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Morphology of the Ovaries in Condition of Inhalation Intoxication with Dust-Saline Aerosols of the Aral Sea in Female White Rats

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Abstract

BACKGROUND: After some clinical studies of the reproductive health of the female population of the Aral Sea region, we concluded that it is necessary to confirm by experiment the theory of the direct influence of dust-saline aerosols of the Aral Sea on the reproductive system of female individuals.

AIM: The purpose of this work is to study the effect of dust-saline aerosols of the Aral Sea on the folliculogenesis process in rats at inhalation intoxication.

METHODS: Inhalation in rats was carried out for 30 days for 4 hours a day for 5 days a week in special inoculating cylindrical chambers with the extra-chamber placement of animals in individual boxes and dynamic aerosol delivery. Morphological changes were assessed using electron microscopy.

RESULTS: Ultrastructural changes in the ovaries of the experimental study group were characterised by the pathology of all structures of the cortical substance of the organ. The accumulation of lutein pigment, the utilisation of lipid inclusions and the destruction of the complete cell reflected the pathology of estrogen production-an important factor in the postovulatory phase of the ovarian cycle.

CONCLUSIONS: Taking into account the proven effect of dust-saline aerosols on the production of estrogen, the violation of the postovulatory phase of the ovarian cycle, we trace the mechanism of folliculogenesis disturbance. This confirms the data of our previous studies on primary and secondary infertility in women living in the Aral Sea region and the necessity of creation and development of preventive measures for the inhabitants of the region.

Introduction

The climate of the Aral Sea region and the bordering regions is sharply continental and is characterised by sharp temperature changes. Winter is cold and severe; summer is hot. There is a deficit of atmospheric precipitation and low air humidity.

A consequence of a sufficiently developed industry of South Kazakhstan, Kyzylorda, and Aktobe regions is a high anthropogenic impact on the environment, which is expressed in the pollution of atmospheric air, soil and large rivers, the accumulation of industrial waste and solid domestic waste. The oil-producing areas are characterised by soil contamination with petroleum. Chromium, boron, phosphorus is the main pollutants for areas with production and processing of minerals, in agricultural areas. The main pollutants are fluorine, phosphorus, etc. [1] [2] [3].

Negative anthropogenic factors become destructive not only for ecosystems but also a real threat to human life and health, an obstacle to sustainable social and economic development. Ecologically dependent shifts and pathological changes occur in various organs and systems under the influence of negative environmental factors-life expectancy decrease, an increase in the degree of psychophysiological and genetic stress, an increase in specific pathology, and the emergence of new forms of environmental diseases [3].

Against the background of global ecological changes affecting all components of the ecosystem, human health deserves more attention, as human resources are the main component of development [4]. The necessity to improve the quality of diseases prevention and treatment that are associated with the impact of adverse environmental factors is dictated by the increased damage that causes environmentally dependent diseases to all of humanity.

According to the monitoring of recent years and scientific research, the complex environmental pollution in the studied regions has been identified and established; it was revealed it negatively effects on the population of the Aral Sea area of Kazakhstan. A high salt load and the effect of some ecotoxicants on the organism of the population in the zones of ecological tension in the Aral Sea region have been established, and it requires an additional systematic study of public health [3] [4] [5].

The results of our study, which was conducted over a period of 3 years and examined 2527 women of reproductive age, who live at least 5 years in the Aral region and engaged in occupations with hazards of no higher than 2nd class, have the following conclusions: in the Aral region, a later attack is observed menarche, over 16 years of age in 36.6% of cases. Perinatal losses according to the history of the population 23.5%, that is, almost every third women have a history of spontaneous abortion and undeveloped pregnancy. According to our study in the Aral Sea region, 3% of women with primary infertility and 5% infertility were identified. Late menarche was observed in the absolute majority of girls, a regular cycle of menstruation is not established for a long time; in a third of them, rare and/or scarce menstruation can persist until the 18th birthday, and in such cases, the primary oligomenorrhea is diagnosed. Later, menarche is most common for girls with body weight deficiency, who have chronic diseases, increased mental and physical stress. Also, the cause of late menarche can be the effect of toxic substances on the immature organism from the environment. Such girls need a careful examination and carrying out activities aimed at preventing disorders of the sexual system in the future, including infertility, complicated course of pregnancy and childbirth, early occurrence of other abnormalities in the reproductive system. After a number of clinical studies of reproductive health of the female population of the Aral Sea region, we came to the conclusion that it is necessary to confirm by experiment the theory of the direct influence of dust-saline aerosols of the Aral Sea on the reproductive system of female individuals [6] [7] [8] [9].

The purpose of this work is to study the effect of dust-saline aerosols of the Aral Sea on the folliculogenesis process in rats at inhalation intoxication.

Materials and Methods

The sampling of the study subjects was realised taken into account the weight, age, content and diet of 30 white female rats in the dioestrus phase. Immediately before the experiment, vaginal smears of animals were studied to determine the phase of the oestrous cycle. The females in the diestrus phase were taken into the experiment. Smears (vaginal smears) were taken with a thin eye dropper, were placed on a slide and examined under a microscope at a magnification of 300 times in a darkened field.

The inhalation effect on rats was carried out for 30 days in special inoculated chambers according to the method of L. B. Borisova by standard modification of N. T. Yelevskaya for 4 hours a day for 5 days a week in cylindrical chambers with the extra-chamber placement of animals in individual boxes and dynamic aerosol delivery. The holes of the boxes are directed by head part inside the chamber. The air compressors are fixed-mounted compressors. Shallow was placed in the distributor for ¾ of its volume. The air was drawn in and out of the chamber at a rate of 50 l/min. A sampling of air to control the concentration of aerosol in the chamber was carried out every 30 minutes on the filters using a portable dust analyser «Prima 01». The euthanasia of the experimental and control animals was carried out by decapitation. The excretion of animals from the experiment was carried out in the diestrus phase for a more accurate result. The work with animals was approved by the Committee on Bioethics at the Karaganda State Medical University and corresponded to the international rules for the use of animals in the experiment.

The material for the study was the ovaries of mature females of white rats. For transmission electron microscopy, pieces of the ovaries were fixed in glutaraldehyde on phosphate buffer and fixed in 1% solution of osmium tetroxide. Semi-thin sections stained with blue toluidine were studied by the light-optical method. Ultrathin sections (30-60nm) were obtained by ultramicrotome Leica U70, after Reynolds contrast, they were examined and photographed on a transmission electron microscope Libra 120 Carl Zeiss.

Results

Electron microscopic examination of the ovaries of experimental animals in the control group showed that the flattened follicular epithelium of the primordial (primary) follicle contained an elongated form of the nucleus with initially located heterochromatin and an enlarged perinuclear space. There was large mitochondrion (M) with an electronically transparent matrix and cristae in the cytoplasm. Well-developed elements of the Golgi complex-a stack of flattened cisterns and small vesicles were near the nucleus. The tubules of the granular endoplasmic reticulum (GER) were enlarged with the flaky material in the lumen (Figure 1). Also in the cytoplasm, there were lysosomes, free ribosomes and large lipid granules. The single-row follicular epithelium had a thin basal membrane and a smooth apical surface.

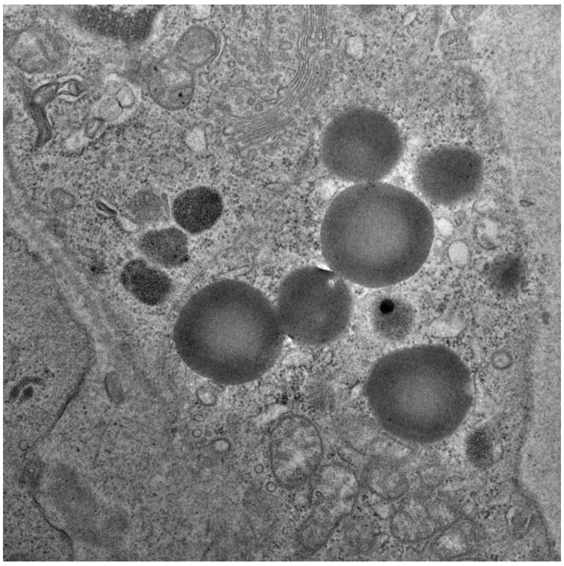

Figure 1: Control; Mitochondria (M), the Golgi complex (GC) and lipid granules (LG) are seen in the cytoplasm of the flattened primary follicle; BM-basal membrane; Electron diffraction pattern; Magnification x4000

Theca interna cells under the epithelium had an elongated shape with thin processes (Figure 2). There were polymorphs in the form of mitochondria and tubules of the GER, small lysosomes in the centre of the cytoplasm. Microtubules and pinocytosis vesicles were visible on the periphery of the cytoplasm.

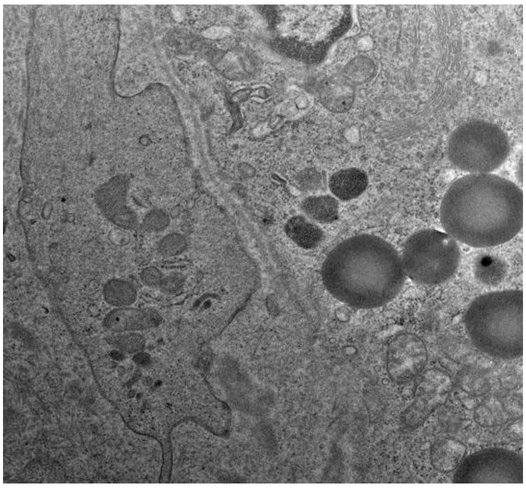

Figure 2: Control; Fibroblast-like theca interna cell; Electron diffraction pattern; Magnification x4000

The follicular epithelium of secondary follicles, represented by several layers of proliferating epithelial cells, was characterised by a large irregularly shaped nucleus with a high proportion of euchromatin and an enlarged perinuclear area. The cytoplasm contained well-developed tubules of the GER and large mitochondria (Figure 3).

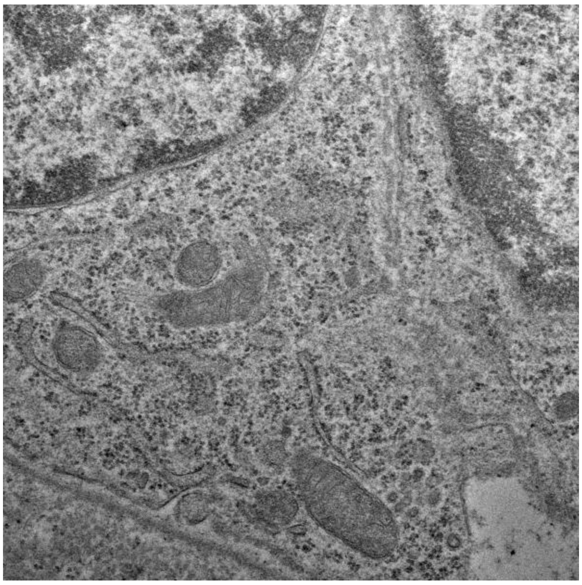

Figure 3: Control; Epithelial cell of the developing follicle; N-nucleus; M-mitochondria, granular endoplasmic reticulum (GER); Electron diffraction pattern; Magnification x4000

Slightly spaced apart follicular fluid cells had a smooth cytoplasmic surface. The apical surface of superficially located cells was characterised by small processes (Figure 4). Near the apical pole of the cell were located the dichotomies of the Golgi complex.

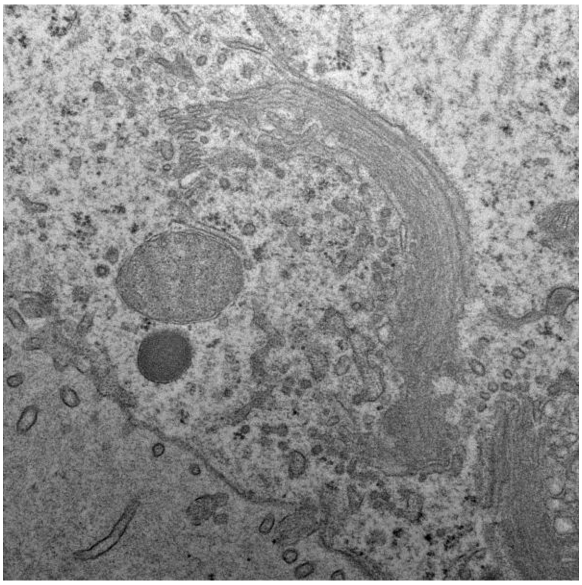

Figure 4: Control; the Apical surface of the follicular cell with the Golgi complex (GC) and microvilli; Electron diffraction pattern; Magnification x8000

Maturing large follicles had a large number of cell layers, marked by the structure of the epithelium. Large nuclei contained euchromatin, the content of mitochondria, GER, and polyribosomes increased sharply in the cytoplasm (Figure 5).

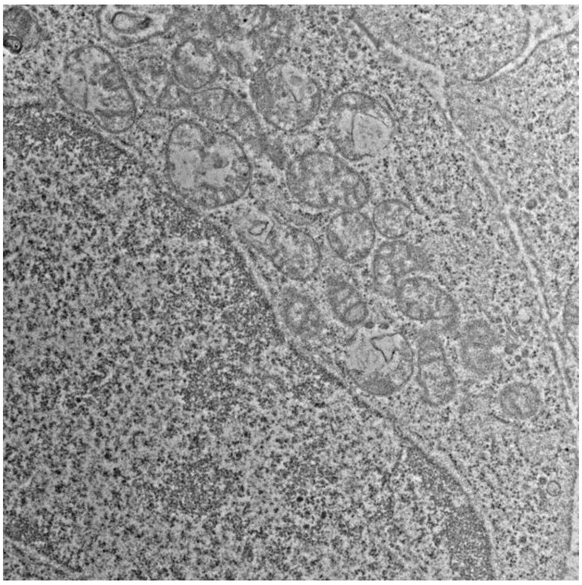

Figure 5: Control; Epithelial cell of a mature follicle; N-nucleus; M-mitochondria; Electron diffraction pattern; Magnification x8000

The ovule located in the centre of the follicles had a large pale nucleus and a cytoplasm with numerous yolk grains. Often the ovum did not fall into the plane of the ultrathin section of the follicle, more often it was found on semi-thin sections. Stroma of the ovary contained numerous spindle-shaped and luteal cells. The latter contained large mitochondria with vesicular crystals and lipid granules of a homogeneous structure of increased electron density (Figure 6).

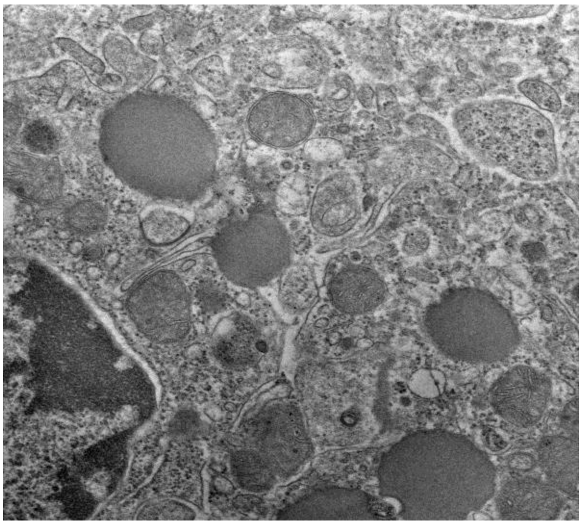

Figure 6: Control; Luteal cell of stroma with large lipid inclusions (L); N-nucleus; Electron diffraction pattern; Magnification x8000

Blood capillaries had preserved endothelial cells with a large nucleus and well-developed organelles (Figure 7). The apical surface was provided with thin cytoplasmic outgrowths.

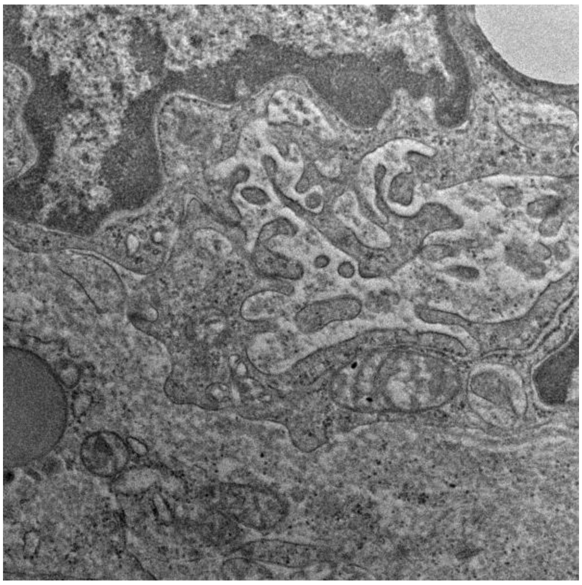

Figure 7: Control; Endothelium of the blood capillary of stroma; N-nucleus, M-mitochondria; CP-cytoplasmic processes; Electron diffraction pattern; Magnification x8000

In the experimental group, the epithelium of the primary follicles was sharply flattened and stretched. In the cytoplasm of the epithelium, secondary lysosomes accumulated, formed on the site of degenerated organelles (Figure 8).

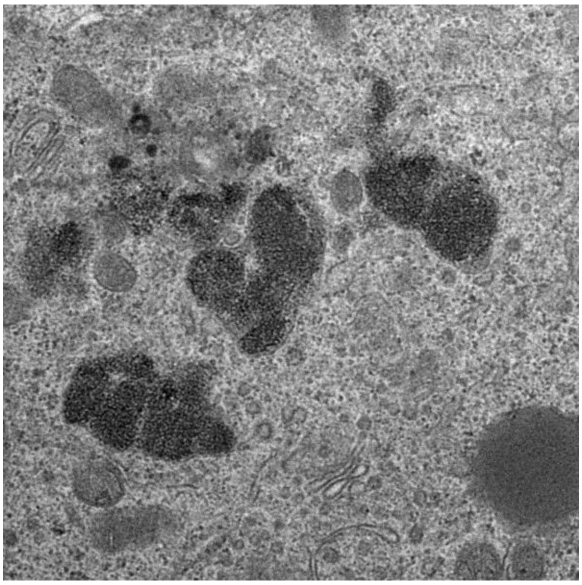

Figure 8: Experiment; Cytoplasm of the primary follicle with lysosomes (LS) and degenerately altered organelles; Electron diffraction pattern; Magnification x8000

Complete destruction of the nucleus and all parts of the cytoplasm of the follicles in the form of ruptures was noted (Figure 9). The fragments of the destroyed cells were seen in the lumen of the follicles. Cellular elements of theca interna «overgrew» with collagen fibrils (Figure 10).

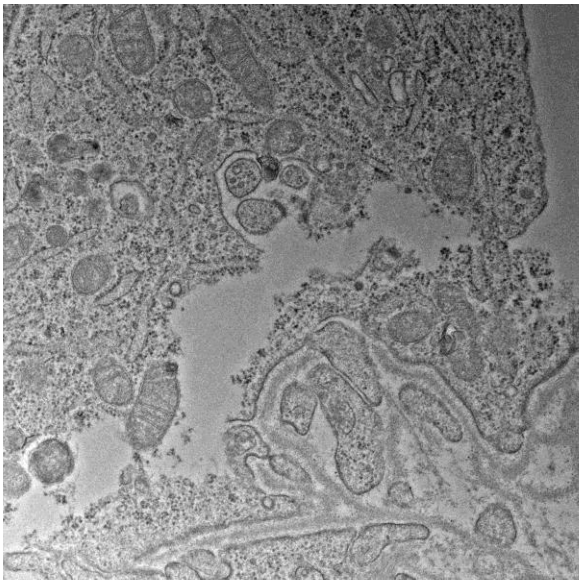

Figure 9: Experiment; Destruction of the cytoplasm of the basal part of the epithelial cell of the follicle in the rupture form; BM-basal membrane; Electron diffraction pattern; Magnification x8000

The wall of fibroblasts was destroyed; the organelles were in a state of necrosis. Numerous bundles of collagen fibrils and fibroblasts were observed in the stroma of the ovaries.

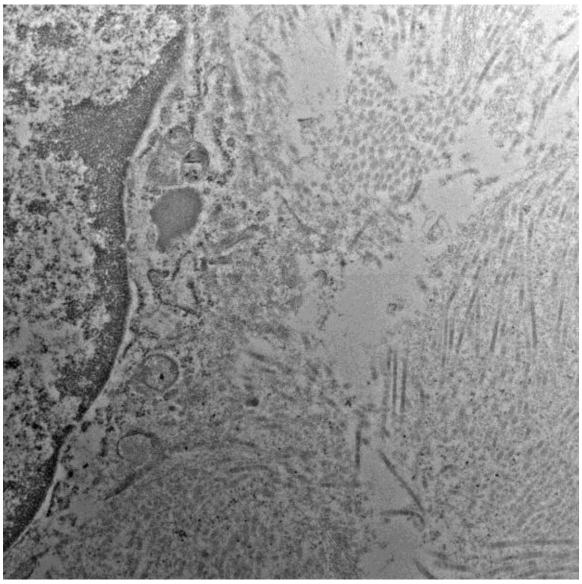

Figure 10: Experiment; Theca interna cell in a state of necrosis; CF-collagen fibrils; Electron diffraction pattern; Magnification x12000

Luteal cells of the stroma were characterised by expressed utilisation of lipids (Figure 11). The luteal pigment accumulated in the cytoplasm. The lipid granules glowed and merged, turning into unformed structures filling almost the entire volume of the cell (Figure 12). Many luteal cells were destroyed (Figure 13). Small blood vessels were necrotic (Figure 14). The capillary wall was a mass of necrotic material of the destroyed organelles. Endothelium of arterioles was perpendicularly elongated concerning the vessel's axis and often necrotic.

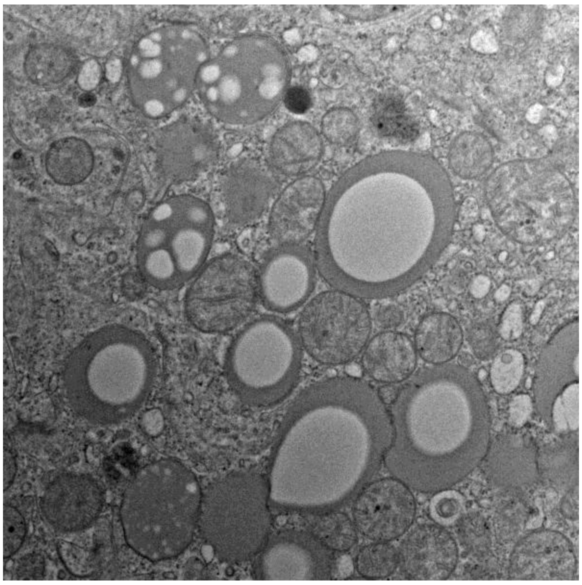

Figure 11: Experiment; Cytoplasm impacted by lipid granules; Electron diffraction pattern; Magnification x8000

Discussion

Ultrastructural changes in the ovaries of the experimental study group were characterised by the pathological changes of all structures in the cortex of the ovary.

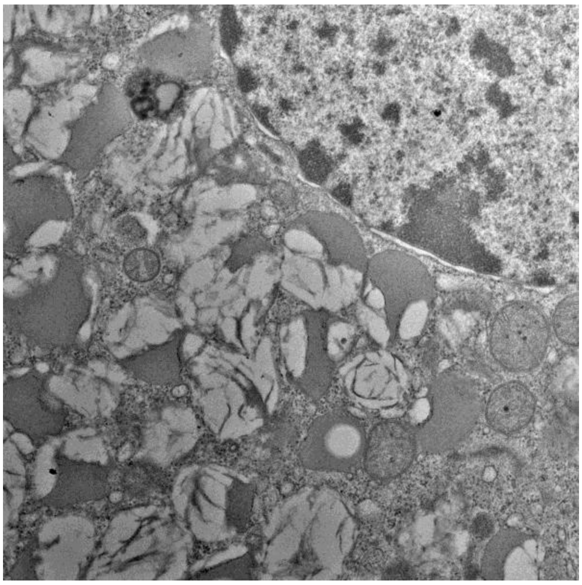

Figure 12: Experiment; Large confluent structures in place of the recycled lipid granules; Electron diffraction pattern; Magnification x8000

Against the background of destruction, necrosis of blood vessels and stromal fibrosis, follicular epithelium of primary follicles underwent dystrophic changes in the form of destabilisation of lysosomal membranes.

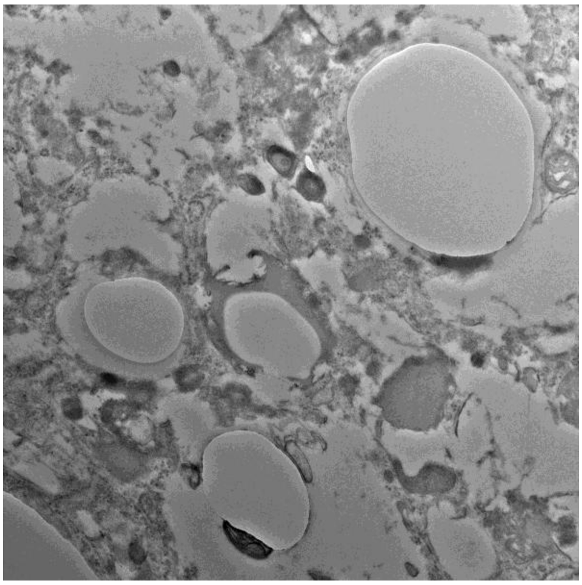

Figure 13: Experiment; Complete destruction of luteal cells; Electron diffraction pattern; Magnification x6000

The destruction of the nucleus and all parts of the cytoplasm in the form of ruptures indicated atresia of the follicles. Theca interna cells, associated with the production of estrogen, were also severely degraded. Ultrastructural changes were noted in the luteal cells of the stroma.

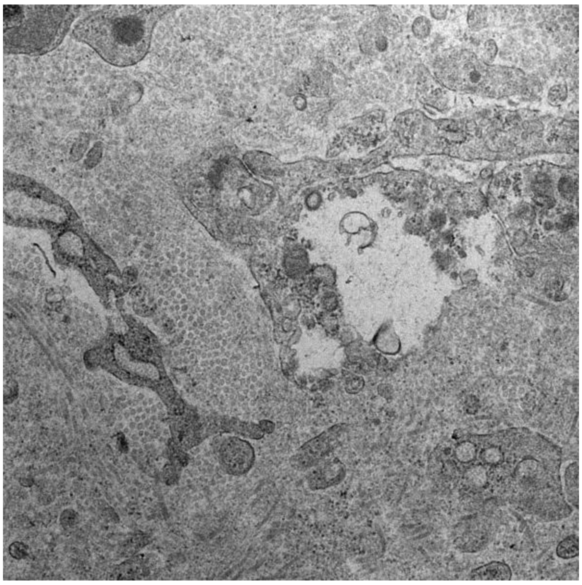

Figure 14: Experiment; Edema, endothelial necrosis of the blood capillary; Electron diffraction pattern; Magnification x6000

The accumulation of lutein pigment, the use of lipid inclusions and the complete destruction of cells reflect a disruption in the production of an estrogens-an important factor in the postviral phase of the ovarian cycle. In the course of the experiment, we concluded that during intoxication of female, white rats with dust-saline aerosols of the Aral Sea, there is a disturbance of folliculogenesis, which manifests itself as a destruction of the follicular composition of the ovaries and blood supply to the ovarian tissue.

The results presented in the course of the research allow us to expand scientific views on the disturbance of folliculogenesis processes when the dust-saline aerosols of the Aral Sea are exposed to the body. The results of the experiment show the expediency of studying the influence of this ecological factor on the reproductive health of the female body to identify possible preventive measures.

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