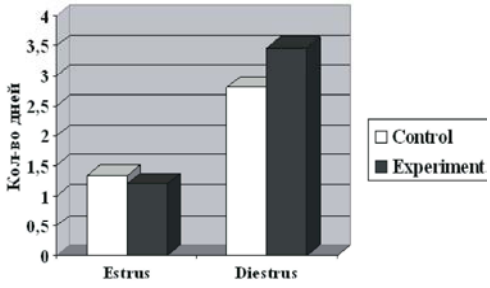


Fig. 11: Duration of estral cycle among albino rate females

The estrus stage shortens by 9.78% ($P \leq 0.05$) and the diestrus extends by 19.31% ($P \leq 0.05$) (Table 3, Figure 11).

CONCLUSIONS

The accomplished studies manifested that the lead acetate leads to negative effect on the rat reproductive system. The ovarian morphofunctional restructuring of the cerebral and brain matter occurs. The thickness thinning covering ovaries outside the one layer epithelium occurs, the protein shell and the ovarian cerebral matter becomes thicker mean while, the cerebral matter expands and thickens.

The cerebral matter has a vividly pronounced follicular atresion. The atresion is more frequent in the secondary growing follicules than in the tertiary follicules.

The cerebral matter contains larger vessels evidenced by intensified blood supply to ovaries.

Due to the ovarian morphological changes, the lead acetate reduces the number of cycles per female, the atrus stage shortens and the diestrus stage prolongs.

CONCLUSIONS

- The lead acetate suppresses the follicogenesis due to reduction of the number of primordial, primary, secondary, tertiary follicules and yellow bodies at the same the number of atrethic follicules grows.
- The lead acetate affects the estral cycle among albino rat females manifested by upset phase alternation and by prolongation of the diestrus duration.

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