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BLOOD LEUKOCYTE FORMULA IN RATS EXPOSED TO ELECTROMAGNETIC RADIATION IN PRENATAL DEVELOPMENT

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Abstract. The study is devoted to the identification of changes in the leukocyte formula of offspring from mothers exposed to non-ionizing electromagnetic radiation of the decimeter wavelength range at different stages of pregnancy. The experiments were conducted on 20 and 30-day-old pups that were exposed to the prenatal effects of 460 MHz EMR during the embryonic, pre-fetal and fetal stages corresponding to 1-6, 7-16 and 17-21 days of pregnancy. The radiation power flux density was 30 μW/cm^2 and the calculated specific absorption rate averaged the whole body was 15 mW/kg. In all three experimental subgroups of two age groups of rat pups, designated as embryonic, pre-fetal and fetal, an increased percentage of lymphocytes and a reduced percentage of monocytes and neutrophils were shown in relation to the corresponding control subgroups. In all three experimental subgroups of two age groups of rats, designated as embryonic, prefetal and fetal, an increased percentage of lymphocytes (for 20-day-old rats in the subgroups by 16.7%, 15.9%, 13.9% at $p<0.001$ and 30-day-old - 5.0%, 23.7% at $p<0.01$, 24.0% at $p<0.001$) and a reduced percentage of monocytes (for 20-day-old rats by subgroups by 73.7% at $p<0.05$, 94.2% at $p<0.001$, 73.7% at $p<0.01$ and 30-day-old rats - 21.6%, 42.8% at $p<0.05$, 52.3% at $p<0.01$) were shown. Neutrophils of both types were not detected in the embryonic subgroup of 20-day-old rats and low levels were observed in the other two subgroups compared to the control subgroups. In 30-day-old rats in the embryonic subgroup, both segmented and band neutrophils exceed the control level by 17.9% ($p<0.001$) and 100% ($p<0.001$), respectively. The age difference was that by the age of 30 days, the level of deviations from the control in the embryonic subgroup was mitigated, unlike in the other two subgroups - pre-fetal and fetal. The exception is an increased percentage of neutrophils (both segmented and bond) in 30-day-old rats exposed during the embryonic period. By the age of 30 days, no metamyelocytes were detected in the blood of prenatally irradiated rat pups and prolymphocytes are less likely to appear in pups irradiated during the fetal period of prenatal development (0.14±0.14%, $p>0.05$). The leukocyte formula in the offspring of rats exposed to non-ionizing EMR during intrauterine development is generally characterized by lymphocytosis, monocytopenia and neutropenia, partially dependent on the period of exposure to EMR, which, apparently, can be considered typical for chronic exposure to this physical factor.

Keywords: *Electromagnetic radiation, rats, in utero exposure, leukocyte formula, oxidative stress.*

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1. Introduction

Non-ionizing radiation is one of the most common environmental factors affecting humans. Electromagnetic radiation (EMR) is widely used in medicine: they are used both for diagnostic purposes and for therapeutic purposes during physiotherapy procedures. On the other hand, electromagnetic radiation, especially non-ionizing radiation, is widely used in everyday human life and information communication in the form of mobile

communication systems. The latter is considered to make the most significant contribution to the electromagnetic environment of modern man. The main attention of researchers into the biological effects of non-ionizing EMR is focused on assessing the effects on the nervous system, although the cardiovascular, endocrine and immune systems also respond to the effects of electromagnetic radiation. Therefore, it is necessary to thoroughly and comprehensively study all changes occurring in organism under such exposure (Liu *et al*., 2024; Gao *et al*., 2025; Shibkova & Ovchinnikova, 2015). The seriousness of the situation with the biological effects of non-ionizing EMR is indicated by the fact that a number of national and international organizations have developed guidelines setting limits on exposure to EMF in occupational and residential areas. The limits of exposure to electromagnetic fields have been developed by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) based on the analysis of numerous scientific literature on assessments of biological effects with health consequences.

The negative effects of electromagnetic radiation on the organism manifest at physiological, biochemical and morphological levels. Embryonic cells are especially sensitive to EMR, as they actively proliferate and have a high metabolic rate. Exposure during prenatal development may lead to intrauterine death, congenital malformations or altered developmental programming.

It is now well-established that even low-intensity electromagnetic fields can adversely affect pregnant women. Scientific literature on the effects of EMR on human and animal development shows conflicting results (Liu *et al*., 2024), though most researchers agree that mobile phone radiation negatively affects the reproductive system (Jargin, 2018; Khorseva *et al*., 2017, 2018). For instance, studies have shown that mobile phone EMR negatively affects the development of chicken embryos (Pawlak *et al*., 2016). Other studies, however, reported that 2-hour daily EMR exposure over 10 months had no effect on testicular tissue apoptosis in rats (Assefa & Abdu, 2025). The central nervous system is considered highly sensitive to EMR (Alkis *et al*., 2019), yet other authors found no evidence that low-intensity pulsed radio frequency from standard mobile phones has negative effects (Vijayalakshmi & Nirmala Devi, 2020). Thus, studying how the human and animal organisms respond to constantly changing environmental electromagnetic backgrounds remains relevant.

The aim of this study was to evaluate hematological parameters of peripheral blood in rat offspring after exposure to electromagnetic radiation during intrauterine development.

2. Research Methods

Subjects: Experiments were conducted on 20- and 30-day-old rat pups born to female Wistar rats exposed to EMR during different pregnancy stages. The “Volna-2” device (Russia), commonly used in clinical physiotherapy was employed. It is a tube-based EMR generator that allows controlled exposure with a frequency of 460 MHz (±1%). The device's radiator and a plastic cage (15 x 20 x 20 cm) with a pregnant female rat were located inside 110x100x100 cm chamber with side metal screens to protect against background electromagnetic fields. With an output power of 60 W, the radiation power flux density at the location of the object was 30 $\mu W/cm^2$, while the radio frequency electromagnetic background did not exceed 0.01 $\mu W/cm^2$ when the device was turned off. The measurements were carried out using an HT627 electromagnetic field meter

(China). The specific absorption rate (15 mW/kg) was estimated by using the following equation: $SAR=\sigma*E^2/\rho$, where σ is the conductivity (0.87 S/m), E is the magnitude of the electric field (4.5 V/m) and ρ is the mass density of the tissue-equivalent media (1105 kg/m^3). The values σ and p were borrowed from the work (Sirav *et al*., 2021), which contains references to authors who evaluated and modeled the distribution of SAR in biological objects excited by various types of electromagnetic signals. Exposure to EMR lasted 20 minutes daily during: embryonic stage (days 1-6 of pregnancy), pre-fetal stage (days 7-16), fetal stage (days 17-21). Each age group (20-day-old and 30-day-old) was formed from three experimental subgroups of rat pups (8 each) obtained from mothers exposed to radiation in the three above-mentioned stages. As a control for each subgroup, puppies of the appropriate age were taken from the offspring of females exposed to "false radiation" under similar conditions, but with the radiation source turned off. Intact animals were not included in the study because there are no significant differences in the measured parameters in blood between the intact animals and those in the control group (false irradiation) in preliminary experiments (Abbasova & Gadzhiev, 2022). The subgroups were conventionally designated as "Control", "Embryonic", "Pre-fetal" and "Fetal". During the experiment, control pregnant (non-irradiated) and experimental pregnant (irradiated) rats and their offspring were housed in normal vivarium conditions and had free access to food and water.

Sample collection: Blood was drawn from the tail vein. Leukocyte differentials were assessed using Romanowsky-stained smears under light microscopy (200 cells per smear). All animal procedures complied with the Geneva Convention "International Guiding Principles for Biomedical Research Involving Animals" (Geneva, 1990). The protocol of the experiment was approved by the Local Ethical Committee for Animal Experiments at the Garayev Institute of Physiology (protocol no. 4 of 28.11.2017).

Statistical analysis: SPSS for Windows v.22.0 was used. Data distribution was tested with the Shapiro-Wilk test. Paired t-tests compared experimental and control data. Results are presented as "mean ± standard error". A p-value <0.05 was considered statistically significant.

3. Results and Discussion

The results of a study of the leukocyte content of peripheral blood in 20 and 30-day-old rat pups born to mothers who were exposed to radiation during pregnancy at the stages of embryonic, pre-fetal and fetal development are presented in the table.

Studies showed that in 20-day-old pups born to mothers exposed to non-ionizing EMR during various prenatal stages, lymphocytes were higher than in controls: this excess is 16.7% ($p<0.001$) for the embryonic group, 15.9% ($p<0.001$) for the pre-fetal group and 13.9% ($p<0.001$) for the fetal group. Monocytes decreased in all three groups. In the embryonic group, there is a decrease of 73.7% ($p<0.05$) compared to the control group, in the pre-fetal group - 94.2%, ($p<0.001$) and in the fetal group - 73.7 % ($p<0.01$).

Eosinophils were not observed in the embryonic and pre-fetal groups, although they were found in the control group (0.56±0.30). In the fetal group, however, it manifests itself at a reduced level of 75.0% ($p>0.05$) compared to the control group. Neutrophils with segmented nuclei were absent in embryonic group and decreased by 88.4% ($p<0.001$) in pre-fetal group and 81.5% ($p<0.001$) - in fetal group. Band neutrophils also absent in embryonic group and show a decrease of 92.1% ($p<0.001$) in pre-fetal group

and 85.0% ($p<0.001$) in fetal group. No metamyelocytes or prolymphocytes were observed in 20-day-old experimental pups.

Table 1. Changes in the leukocyte formula of blood in 20 and 30-day-old rats exposed to microwave radiation in prenatal development

	20-day-old				30-day-old			
	Control	**Embryonic stage**	**Pre-fetal stage**	**Fetal stage**	**Control**	**Embryonic stage**	**Pre-fetal stage**	**Fetal stage**
Lymphocytes	84,8±2,6	99,0±0,7	98,3±0,8	96,6±0,97	77,1±7,1	81,0±3,8	95,4±2,02	95,6±1,3
		p<0,001	p<0,001	p<0,001		p=0,63	p=0,03	p=0,02
Monocytes	3,81±0,8	1,0±0,7	0,22±0,2	1.0±0,8	3,06±0,5	2,4±0,5	1,75±0,7	1,43±0,4
		p=0,02	p<0,001	p=0,02		p=0,037	p=0,016	p=0,02
Eosinophils	0,56±0,3	0	0	0,14±0,14	0,8±0,3	1,1±0,2	0	0,4±0,3
				p=0,24		p=0,023		p=0,084
Segmented neutrophils	6,75±1,6	0	0,78±0,5	1,25±0,4	8,4±1,3	9,9±2,3	1,25±0,8	1,57±0,6
			p=0,003	p=0,005		p=0,023	p=0,001	p=0,002
Band neutrophils	4,2±0,6	0	0,33±0,24	0,63±0,4	2,1±0,4	4,3±0,5	1,14±0,7	0,71±0,29
			p<0,001	p<0,001		p=0,01	p=0,090	p=0,21

Thus, 20-day-old pups showed increased lymphocytes, decreased monocytes and neutrophils and no eosinophils (except in fetal group where they were reduced).

It was shown that in 30-day-old rat pups from prenatally irradiated mothers, the percentage of lymphocytes in the blood is increased relative to control animals: in the embryonic group, the increase is 5.0% ($p<0.01$), in the pre-fetal group - 23.7% ($p<0.01$), and in the fetal group - 24.0% ($p<0.001$). At the same time, monocytes decreased in all three experimental groups: by 21.6% ($p<0.05$) in embryonic group, 42.8% ($p<0.05$) in pre-fetal group and 52.3% ($p<0.01$) in fetal group. There were 37.5% more eosinophils ($p<0.05$) in the embryonic group compared to the control group. In the pre-fetal group, they were not found, while in the fetal group, the level was 50% ($p<0.05$) less than in the control group.

Both types of neutrophils are found in the embryonic group, which belongs to the 30-day category. Here, the percentage of segmented neutrophils is 17.9 % ($p<0.001$) higher than in the control group. In the pre-fetal group, the percentage of segmented neutrophils decreases by 85.1% ($p<0.001$) in the experimental group compared to the control group and by 81.3% ($p<0.001$) in the fetal period. While band neutrophils were more abundant in the embryonic group than in the control group, a low level was revealed in the pre-fetal and fetal groups. It was shown that the percentage of band neutrophils in the embryonic group is 2.0 times ($p<0.001$) more than in the control group and in the pre-fetal and fetal groups it is 45.7% ($p<0.05$) and 66.2% ($p<0.01$) less, respectively. No metamyelocytes were found in all three subgroups of both age groups. A small percentage of prolymphocytes was found only in the fetal subgroup of the 30-day age group, the statistical reliability of which is very low (0.14 ± 0.14, $p=0.355$). The entry of immature lymphocyte progenitor cells into peripheral blood may indicate isolated cases of pathological processes after exposure to EMR, which requires more reliable data.

30-day-old pups showed increased lymphocytes percentage in all subgroups, but decreased monocytes. No basophils or metamyelocytes were seen. Neutrophils increased in embryonic subgroup only. Prolymphocytes were observed only in the 30-day group during fetal stage radiation.

A review article by Kashani et al. (2023), devoted to the results of a meta-analysis of epidemiological studies of the effect of electromagnetic non-ionizing radiation on intrauterine and childhood abnormalities, indicates the association of exposure to EMR in mothers during pregnancy with fetal complications, such as a significant increase in

the level of oxidative factors, a decrease in antioxidant protective capabilities and an increase in DNA damage parameters, as well as changes in protein expression in umbilical cord blood genes.

In recent years, studies have often been carried out to investigate the possible oxidative effects of radio- and microwave radiation. In particular, data are published on the occurrence of pathophysiological changes associated with oxidative stress in various organs and tissues, including the reproductive system, during the influence of radiation used in cellular communications on the body. In rats irradiated with microwaves (2.1 GHz) for a long time, the effect of causing oxidative damage to liver, lung, kidney, heart tissues was observed Sirav et al. (2021). Possible histopathological changes in the tissues of the heart and spleen under the influence of microwave radiation of the same frequency were studied in experimental studies of Yavaş et al. (2022). They concluded that short-term irradiation does not lead to serious morphological changes and the reason for this is the body's antioxidant resistance. Studies by Alkis and others on rats show that microwave radiations of 900, 1800 and 2100 MHz emitted by cell phones can cause oxidative damage to the brain. Irradiation can lead to an intensification of the process of lipid peroxidation, oxidative damage to DNA in the frontal lobe of the brain (Alkis *et al*., 2019).

Previously, we conducted a number of studies on the effect of decimeter electromagnetic radiation on the state of oxidative stress in various organs and tissues. It has been shown that irradiation of rats for up to four weeks for 20 minutes per day causes shifts in the intensity of tissue respiration of brain structures, correlating with changes in the rate of formation of reactive oxygen species in mitochondria and in the intensity of lipid peroxidation (Gadzhiev *et al*., 2016). In our other experiments, we observed the pro- or antioxidant effects of radiation in the lens of the eye in rats, depending on the intensity of radiation (Gadzhiev & Ibrahimova, 2023). These effects were observed in the area of non-thermal EMR intensities. Prolonged exposure to electromagnetic radiation with a frequency of 460 MHz caused changes in the level of lipid peroxidation and total antioxidant activity in the blood of rats (Abbasova & Gadzhiev, 2022).

In another study on mice (Hormozi *et al*., 2021), it was shown that the number of white blood cells in the blood increases due to exposure to electromagnetic radiation, while in the leukoform percentage changes manifest themselves only in a significant decrease in neutrophils. It is important to note that the study does not reveal changes caused by exposure to radiation on the background of vitamin C and this indicates an increased antioxidant protection against the oxidative effects of radiation.

If disorders in the system of oxidant-antioxidant relationships and their connection with various pathophysiological changes in cells, tissues and organs in an organism directly exposed to non-ionizing electromagnetic radiation can be considered sufficiently well-reasoned, then in the case of radiation during intrauterine development this cannot be argued, since there is insufficient relevant research. There are separate experimental studies on the intrauterine effects of electromagnetic fields of the global mobile communication system on the state of oxidative stress, apoptotic and inflammatory changes in the liver of rats during intrauterine development (Salameh *et al*., 2023), the effect of electromagnetic radiation caused by a mobile phone (900-1800 MHz) on the redox status of the heart, liver, kidneys, cerebellum and hippocampus of females mice and their offspring (Bahreyni Toossi *et al*., 2018), behavioral activity of rats exposed in utero to low-intensity electromagnetic radiation in the centimeter range (Denisenko, 2009; Zhang *et al*., 2015).

We have previously shown that prenatal irradiation of rats with an ultra-high frequency electromagnetic field (460 MHz) leads to changes in the basic levels of lipid peroxidation and the activity of antioxidant enzymes catalase and superoxide dismutase in erythrocytes in early postnatal ontogenesis (Guliyeva *et al*., 2024). It is assumed that the detected cross-activation of these two enzymes of the antioxidant system, against the background of increased level of lipid peroxidation in rat offspring, may be associated with EMF-induced tissue hypoxia. The nature of these changes depends on the period of prenatal development (embryonic, prefetal or fetal) in which exposure is performed. It should be noted that a study under the same conditions showed a reduced hemoglobin level in the blood of offspring of rats exposed during the embryonic period of gestation compared to the control group and an increased level when irradiation occurred during the fetal period, which may be due to hypoxia (Kulieva, 2021).

Interesting data were obtained in the work of Burlaka et al. (2022) where the effect of low-intensity microwave radiation with a frequency of 1800 MHz on quail embryos was studied as a biological model of development *in ovo*. It was shown that irradiation led to an increase in the content both of reactive oxygen and nitrogen species, TBA-reactive products, DNA integrity damage in embryo cells and increased embryo mortality. These data indicate the oxidative nature of the biological action of EMR, at the same time, they allow for the occurrence of an effect in the absence of a maternal-fetal relationship.

Significant changes in the bone marrow, spleen, thymus and peripheral blood of experimental animals (mice) and their offspring occurred when exposed to radio frequency EMR (Shibkova *et al*., 2019). These data indicate the development of compensatory and adaptive reactions in the blood of experimental animals. The manifestation of abnormalities such as lymphopenia and neutrophilia indicate that radiation exposure is a serious stress factor including oxidative stress. Oscillations in blood parameters likely reflect adaptive reorganization. Persistent blood abnormalities indicate serious hematopoietic disruptions with potential long-term consequences.

4. Conclusion

Analyzing our own results and literature data on the biological effects of non-ionizing electromagnetic radiation (fields), we can conclude:

1) The changes in the leukocyte component of the immune system in offspring from the maternal organism exposed to radiation during pregnancy occur against the background of EMF-induced oxidative stress;

2) Due to the fact that the nature of early postnatal immunological and oxidant-antioxidant changes (disorders) in offspring depends on the period of radiation exposure during intrauterine development, it is possible to use these data in the development of biomarkers for the early detection of metabolic disorders in the body.

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