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An Ultrastructural Study of the Antioxidant Effects of Vitamin E and Fennel Extract on Zona Pellucida Cell Changes of Rat Ovaries Under Non-Ionizing 50Hz Electromagnetic Fields

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Abstract

Objective: Everyday use of various electronic tools and appliances has caused a large number of people to constantly be under the influence of electromagnetic fields (EMFs).

Materials and Methods: For the purpose of this study, 40 female rats were randomly selected from the animals' laboratory. The rats chosen for the study were 3 months old and weighted 20 + 200 g. The animals were then randomly divided into 4 groups; Control (n = 10), Experiment 1 (n = 10), Experiment 2 (n = 10), and Experiment 3 (n = 10). During the experiment, all 4 groups were maintained in the same conditions and received the same feeding procedure. Test groups 1, 2, and 3 were under the influence of a 50 Hz EMF for 8 weeks. Subsequently, the second and third groups were kept away from the effects of EMF for another 8 weeks. At the end of the study, after removing the ovarian using glutaraldehyde, they were prepared for electron microscopy study. Ex2 group rats were not sacrificed, and were maintained for another 8 weeks in normal laboratory environment away from the impacts of EMF. The rats were fed vitamin E (100 mg/kg) and fennel extract (1.5 gr/kg/body weight) was added to their daily food. Samples were taken from this group at the end of the second 8 weeks. Samples from the Ex3 group were taken at the end of the second 8 weeks which were maintained in normal conditions without the use of vitamin E and fennel extract. The 10 rats from the control group were biopsied simultaneously with the Ex1 group sampling.

Results: This study showed that in the groups that had been exposed to electromagnetic radiation, zona pellucida cells had lost their microvilli and mitochondrial crystal structure. In the groups that were exposed to vitamin E and fennel extract, these changes were reduced.

Conclusion: The use of vitamin E in combination with fennel extract can reduce the damaging effects of non-ionizing radiation with 50 Hz frequency on the zona pellucida cells of rat ovaries.

Keywords: Electromagnetic Field (EMF), Fennel, Ovary, Vitamin E

Introduction

Electromagnetic waves were first predicted by Maxwell and Heinrich Hertz's experiments prove their existence. After completion of Maxwell's theory of electromagnetism, from the equations of this theory, a form of the wave equation was obtained; thus, he showed that electric and magnetic fields can also have wave-like behavior. The speed of propagation of electromagnetic waves from Maxwell's equations was precisely equal to the speed of light, and Maxwell concluded that light must also be an electromagnetic wave (1). Since the early twentieth century, with increasing use of items such as radio, television, radar, computers, medical diagnostic devices (such as magnetic imaging, ultrasound, and laser) and high voltage power stations, the amount of electromagnetic radiation, especially low-frequency radiation, has increased in

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the environment around us (2). The cellular and molecular rate of changes induced by radiation waves depend on the duration of irradiation, the degree of permeability in the tissue, and heat production. These factors also depend on the severity and frequency of the waves. Cellular responses differ with respect to wave characteristics, such as waveform (sinus or square), rate of change, the biological effects, and the type of cells exposed to radiation (3).

Exposure to low frequency electromagnetic fields (EMFs) of 200 to 300 Hz can alter the translation and replication of genes, which changes the speed of cell differentiation and enzymatic activity. It has also been shown that exposure to EMFs with 0.1 mT power for 24 hours causes increase in single-stranded and double-stranded DNA fragmentation. The effects of 3 mT magnetic field strength on ovarian follicles have not been studied (4,5). Therefore, the aim of the present study was to investigate the effects of long-term exposure to EMFs on rat follicle and the possible effect of vitamin E and fennel extract on eliminating its destructive effects.

Materials and Methods

For the purpose of this study, 40 female rats were randomly selected from the animals' laboratory. First, their age and weight were determined. The rats chosen for the study were 3 months old. Then, they were randomly divided into 4 groups; Control = 10, Experiment 1 (EX1) = 10, Experiment 2 (EX2) = 10, and Experiment 3 (EX3) = 10. During the experiment, all groups were maintained in the same conditions and had the same feeding procedure. Test groups 1, 2, and 3 were under the influence of a 50 Hz EMF. The first, second, and third groups were under the influence of EMF for 8 weeks. Then, the second and third groups were kept away from the effects of EMF for another 8 weeks. During the mentioned periods the rats were sacrificed with chloroform. After sacrificing the rats, the ovaries were removed and placed in fixative and the samples were prepared for electron microscopy study. Ex2 group was not sacrificed, and was maintained for another 8 weeks in normal laboratory environment without EMF effects. The rats were fed vitamin E (100 mg/kg) and fennel extract (1.5 /gr/kg/body weight) orally and in combination with their food every day. Samples were taken from this group at the end of the second 8 weeks. The food product with the brand name Fennelin, product of Barij Essence Pharmaceutical Company (Tehran, Iran) was used in this study. Each milliliter of this product contains 4.11 mg of fennel extract. This extract is available in the form of a drop in pharmacies. Samples were taken at the end of the second 8 weeks from Ex3 group which were maintained in normal conditions without the use of vitamin E and fennel extract. Biopsies were performed simultaneously on 10 rats of the control group and the Ex1 group. To avoid the influence of environmental variables such as temperature, a fan was placed in the upper part of the field generating device. When placing the animals in the field, the fan with its constant functioning would prevent the increase in the device's temperature. In order to permanently and continuously control the inside of the machine, a temperature gauge was installed and continuously controlled by a technician and project manager. In this study, there were no other important confounding variables except the heat from the machine performance, which, as noted, was controlled. At different times, by measuring the outside and inside temperature of the machine, the ineffectiveness of the temperature was determined. Even if there were other confounding factors, due to maintaining the control group in the same conditions as the EX groups, their possible impacts on both groups would be the same. Therefore, they would have no effect on the results of the study.

In this study, an EMF generator that generated a field of 50 Hz was used. The EMF generating device was made according to Helmholtz's theory. The Helmholtz windings had a diameter of 20 cm and contained 200 windings of 0.8 mm copper wire placed at a distance of 10 cm from each other. An AC power supply with a voltage of 0 to 250 volts was also used. A two-channel oscilloscope was used to study the parameters and the waveform of the input and output windings. A multimeter was used to measure the current voltage. An AC and DC Teslameter with an accuracy of 0.001 mT, a thermometer with an accuracy of 0.1 °C, and an incubator device were also used. In selecting these devices, issues such as the need to achieve a uniform field with certain intensity were carefully considered.

Fennel seeds were purchased from local markets and authenticated by a botanist (School of Pharmacy, Tabriz University of Medical Sciences, Iran). The extract was prepared according to the World Health Organization (WHO) protocol for preparation of an alcoholic extract.[7] Briefly, 100 g of fruit was shed-dried, powdered, and added to 1000 ml of 70% ethanol (v/v) and left to macerate at room temperature for 20 hours. The basin was slowly rotated during this time. After filtration, ethanol was evaporated at low pressure at 30 °C.

Preparation of samples for electron microscopy studies: Ovarian tissue samples were transferred on a clean surface and on a plate containing washing solutions (phosphate buffer pH = 7.4). They were washed several times to remove blood clots and tissue debris and clean any blood stains and clot adhesion. Then, the samples were sectioned into 0.5 mm^3 pieces. subsequently, the samples were placed into glutaraldehyde 2.5% solution and were washed for 6 hours in a phosphate buffer solution 0.1 M (pH = 7.4). Next, they were maintained in osmium tetroxide 1% for 2 hours. Later, washed 3 times with phosphate buffer 0.1M (pH = 7.4). In order to extract hydration, alcohol (ethanol) was used with

increasing concentration gradient. Then, the replacement operation was performed using propylene oxide. For molding of the samples, Epon 812 resin was used. Trimmed samples were installed on the ultramicrotome device (Reichert–Jung, Germany). Semi-thin sections with 500-700 nm thickness were prepared with a rate of 2.5 mm per second and were stained with toluidine blue solution. After preparing ultra-thin sections, to stain the grades, uranyl acetate solution 3% and lead citrate were used for 1-2 hours. This stage of the study was conducted at the research center of Tabriz University of Medical Sciences. To evaluate changes, 100 microscopic sections were used.

Statistical analysis

Data analysis was performed using ANOVA and SPSS software (version 16, SPSS Inc., Chicago, IL, USA). All P values of less than 0.05 were considered significant.

Results

Electron microscopy of ovary samples of experimental groups

In the control group samples, a large round oocyte was seen enveloped by follicular cells. The nucleus of oocyte was large and eccentric with a prominent nucleolus. Most of the chromatin was dispersed, but there were a few clumps of heterochromatin at the periphery of the nucleus.

Control group

Mitochondria were densely packed and spherical in shape. Most of the cell organelles were seen clustered in this paranuclear complex, only a few organelles were located in the rest of the cytoplasm. The zona pellucida, a homogenous pale structure, was invaded by cytoplasmic processes that extended from the surface of follicular cells (Figure 1).

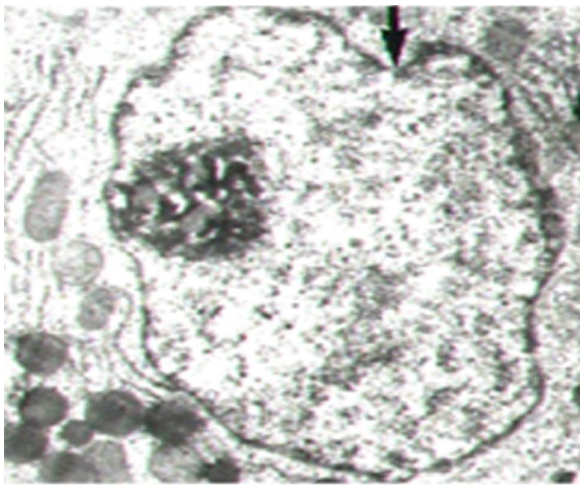

Figure 1. Heterochromatic and chromatic masses located at the periphery

Experimental group exposed to 50 Hz EMF for 8 weeks

Oocyte showed infolding of nuclear membrane, an early sign of degeneration. Nucleus was more heterochromatic and chromatin masses were located at the periphery. In the cytoplasm, there were some mitochondria and several vacuoles. Some vacuoles appeared as empty vesicles and others were filled with low-density material. Greater cytoplasmic vacuolation was a sign of late degeneration. Balbiani's vitelline body was seen next to the nucleus. The granulosa layer was composed of polyhedral cells with irregular dark nucleus. Mitochondria were seen with few lamellar cristae (Figure 2).

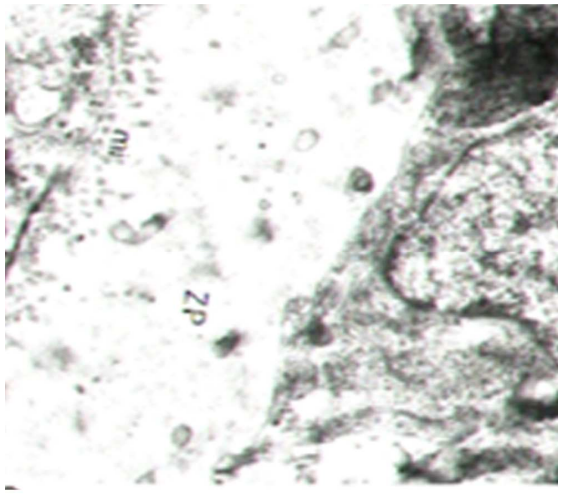

Figure 2. The zona pellucida (a homogenous pale structure) invaded by cytoplasmic processes

Experimental group exposed to 50 Hz EMF for 16 weeks

All results in this group were similar to that of the group exposed to 50 Hz EMF for 8 weeks, but the main important problem was seen in zona pellucida. Zona pellucida did not show any microvilli from oocyte toward the corona radiata or any cytoplasmic indentation or filopodia from follicular cell toward oocyte (Figure 3).

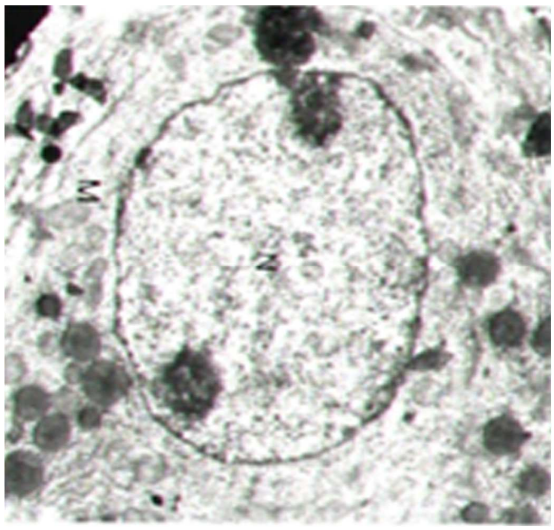

Figure 3. Early signs of degeneration and infolding of nuclear membrane in oocyte

Experimental group that was exposed to 50 Hz EMF (8 weeks) and received 100 mg/kg vitamin E and 1.5/gr/kg/body weight fennel seed extract

Oocyte had an ovoid to spherical, euchromatic nucleus. In addition, 1 or 2 large nucleoli were observed. Mitochondria were spherical in shape and densely packed. Balbiani's vitelline body was seen at one pole of the cytoplasm. The number and size of lipid droplets decreased in comparison to other groups. No vacuoles were seen in the cytoplasm. Metarteriolar endothelial cells were either spindle or irregular shaped. They were less swollen, with an irregular surface, and the nucleus was less heterochromatic, with a prominent nucleolus. Lumen was star shaped and larger than that of samples of other groups (Figure 4).

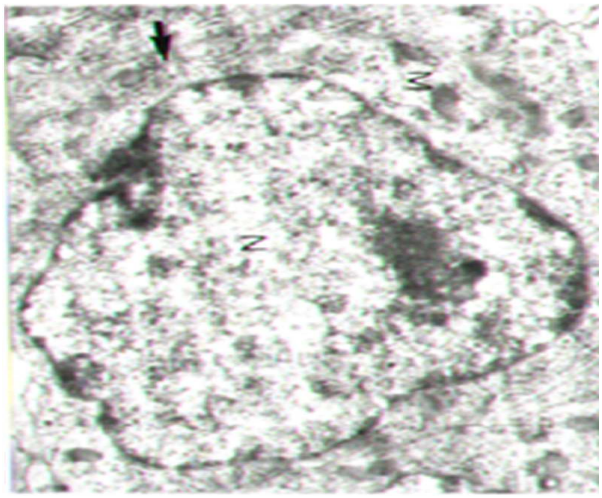

Figure 4. Oocyte with an ovoid to spherical, euchromatic nucleus

Discussion

Exposure to EMFs of 0.13 microtesla for 2 and 4 hours during 5 days increased the density of glycogen in the liver, atrophy of the seminiferous tubules, and interstitial tissues, and decrease the Leydig cells in the testes and cell necrosis, urinary tract, and swelling of the epithelial cells of the kidney (6). The mechanism of action of low-frequency EMFs produced by most electrical appliances in the frequency range of 50 to 60 Hz affects live cells. Most studies have been conducted on the effects of electromagnetic waves on living organisms in this frequency range (7,8). However, macrophages exposed to extremely low frequency electromagnetic waves (ELF-MF), by releasing cytokines and free radicals of active oxygen species and active nitrogen species, caused instability in biological molecules. These materials have impact on intracellular signaling pathways, regulation of gene expression for inflammatory response, cell growth, differentiation, redistribution, and cellular stress response (9). Recent findings suggest that free radicals generated from ELF-MF can increase cell survival. Free radicals, reactive oxygen species, and reactive nitrogen species (RNS), are important factors in oxidative stress. The cellular structure damage is caused by molecules such as fats, proteins, and nucleic acids. Polyunsaturated fatty acids in biological membranes that are rich in cellular structures are susceptible to attacks by free radicals. Free radicals react with unsaturated fatty acids in cell membranes, resulting in the lipid peroxidation process (10).

Vitamin E is a fat-soluble vitamin. This vitamin, like vitamin C, has antioxidant properties and destroys the damaging effects of chemicals on the body's tissues. Vitamin E was isolated from wheat germ and was called alpha-Tocopherol. This name is derived from the Greek word "tokos", meaning child birth and the word "phereim" meaning giving birth. OL indicates its alcoholic structure. This vitamin exists in the fat layer of the cell wall and within the cell, and prevents the degradation of cell wall. Vitamin E is also a name for a group of molecules that have similar effects as that of alpha-Tocopherol (11).

The results of this study confirmed that of previous studies that showed that in the group exposed to electromagnetic radiation, zona pellucida had lost their microvilli, mitochondria had lost their crystal structure, vacuoles were formed on the surface of the ovary cells, and watery swellings were detected on the cell surface. Moreover, these changes were less observed in the groups that used vitamin E and fennel extract (12,13).

Conclusion

Given the irreversible changes caused in ovary cells due to EMFs and the role of antioxidants, such as vitamin E and fennel extract, in the protection of the zona pellucida, it was concluded that protecting zona pellucida resulted in the protection of oocytes, and prevention of polyploidy cell formation. Nevertheless, the duration of exposure to EMFs can also be decisive.

Ethical issues

Ethical of this research work was approved by Tabriz University of Medical Sciences.

Conflict of interests

We declare that we have no conflict of interests.

Acknowledgments

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