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THE CONSEQUENCES OF ELECTROMAGNETIC FIELD EXPOSURE ON THE SPERMATOGENESIS PROCESS IN RAT'S TESTES

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There are factors that adversely affect the health of workers at the modernized enterprises of the metallurgical, chemical, mining industries of the Ukraine. The electromagnetic field is one of the possible harmful factors. The purpose of this study was to determine the effects of the electromagnetic field of high voltage and low frequency on the genitals of male rats, namely, to determine the number of degenerative forms of spermatozoa forming as a result of mitotic and meiotic disorders at the stage of late spermatids. Male rats in the amount of 50 were exposed to a 750 kV electromagnetic field with a frequency of 50 Hz and a power of 10 kV/m at the "Dniprovsk" electrical substation in the city of Dnipro. The chambers with animals were located at a distance of 75 m from the surface of the earth. The control group was consisted of 10 rats. Animals were extracted on the 14th, 30th, 45th, 90th, 120th day after the end of the experiment. Histological preparations were made from the blocks, the morphological evaluation of the results of which was carried out on a LEICA CME microscope. Special attention was paid to the evaluation of germ cell polymorphism, since the degeneration of sperm cells is directly related to their modification, which is the result of aberrations of cell differentiation. The percentage of normal and degenerative forms of spermatozoa was calculated per 100 cells. Pathological processes are developed in the testicles of rats in the process of spermatogenesis under the influence of high-voltage EMF of low frequency, that led to the modification and degeneration of spermatozoa due to impaired mitotic and meiotic divisions. Reproductive function in experimental animals declines. This effect correlates with the "horizontal transfer" of genes, which explains a valid theory in modern evolutionary biology. In the course of the study, it was proved that with an increase in the time of irradiation by the electromagnetic field the number of degenerative cells increased from 20 to 83 %, which was confirmed by statistical confidence. Degenerative forms of spermatozoa looked like cells with double centrioles and double flagella, which is explained by an increase in the DNA content in the heads of germ cells and an imbalance in the apparatus of pressure at the level of chromosomes. The action of the electromagnetic field of high voltage and low frequency in the testicles of rats led to edema of the interstitial tissue due to impaired blood circulation of the organ. Interstitial edema entailed a decrease in the average diameter of the testicular tubules from 0.241 ± 0.063 to 0.232 ± 0.081 mm and a decrease in the total area of spermatogenic tissue, which was also statistically confirmed. The obtained results a negative effect of the indicate electromagnetic field on the morphology of the genital organs of male rats in general and on the process of spermatogenesis in particular.

Key words: spermatogenesis, sperm cell, electromagnetic field, polymorphism, rat.

Шарапова Олена. Наслідки впливу електромагнітного поля на процес сперматогенезу в яєчках щурів

Фактори, що суттєво впливають на здоров'я робітників модернізованих підприємств металургійної, хімічної, гірничодобувної галузей України, представлені в даній роботі. Електромагнітне поле є одним із таких шкідливих факторів. Метою даного дослідження було визначення дії електромагнітного поля високої напруги низької частоти на статеві органи щурів-самців і, відповідно, кількості дегенеративних форм сперматозоїдів, що формуються в результаті мітотичних і мейотичних порушень на стадії пізніх сперматид. Щури-самці в кількості 50 особин були опромінені електромагнітним полем напругою 750 кВт, частотою 50 Гц і потужністю 10 кВ/м на електропідстанції «Дніпровська» в м. Дніпрі. Клітини з тваринами були розташовані на відстані 75 м від поверхні землі. Контрольна група склала 10 тварин. Тварини були виведені з експерименту на 14-тій, 30-тій, 45-тій, 90-тій, 120-тій добі. Гістологічні препарати були виготовлені з блоків, морфологічне пояснення результатів яких виконано на мікроскопі "LEICA CME". Особливу увагу приділено оцінюванню поліморфізму статевих клітин, тому що дегенерація статевих клітин залежала від їх модифікації та відбувалася в результаті клітинної диференціації. Відсоток нормальних і дегенеративних форм сперматозоїдів підраховувався на 100 клітин. Патологічні процеси розвивалися в яєчках щурів у процесі сперматогенезу під впливом ЕМП високої напруги низької частоти, що призвело до модифікації та дегенерації сперматозоїдів внаслідок дискординованих мітотичних і мейотичних ділень. Репродуктивна функція в експериментальних тварин була порушена. Це є ефектом кореляції з «горизонтальним переносом» генів, що пояснюється діючою теорією в сучасній еволюційній біології. У процесі дослідження доведено, що при збільшенні терміну опромінення електромагнітним полем кількість дегенеративних клітин збільшилась від 20 до 83 %, що було підтверджено статистично. Дегенеративні форми сперматозоїдів виглядали схожими на клітини з подвійними центріолями та подвійними джгутіками, що

пояснювалося збільшенням вмісту ДНК у голівках статевих клітин і дисбалансом в апараті тиску на рівні хромосом. Дія електромагнітного поля високої напруги низької частоти в яєчках щурів призвела до набряку інтерстиціальної тканини внаслідок дискоординованої циркуляції крові в органі. Інтерстиціальний набряк призвів до зменшення середнього діаметра сім'яних каналців від $0,241 \pm 0,063$ до $0,232 \pm 0,081$ мм і до зменшення загальної площини сперматогенної тканини, що також було підтверджено статистично. Одержані результати показали негативний ефект електромагнітного поля на морфологію статевих органів щурів-самців взагалі та на процес сперматогенезу зокрема.

Ключові слова: сперматогенез, сперматозоїд, електромагнітне поле, поліформізм, щур.

Introduction. The impact of harmful factors in the external environment on the human body remains a relevant issue today. Electromagnetic fields are one such factor, and their harmful effects can be experienced by people in domestic and industrial settings.

The electromagnetic field affects workers in industries such as mining, metallurgy, and chemistry, affecting both the overall organism and specific systems and organs [1, p. 346–348; 2, p. 33–34].

The most vulnerable systems in humans are the cardiovascular, genitourinary, and sensory organs. Previous experimental studies [3, p. 160–161; 6, p. 110–112] have revealed a range of factors indicating disruptions in reproductive function in humans and experimental animals. Animals exposed to electromagnetic fields (EMFs) experienced mutations, embryonic suppression, reduced offspring viability, and even complete cessation of reproductive function in sexually mature animals [4, p. 67–68; 5, p. 10–12].

The aim of our study was to determine the changes occurring in the testes of rats exposed to high-voltage low-frequency electromagnetic fields, with a focus on determining the ratio of pathological forms of germ cells and identifying possible causes of degenerative forms of spermatozoa.

Materials and methods. Fifty male rats were exposed to an electromagnetic field with a voltage of 750 kV, a frequency of 50 Hz, and an EMF intensity of 10 kV/m at the "Dniprovsk" electric substation in Dnipro city. The animal chambers were placed under power transmission lines located 75 m from the ground surface. The control group consisted of 10 rats kept in the basement of the vivarium, which had reinforced concrete floor slabs. The animals were removed from the experiment on the 14th, 30th, 45th, 90th, and 120th days after the experiment ended. Histological preparations were made from the wax blocks.

Morphological evaluation of the results was performed using a "LEICA CME" microscope at a maximum magnification of $\times 400$. The study involved counting normal and pathological forms of spermatozoa, measuring the large diameter of convoluted seminiferous tubules, and statistically analyzing the data. Degenerative changes were assessed based

on the counting of normal and atypical cells at the late spermatid stage. Special attention was given to evaluating the polymorphism of germ cells, as spermatozoa degeneration is directly related to their polymorphism, which is a result of cell differentiation aberrations. The percentage ratio of atypical and unchanged spermatozoa was calculated per 100 cells.

Discussion. The study revealed that in the seminiferous tubules of the testes in the control group of rats, the normal spermatogenic process occurred with varying degrees of differentiation of germ cells in different areas of the tubules. During the experiment, alongside normally formed spermatozoa, pathological forms were found in the seminiferous tubules. Degenerative changes in spermatozoa were characterized by deformation of the heads, such as doubling, appearance of spermatozoa with duplication or absence of flagella.

On the 14th day of EMF exposure, inside of the seminiferous tubules, epithelial cells at all stages of the spermatogenic cycle were observed, along with the appearance of diploid forms of spermatozoa with two or three flagella. 80 % of germ cells were formed through normal spermatogenesis and did not exhibit morphological anomalies, while 20 % of cells showed centriole duplication in the heads of spermatozoa. The average value of the large diameter of convoluted seminiferous tubules was 0.241 ± 0.063 mm ($p \leq 0.05$).

On the 30th day of EMF exposure, a significant amount of seminal fluid was observed inside of the seminiferous tubules, with degenerative spermatozoa accounting for 56 % and normal spermatozoa for 44 %. Among the pathological forms of germ cells, cells with duplicated centrioles and two flagella were observed. The large diameter of convoluted seminiferous tubules reached a value of 0.245 ± 0.062 mm ($p \leq 0.05$).

On the 45th day of EMF exposure, in the seminal fluid of rat testes, spermatogenic epithelial cells at all stages of development were observed. At this stage of the experiment, the percentage of atypical forms of spermatozoa increased to 64 %, with cells with two flagella being the most prevalent, while the percentage of normally formed cells decreased to 36 %. The

large diameter of convoluted seminiferous tubules was measured at 0.231 ± 0.037 mm ($p \geq 0.05$).

On the 90th day of the experiment, the number of pathological forms of spermatozoa continued to increase. They accounted for 78 % in relation to the number of fully formed germ cells (22 %), with the mean value of the large diameter of seminiferous tubules at 0.233 ± 0.061 mm ($p \leq 0.05$).

At the end of the experiment, in the seminal fluid of the rats exposed to EMF for 120 days, the proportion of germ cells with genetically unformed sets of chromosomes continued to increase, reaching 83 % compared to normally formed cells (27 %), with the mean value of the large diameter of convoluted seminiferous tubules measured at 0.232 ± 0.081 mm ($p \leq 0.05$).

The observed morphological changes indicate that under the influence of EMF, the interaction of centrioles, which normally separate from each other during cell division and act synergistically during the formation of spermatozoa flagella, is disrupted. In cases of atypical development, duplication of the proximal part of the flagella occurs, resulting in the formation of two and more flagella. Alongside pathological changes in centrioles, the most likely cause of the appearance of abnormal spermatozoa is an imbalance in the pressure apparatus at the chromosomal level.

The increase in size and deformation of spermatozoa heads indicate an increase in DNA content in the heads of diploid spermatozoa, indicating abnormalities in conjugation during meiosis and disturbances during the second stage of division in the maturation of germ cells.

Results. Under the influence of high-voltage low-frequency EMF, pathological processes occurred in the testes of rats during spermatogenesis, leading to degeneration and polymorphism of spermatozoa, which are consequences of mitotic and meiotic divisions disruptions, resulting in decreased reproductive function in the experimental animals. This effect correlates with the "horizontal gene transfer," which is a well-grounded theory in modern evolutionary biology. With the increase in duration of the experiment, the number of degenerative spermatozoa in the testes of animals increased from 20 % to 83 %, which can be explained by disruptions in mitotic and meiotic divisions during spermatogenesis. The diameter of seminiferous tubules in the rat testes decreased from 0.241 ± 0.063 to 0.232 ± 0.081 mm, which was also a result of the negative effect of the electromagnetic field.

In the future, it may be possible to determine the amount of unchanged and degenerative forms of spermatozoa after administering immunomodulatory drugs to the animals exposing EMFs.

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