

## **BIOLOGY**

### **MORPHOFUNCTIONAL CHANGES OF THE RATS PLACENTA AND OVARIES UNDER THE INFLUENCE OF LEAD ACETATE AND ITS COMBINATION WITH METAL CITRATES**

*Vira Maior, Candidate of Biological Sciences,  
Iryna Kolosova, Candidate of Biological Sciences,  
SE «Dnipropetrovsk Medical Academy of Health Ministry of Ukraine»*

**Annotation.** *The formation of the rats fetus and placenta, functional state of rats ovaries under injection of lead acetate and its combined injection with citrate metals (gold, silver) during pregnancy was researched. Embryotoxic, gonadotoxic and placentotoxic effect of lead acetate in isolated injection was found, modifying effect of citrate metals on toxicity of lead acetate in combined injection was found.*

**Key words:** *placenta, ovaries, embryotoxicity, lead acetate, gold citrate, silver citrate.*

**Introduction.** The negative impact of anthropogenic factors on the environment and population of urban areas increases with the development of scientific and technological progress. The most dangerous are the heavy metals that enter the atmosphere, soils and reservoirs as a result of industrial emissions, followed by inhalation, oral or transcutaneous intake to the human body [12]. Heavy metals, even in microdoses, can cause damage to sensitive body systems [14], as they are characterized by high toxicity and biochemical activity. One of the most common heavy metals is lead and its compounds [1, 16]. The effect of lead on the human body is accompanied by a dysfunction of all organs, most sensitive to the lead influence are the circulatory, nervous, cardiovascular, digestive and urinary systems [2, 9]. Lead compounds also have an effect on the reproductive system by reducing fertility and occurrence of infertility [6, 13, 18]. Analysis of scientific literature data has shown that exposure to lead compounds increases the risk of prenatal and postnatal lesions to the organisms, leading to stillbirths, miscarriages, structural and functional alterations of the female reproductive system [8, 11]. The issue about the influence of lead low doses on the process of embryogenesis, structure and functioning of generative system organs and the condition of the fetoplacental complex remains incomplete.

The placenta is a temporary extraembryonic organ with various functions that provides the connection of the fetus with the parent organism. The study of morphofunctional changes in the placenta, as an organ that has protective properties, prevents the transport of toxic substances to the fetus and promotes their deactivation [3, 5] is an actual problem.

The search for substances that contribute reducing or neutralizing the negative action

of lead in the body is an important issue; one of the most promising areas in modern conditions is the applying of nanotechnologies. The gold and silver nanoparticles that are used in the manufacture of cosmetics, pharmaceuticals and agriculture are of greatest interest [4, 7, 15, 17, 19]. At the same time, the literature does not contain information about the interaction of nanoscale gold and silver with toxic metals, their joint effect on the development of the fetus and placenta and on the structure and function of ovaries, as a key link in the reproductive system. In this regard, the in-depth study of the morphofunctional state of ovaries under the action of heavy metals and detection of their antagonists in the context of environmental pollution is extremely urgent and modern problem.

The search of lead acetate bioantagonists among nanomaterials in the future may contribute to the development of biodrugs of directed action using nanobiotechnologies to reduce lead toxicity.

The purpose of the study is to detect the features of morphofunctional changes of rat placenta and ovaries under isolated injection of lead acetate and the combined injection of lead acetate with citrates of metals (gold, silver) and to determine the effect of the studied substances on the embryogenesis.

**Materials and methods of research.** In the experimental research, a complex of adequate methods and research material was used that made it possible to determine the effect of lead acetate in isolated administration and in combinations thereof with gold citrate or silver citrate on the morphofunctional state of the placenta and ovaries of experimental animals and the development of the fetuses.

In an experimental study, sexually mature female rats from the Wistar line were used. At the beginning of the experiment, the study of the estral cycle of the rats was carried out by studying the vaginal smears to determine the stage of the estrus (vesicle), which was followed by pairing. The first day of pregnancy was determined by the presence of sperm in the vaginal smears, after that the animals were divided into groups and the injection of the studied substances - lead acetate and citrates of gold and silver were started.

In the experiment, 96 female rats were examined, which were divided into 4 groups (of 24 individuals) with 3 subgroups (of 8 individuals) in each group. Group 1 (experimental group «E №1») – animals that were injected with a solution of lead acetate in a dose of 0.05 mg / kg; group 2 «E №2» – animals that were injected with a solution of lead acetate in a dose of 0.05 mg / kg and a solution of gold citrate in a dose of 1.5 mg / kg; group 3 «E №3» – animals that were injected with a solution of lead acetate in a dose of 0.05 mg / kg and a solution of silver citrate at a dose of 2 mg / kg; group 4 – control, animals that were injected with distilled water. Solutions of metals and citrates of metals were injected to females through the probe once a day, at the same time, from the first day of pregnancy to a certain period of the study: in the first subgroup of each group up to the 11th day of pregnancy, in the second – up to the 15th day and in the third – up to the 19th day of pregnancy, respectively on the 12th, 16th or 20th day of pregnancy the animals were withdrawn from the experiment by injection of the overdose of etheric

anesthesia.

Fetuses and placentas were taken for further research, counting the number of yellow bodies in the ovaries. The placentas and ovaries after the measurement of the mass and size were fixed in a neutral 10 % solution of formalin, followed by the preparation of histological slides. A total of 862 fetuses, 192 ovaries and 594 placentas were obtained. The placenta was studied on 16th and 20th days of pregnancy. At 12th day of pregnancy the placenta can not be separated from the uterus, therefore, 2 control periods were selected.

Estimation of the results of the influence of metal solutions on embryogenesis was carried out on the indicators of general, preimplantation, post implantation mortality of embryos, fetus and placenta mass, fetus-placental coefficient. The teratogenic effect of lead acetate and its combinations with metal citrates was determined by the method of J.G. Wilson.

In accordance with the goals and objectives, an adequate set of methods of morphological research was used: anatomical, macroscopic examination, histological, morphometric, biostatistics.

**Results of the research and their discussion.** It is known that the compounds of heavy metals can cause metabolic changes, morphofunctional rearrangements of organs and systems in organism, due to their polythropicity [10, 16]. In actual experimental study, it has been shown that lead acetate in a dose of 0.05 mg/kg in daily injection to pregnant rat females causes morphofunctional changes in reproductive organs, namely ovaries and placenta, and has embryotoxic properties.

The analysis of the data obtained as a result of the experimental study showed that the injection of lead acetate in rat during pregnancy leads to an increase the level of embryonic mortality twice, a reduction in the number of live fetuses per 1 female by 23.0 % ( $p < 0.05$ ) and a decrease in the weight of the fetuses by 14.6 % ( $p < 0.05$ ), found the embryotoxic effect of lead acetate in the dose 0.05 mg / kg.

In combined injection of lead acetate with gold citrate, a decrease in the level of embryomortality was detected to control values in the absence of post-implantation mortality; there was an increase in the number of live fetuses per female on average by 44.5 % on the studied terms of pregnancy compared with the lead exposure group; The fetal-placental coefficient was equal to the control value.

Under the conditions of the injection of lead acetate combined with silver citrate, the tread effect of silver citrate on embryogenesis is manifested. An increase in the number of live fetuses per 1 female was detected by 40.7 %, a decrease in the total embryonic mortality by 60.8 % on average during the studied pregnancy compared with the isolated injection of lead acetate, at the same time, the fetal-placental coefficient corresponded to the norm.

The teratogenic effect of all test substances was not detected.

It was determined that the effect of lead acetate on intragastric injection is reflected on the morphofunctional state of the rats placenta, promotes the development of a circulatory disorders complex and quantitative changes in the histological elements of

the placenta, and the lag in the development of the placenta of this experimental group is determined. Thus, a significantly lower mass of placenta in the 16th day of pregnancy was detected, while simultaneously reducing the overall placenta thickness with a change in the percentage of maternal and fetal layers of the placenta, which was in the norm 30 / 70 %, in the lead acetate exposure group 34 / 66 % ( $p < 0.05$ ).

In the placenta of the 20th day of pregnancy, an increase at 12.6% ( $p < 0.001$ ) in the total thickness of the placenta was observed, due to the expansion of the capillaries of the labyrinth section as a response to hypoxia caused by the injection of a solution of lead acetate and accompanied by an increase in relative volume Kashchenko-Hofbauer cells that take part in the processes of germination of villi and the development of placenta vessels.

The presence of discirculatory disorders was noted in the group E №1, which was manifested in a decrease in the relative volume of maternal lacunae, allantoic vessels, and dilation of the central placental vessels. There was a violation of the trophic function of the placenta, as evidenced by an increase in the relative volume of «glycogen» cells in both gestational periods, with a significantly lower amount of glycogen granules in them compared to the control group.

The aforementioned changes in the aggregate are signs of placental insufficiency, which occurs on the background of hypoxia, caused by the effect of low doses of lead acetate solution during pregnancy.

The analysis of macroscopic and microscopic placental restructure showed the emergence of compensatory and adaptive reactions in this group of rats, as evidenced by a complex of morphometric changes. Thus, the mass of placenta in the 16th day of pregnancy exceeded the values of the group E № 1 by 9.2 % ( $p < 0.05$ ), and at the 20th day it was not significantly different from the placenta of the control group with simultaneous increase of the total thickness of the placenta with the growth of all its layers. The relative volume of giant cells at the 16th day of pregnancy increased by 50.2 % ( $p < 0.05$ ) relative to the group of isolated lead acetate injection and did not differ significantly from the control group, and on the 20th day it was equal to it. «Glycogen» cells contained a large number of glycogen granules, in contrast to the isolated lead acetate injection group.

In the group E № 2, enhanced blood filling of lacunae and capillaries was observed, an increase in the relative volume of maternal lacunas in the 16th day of pregnancy compared with the lead acetate introduction group was noted at 58.8 % ( $p < 0.01$ ). The average diameter of the fetal vessels did not have a significant difference with the control group in both terms of pregnancy.

The diameter and mass of the placenta of the group E № 3 were within the statistical norm in both investigated terms of the pregnancy. The total thickness of the placenta of this group was higher than that in control at the 20th day of pregnancy by 14.7 % ( $p < 0.001$ ), but the ratio of layers did not change due to the uniform growth of all layers. At the 20th day of pregnancy there was a normalization of the relative volume of giant cells, an increase in the relative volume of «glycogen» cells at high levels of glycogen in it.

The effect of lead acetate combined with silver citrate leads to full-blooded of the lacunae, fetal vessels and capillaries, a decrease in the relative volume of maternal lacunas and a decrease in the mean diameter of the vessels compared to the lead acetate injection group (22.8 % ( $p < 0.01$ )) at the 16th day and 30.3 % ( $p < 0.001$ ) at the 20th day of pregnancy) with a normal index of their relative volume.

Consequently, the injection of gold and silver citrates against the background of lead effect contributes to the formation of a number of adaptations in the placenta, which enable to provide the developing fetus by necessary substances and contribute to the normal functioning of the placenta.

The completed research have established that the injection of lead acetate in the dose of 0.05 mg/kg over the course of 12, 16, 20 days negatively impacts on the morphological-functional state of ovaries and affects both organometric and histological-structural characteristics. Thus, in animals receiving lead acetate, average values of absolute (+13.1 %) and relative (+7.3 %) mass of ovaries reliably decreased ( $p < 0.05$ ) as well as mass index of the investigated organ (+7.4 %). Index of influence of solutions of metals (I) of the test compound below the unit value from ( $0.78 \pm 0.033$ ) c.u. to ( $0.93 \pm 0.045$ ) c.u., is the objective verification of the negative effect of lead acetate on mass-metric indices, which indicates to the inhibition of gonads development.

It has been found that injection of low doses of lead acetate (0.05 mg/kg) leads to: accelerated and active atresia of follicles, which is manifested by the decrease in the total number of primordial follicles (on average by 24.3 %,  $p < 0.01$ ) in the increase of atretic (on average by 2.8 times,  $p < 0.001$ ) in the ovary of rats; decrease in the number (on average by 7.3 %,  $p > 0.05$ ) and sizes of yellow bodies (an average by 18.0 %,  $p < 0.05$ ), their premature regress, degeneration and reduction of luteocytes content, enlargement of organ stroma, hemodynamic disturbances which contribute to the enhancement of alternating effect of lead acetate.

By the analysis of organometric indices in the research groups of combined effect of lead acetate with citrates of gold / silver in comparison with the group of lead exposure, there was established the increase in indices of absolute mass of ovaries according to investigated gestation terms (12th, 16th, 20th day): E № 2 – by 28.2 % ( $p < 0.01$ ); 25.0 % ( $p > 0.05$ ); 15.8 % ( $p < 0.05$ ); and E № 3 – by 31.2 % ( $p < 0.001$ ), 25.7 % ( $p < 0.001$ ), 10.4 % ( $p < 0.05$ ), and relative mass of ovaries: E № 2 – by 20.1 % ( $p < 0.001$ ); 20.4 % ( $p < 0.001$ ); 15.8 % ( $p < 0.05$ ); and E № 3 – by 27.9 % ( $p < 0.001$ ); 22.3 % ( $p < 0.001$ ); 10.4 % ( $p < 0.05$ ), increase in total parameters of ovarian volume with simultaneous decrease in specific gravity.

Thus, completed research have determined that injection of lead acetate with citrates of gold and silver into the organism of pregnant rat female reduces toxicity of lead and positively influences on mass-metric indicators of ovaries and testifies to the presence of compensatory reactions in the organ under the influence of citrates of metals in terms of introduction of lead acetate, this is confirmed by the positive average values of the index I, which makes up ( $1.02 \pm 0.022$ ) – ( $1.14 \pm 0.062$ ) c.u.

It has been found that in experimental groups of combined injection of lead

acetate with citrates of metals, generative and stromal elements of ovaries did not have significant differences from the control group. However, in rats receiving lead acetate in the groups undergoing combined injection, average indices of number of primordial follicles increased, they were arranged reliably ( $p < 0.01$ ) in decreasing order: E № 3 (+ 38.5 %) > E № 2 (+ 27.6 %), and the number of atretic follicles reliably ( $p < 0.001$ ) decreased, the latter were arranged as follows: E № 3 (-65.5 %) > E № 2 (-62.9 %), testifying to a larger follicular reserve and a slower process of atresia in ovaries of rats in combined administration of lead acetate with citrates of metals.

It has been established that in groups of combined action of lead acetate with citrates of gold / silver in relation to the group of isolated injection of lead acetate, both indices of the number of yellow bodies: E № 2 (22.2 %) > E № 3 (+ 15.7 %), and indices of the relative area of yellow bodies: E № 3 (+66.2 %) > E № 2 (+ 64.7 %) had more higher values on average.

It has been found that in groups of combined action of lead acetate with citrates of gold / silver in relation to the group of isolated injection of lead acetate, indices of yellow bodies on average had more higher values: E № 2 (22.2 %) > E № 3 (+ 15.7 %), this was also typical for indices of the relative area of yellow bodies: E № 3 (+66.2 %) > E № 2 (+ 64.7 %).

The results of the study make it possible to predict the degree of embryonic toxicity of low doses of lead acetate, its influence on morphofunctional state of mammals' ovaries, being an important prerequisite for developing a framework of preventive and corrective measures in lesions to the genital sphere by lead compounds.

**Conclusions.** 1) On the basis of the complex of morphological and morphometric studies, it has been proved that experimental animals administered during pregnancy citrate metals against the backdrop of lead exposure the 20th day of pregnancy forming compensatory adjustment in the placenta which leads to improvement of morphofunctional development rates compared with the isolated lead introduction group, namely: the growth of the labyrinth, increased blood supply to the placenta, the normalization of the relative volume of giant cells and the increase in the amount of glycogen in the «glycogen» cells. The combination of these adaptations contributes to form a live fetus during pregnancy, accompanied by the influence of lead compounds.

2) Isolated injection of lead acetate negatively impacts on morphological-functional condition of rats' ovaries and leads to the changes of organometric indicators of gonads with accelerated and active atresia of follicles, reduction of the corpora lutea sizes, their premature regression, proliferation of ovaries stroma, hemodynamic disturbances. In the groups of animals with combined impact there was revealed compensatory effect of metal citrates on gonadotoxicity of lead acetate, which was accompanied by the increase in the number of primordial follicles on a background of reduction of atretic and increase in the number and relative area of corpora lutea in all investigated stages of gestation.

3) Performed experimental research allowed to determine modifying properties of gold / silver citrates for the negative impact of lead on morphofunctional state of placenta and ovaries of rats and processes of embryogenesis, the most pronounced biological

antagonism was observed in the group of combined influence of lead acetate and silver citrate.

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