Thesis abstract for PhD degree
"Assessment of folliculogenesis and genomic instability in female white rats and their offspring under inhalation exposure to chemical pollutants (experimental study)"

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Relevance

Many studies have shown that the air pollution is associated with numerous pathologies, usually cardiovascular and respiratory diseases. The atmosphere is a shared resource, therefore the scale of air pollution consequences can vary from local to transboundary levels[1].

Many air pollutants are known to be harmful at trace concentrations, while the potential effects of chronic exposure to low concentrations of certain types in the atmosphere have not yet been determined. This requires the constant development of sensitive research methods for recording possible violations in all organs and systems in order to develop a scientific understanding and prevention system in healthcare [1,2].

The reproductive potential of a country includes the ability of a population to grow under optimal conditions in a stable age composition [3,4]. Consequently, the state of follicular stock and the process of folliculogenesis as a whole directly affects the reproductive potential. According to some authors, environmental pollutants are one of the etiological factors of premature ovarian exhaustion. The main mechanism of the exhaustion is the increase in the number of atresized follicles [5-7].

The main indicators of reproductive health are the ability to conceive, fertility, reproductive losses. Temporary indicators of the onset of menarche, early or premature menopause can also be considered as an indicator of reproductive health.

Perhaps a negative effect on the reproductive system is mediated through an imbalance in the lipid peroxidation and antioxidant protection system.

Lipid peroxidation modifies lipids and fatty acids in the membranes of sperm and oocytes. These modified by-products greatly affect the viability and overall quality of reproductive cells, increasing the likelihood of infertility. Oocytes contain a large amount of lipids, which can be affected by lipid peroxidation.

Previous research shows that more than 30% of embryos die before birth due to the presence of genetic defects incompatible with life [8]. This group also includes patients with a history of repeated miscarriages, non-developing pregnancies and the birth of children with multiple malformations. It is possible that damage to the maternal genome occurs during a period of prolonged rest, before meiosis is reactivated during folliculogenesis at the stage of primordial follicles, or damage to the oocyte occurs under the influence of oxidative stress in the last stages of growth.

It is believed that the same anthropogenic factors can have different influence based on climate conditions.

Dust-salt aerosols of the Aral Sea in their composition are a complex multicomponent mixture of chemicals that spreads over thousands of kilometers and is inhaled for decades by people living in the Aral Sea region and beyond. Particles of dust settle on plants, enter the soil and, through the use of plant food and water, also enter the human body. Foreign substances accumulating in the body have a damaging effect on the formation of reproductive function, and egg maturation [9-11].

The individual components of these aerosols were studied in the context of exploring the effects of mining and other industries [12]. There is previous research on the study of the accumulation of harmful substances in hair and breast milk in the population living in the Aral region [13]. There are also a number of experimental works devoted to the study of the influence of environmental factors on the male reproductive system [14,15].

In order to assess the health of the population living in an ecologically unfavorable region, a large-scale clinical and laboratory study was conducted as part of the STP "Integrated approaches

to managing the health status of the Aral Sea population" 2014-2016. According to the study results, we found that women living in the Aral Sea crisis and catastrophe have a tendency to early or premature menopause, and later to menarche. The average age of menopause in residents of the Aktobe region was 41.5 years according to our results [16.17].

Despite numerous clinical and experimental studies from the literature review, we concluded that there is no complete picture of the effect of salt aerosols on the body, and the reproductive health of the population at the molecular-cellular level, traced over generations.

However, it is not always possible to assess the morphological changes in the reproductive organs at the tissue and cellular levels. In the scope of one study with the participation of people, it is impossible to study several generations, due to the duration of such a study. Experimental work is a suitable answer to these tasks, making it possible to assess whether the environmental factor has mutagenic activity and reproductive toxicity, reducing the fertility of organisms. Also, the study of folliculogenesis in generations gives us the opportunity to obtain deeper knowledge to solve gynecological problems and the ability to predict the reproductive potential of the population.

Based on the foregoing, we have conducted an experimental study of the influence of dust-salt aerosols of the Aral Sea on the body of experimental animals.

Objective: To assess the state of folliculogenesis, genomic instability, and oxidative stress in female white rats and their offspring when exposed to chemical pollutants.

The objectives of the study

1. To study folliculogenesis in female white rats and their offspring under chemical pollutants' exposure
2. To study the effect of chemical pollutants on the hereditary material of female white rats and their offspring at the chromosomal and cellular levels
3. To assess the effect of chemical pollutants on oxidative stress in the blood and ovarian homogenate of female white rats and their offspring

Key points for defense

1**.** The accumulation of reproductive toxic heavy metals is found in the dust-salt aerosols of the Aral Sea in the tissues of rat ovaries upon inhalation exposure.

2. Quantitative and qualitative changes in the follicular and ovarian conditions of the ovaries occur under the influence of chemical pollutants included in the dust - salt aerosols of the Aral Sea. Dust-salt aerosols of the Aral Sea lead to a decrease in the number of primary follicles and yellow bodies, with an increase in the severity of these processes in the generation.

3. Genomic instability, characterized by a chromosomal imbalance at the level of qualitative and quantitative indicators of chromosomal aberrations, has been established. The mutagenic effect of chemical pollutants on the body of female rats was studied, with an increase in the number of cells with micronuclei in the bone marrow of animals.

4. Multidirectional changes in the level of products of oxidative destruction of lipids were revealed, as well as the decrease in the activity of antioxidant enzymes, leading to the development of molecular mechanisms of disturbance at the cellular, organ, and system levels when exposed to dust and salt aerosols of the Aral Sea for 30 days.

Scientific novelty

For the first time, an experimental study was conducted to study the effect of dust - salt aerosols of the Aral Sea on the body of female white rats and their offspring.

For the first time, by the method of atomic absorption spectrometry, the accumulation of salts of heavy metals, included in the dust-salt aerosols of the Aral Sea in the tissues of rat ovaries upon inhalation exposure, was determined.

For the first time, a characteristic is given to the process of folliculogenesis under the influence of chemical pollutants in generations under experimental conditions. Consequently, it is established that dust-salt aerosols of the Aral Sea lead to a significant decrease in primary follicles, yellow bodies, an increase in the number of atretic follicles, and also aggravation of these processes in generations.

For the first time, the effect of chemical pollutants on oxidative stress indices in ovarian homogenate in rats and their offspring was studied.

For the first time, an assessment of the influence of dust-salt aerosols of the Aral Sea on genomic instability is given at the cellular and chromosomal levels in generations under experimental conditions.

Practical significance of the research

A certificate of registration of copyright was obtained based on the thesis materials (No. 2721 dated November 17, 2017 “Assessment of oxidative stress in the blood and tissues of experimental animals exposed to dust and salt aerosols from the Aral Sea”). There is an act of introducing the results of scientific research into practice:

- Institute of Public and Occupational Health, Professional Health Clinic “Methodology for conducting biochemical methods of tissue research (Appendix G).

This implementation can improve the skills of practicing doctors in performing biochemical and cytogenetic studies.

Besides, the main thesis results are introduced into the educational process of the Karaganda Medical University:

- Department of Morphology and Physiology “Methodology for conducting a structural assessment of the follicular state of the ovaries in experimental animals” (Appendix F)
- Department of Biology “Methodology of cytogenetic study of bone marrow smears of laboratory animals" (Appendix E)

These implementations expand students’ knowledge in the study of the discipline of morphology and physiology, medical genetics, as well as improve the qualifications of undergraduates in carrying out research work, help to systematize material for research work, and expand knowledge in the direction of the influence of environmental factors on the reproductive system.

Research testing

The main provisions and results of the thesis were presented at:

XXII International Scientific Conference “Relevant Scientific Research in the Modern World”, Pereyaslav-Khmelnitsky, Ukraine, January 26-27, 2017;

The OCC World Congress and Annual SFRR Conference Metabolic Stress and Redox Regulation, Berlin, Germany, June 21-23, 2017;

The International Scientific and Practical Conference “Relevant Issues of Fundamental and Applied Morphology of Man and Animals” dedicated to the 80th anniversary of Academician of the Academy of Medical Sciences of the Republic of Kazakhstan, Doctor of Medical Sciences, Professor Turakbay Umbetov, Aktobe, May 31, 2018;

The international conference “Relevant issues of occupational medicine in Kazakhstan “chrysotile and health””, Karaganda November 1-2, 2018;

The XIV Congress of the International Association of Morphologists, Astrakhan, Russia, September 19-22, 2018;

The international conference “The Leiden International (Bio) Medical Student Conference”, Leiden, the Netherlands, March 13-17, 2019.

List of publications on the thesis topic

Based on the thesis material, 5 articles and 8 abstracts were published in Russian, Kazakh and English languages. There are 4 publications recommended by the Committee for control in

the field of education and science of the Ministry of Education and Science of the Republic of Kazakhstan. 2 publications, including 1 article and 1 abstract, have been published with a non-zero Impact Factor Journals, included in the Scopus database. The work was tested at 7 international conferences, and 1 republican conference with international participation. There are 1 certificate of state registration of rights to the copyright object, 1 act of implementation in practical health care, and 2 acts of implementation in the educational process.

Volume and structure of the thesis

The dissertation contains 140 pages of typewritten text, consists of an introduction, a literature review, the main part (materials and research methods, chapters of own research), conclusions, practical recommendations, 40 tables, 43 figures and a list of references, including 209 sources, 7 appendices.

Materials and methods:

On the basis of the biological laboratory of the Department of Biology of the Karaganda Medical University (KMU), the vivarium, and the KMU laboratory for the collective use, an experimental study was carried out. The study included the setting up of an experiment on female outbred rats and their offspring, morphological study of ovarian tissue, cytogenetic study of animal bone marrow and peroxide parameters oxidation of lipids and antioxidant enzymes in the blood and ovarian homogenate of experimental animals. A total of 4 groups of animals were studied: control, initial animals of the experimental group, first generation and second generation. The final sample included 92 animals. Preliminary the dose for inhalation inoculation of the rat was calculated on the basis of hygienic standards for atmospheric air in urban and rural settlements in Kazakhstan. The accumulation of chemicals in the ovarian tissue was evaluated by atomic absorption spectrometry. Morphological examination of the ovaries included the study of tissue at the electron microscopic level and light-optical level (indicators of ovarian size, quantitative and qualitative characteristics of folliculogenesis). A cytogenetic study of bone marrow included counting micronuclei in polychrome bone marrow erythrocytes according to the method of J. A. Headdle and W. Schmid, as well as analysis of metaphase plates with calculation of chromosomal and chromatid aberrations. The lipoperoxid cascade indices were evaluated by the level of malondialdehyde and the level of glutathione peroxidase and catalase enzymes in the blood, and ovarian homogenate of experimental animals. Statistical processing of the results was carried out using the package “Statistics 8”. When testing statistical hypotheses, a 5% significance level was adopted.

Based on the experimental study results, **the following conclusions** can be drawn:

1. The accumulation of salts of heavy metals belonging to the class of reproductive toxicants was established in the tissues of rat ovaries under inhalation exposure to dust-salt aerosols of the Aral Sea, leading to changes at the cellular, tissue and organ levels.

2. As a result of the inhalation effect of dust-salt aerosols of the Aral Sea on folliculogenesis in female white rats and their offspring, statistically significant qualitative and quantitative changes in the cortical and brain layers of the ovary of the experimental groups are observed in comparison with the control group.

A decrease in the area of the cortical layer and follicular reserve of the ovaries in the first and second generations of the experimental group was revealed in comparison with the control group and the initial experimental group ($p < 0.05$).

Structural qualitative changes in the ovaries from rats of the experimental groups were associated with severe dystrophic changes, characterized by vacuolization of the cytoplasm, necrobiotic changes in the structurally functional cells of the corpus luteum and follicles in comparison with the control group. More severe changes are detected in subsequent generations in comparison with the original group.

An inverse close and inverse average correlation was established between an increase in the level of malondialdehyde in the blood and in the homogenate, respectively, and the number of primary follicles in the ovaries of experimental animals ($r = -0.78$, $r = -0.71$ at $p < 0.05$).

3. When studying the effect of chemical pollutants that are part of the dust-salt aerosols of the Aral Sea on hereditary material in female white rats and their offspring, an increase in the number of hyperchromatophilic red blood cells in bone marrow smears in the original animals of the experimental group was 3.5 times higher than in the group control (6.9 ± 0.4 and 2.0 ± 0.2 at $p < 0.05$). In generations, there is an increase in the number of cells with micronuclei in the first generation of animals of the experimental group compared with the original group by 22% (8.8 ± 0.2; $p < 0.05$).

There is an increase in chromosome aberrations in the initial animals of the experimental group by 2.8 times compared with the control group (4.7 ± 0.9 and 1.7 ± 0.7, respectively, at $p < 0.05$). Growth is due to chromatid type aberrations. The number of chromosomal aberrations in animals of the first generation increases by 16%, and in animals of the second generation by 23%, compared with animals of the original group (5.6 ± 0.9 and 6.1 ± 0.8; $p < 0.05$)

These indicators have a strong correlation with the level of malondialdehyde in the blood of animals ($r = 0.93$ for the number of cells with micronuclei and protrusions, and $r = 0.87$ for the number of chromosomal aberrations, at $p < 0.05$).

4. The level of malondialdehyde in the blood of the original animals of the experimental group is 2.4 times higher than in the control group (1.79 ± 0.15 and 0.7 ± 0.06 $p < 0.05$). In generations, this indicator increases by 14% in the first and 13% in the second generation compared to the original animals of the experimental group. The level of malondialdehyde in the ovarian homogenate in the initial animals of the experimental group exceeded the control value by 2.6 times (5.3 ± 0.3 and 2.05 ± 0.2; $p < 0.05$).

Catalase activity decreased in the blood of the original animals by 2.5 times in comparison with the control group, by 3 times in the first and second generation groups. In the homogenate of the initial group of animals, catalase activity is 2.3 times less than in the control group.

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