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The electromagnetic fields with an extremely low frequency as a factor affecting the aromatase synthesis in the uterine tissues of European roe-deer (*Capreolus Capreolus* L.)

Abstract: *This paper presents the results of the impact of the extremely low frequency electromagnetic field for the production of the aromatase enzyme in the European roe-deer endometrium (Capreolus capreolus L.). The uterine tissuse were subjected to an impact of an electromagnetic field with a frequency of 50 Hz for 2 or 4 hours. The conducted immunohistochemical reaction confirmed the increase in the production of aromatase enzyme by the tissue of the endometrial roe-deer after the influence of the field. The high amount of an aromatase can lead to increased estrogen production. Such hormonal disorders can have serious health effects*

Streszczenie: *W niniejszej pracy zostały przedstawione wyniki badań oddziaływania pola elektromagnetycznego ekstremalnie niskiej częstotliwości na produkcję enzymu aromataza w endometrium macicy sarny europejskiej (Capreolus capreolus). Skrawki tkanki macicy zostały poddane oddziaływaniu pola elektromagnetycznego o częstotliwości 50 Hz przez 2 lub 4 godziny. Przeprowadzona reakcja immunohistochemiczna potwierdziła wzrost produkcji enzymu aromatazy przez tkankę endometrium macicy sarny. Wysoka ilość aromatazy może prowadzić do zawyżonej produkcji estrogenów. Takie zaburzenia hormonalne mogą mieć poważne skutki zdrowotne.****(Pola elektromagnetyczne o ekstremalnie niskiej częstotliwości jako czynnik oddziałujący na syntezę aromatazy w tkankach macicy sarny europejskiej (Capreolus capreolus L.)*

Keywords: electromagnetic field of extremely low frequencies; aromatase enzyme; uterus; European roe-deer.
Słowa kluczowe: pole elektromagnetyczne ekstremalnie niskiej częstotliwościach; enzym aromataza; macica; sarna europejska.

Introduction

In the electromagnetic field, a specific force works for each electric charge or magnetic dipole. The definition of the electromagnetic field shows that it is a system of two interrelated fields: electric field and magnetic field. It is difficult to imagine our functioning without the universal electricity network and devices supporting us in everyday activities. All these devices are a source of electromagnetic field emissions of an extremely low frequency. The number of sources of the electromagnetic field increases with the development of technology in recent years rapidly. Civilization development and technological revolution of the 20th and 21st centuries caused a significant increase in the combination of an electromagnetic fields of various frequencies.

The spectrum of the electromagnetic field frequencies is very extensive and is divided into not ionizing and ionizing. To the former, we count radiation with extremely low frequency, low -frequency, radio waves or microwaves. The ionizing frequencies include high ultraviolet radiation frequencies, X radiation as well as gamma radiation. (Presman, 2013).

The 50 Hz electromagnetic field is constantly produced by most electrical devices surrounding human every day. There was conducted the research study on the influence of the field on cells (Koziorowska et al. 2017, 2018) and tissue (Koziorowska at al. 2020) culture. Many studies indicate that they have caused side effects such as DNA damage, slowing the rate of fetal development (Drzewiecka et al., 2021), as well as the impact on the transcription and translation of protein (Kozlowska et al., 2022). Cytogenetic tests carried out towards the impact of such electromagnetic field frequencies indicate an increase in chromosomal aberrations in people who are still exposed to its action. However, such studies are very difficult to interpret, because exposure to the field may not be even, and other factors, such as smoking, can be involved in it. This can effectively disturb the results (Winker i in., 2005). It is suggested that the operation of an electromagnetic field with a frequency of 50 Hz reduces oxidative stress and its negative effects with brain ischemia. The electromagnetic field with extremely low frequency has anti-inflammatory, analgesic or regenerative effects, and also promotes cell proliferation, ion transport or protein synthesis, and can affect cell signaling, which is why it is often used in the therapy of people after stroke (Cichoń i in., 2018). The positive effect of the electromagnetic field on the body is also important in the treatment of inflammation or skeletal muscle tension, which is why an electromagnetic field with extremely low frequency also works in physical therapies as a complement to surgical and pharmacological treatment (Ciejka i in., 2014). Magnetotherapy is characterized by the ability to deeply penetrate the treated tissue, and its mechanism consists in the flow of electric charges that cause ion flow, and thus cell reconstruction, ensuring cytoprotection and synthesis of growth factor (Kanat et al., 2013). Such treatment is carried out using a camera called magnetronics (Ciejka et al., 2011), and the frequencies used for such therapies are in the range of 1-120 Hz, with magnetic induction value up to 20 mT (Łada-Tondyra and Krawczyk, 2017) . Magnetotherapy is used in the treatment of many diseases.

European roe-deer are small representatives of the *Cervidae* deer family, reaching a mass of up to 32 kg. It is a species widespread in most Europe, which adapts very well to the environment in which it stays (Randi i in., 2004). Deer are animals living in a moderate climate, which are characterized by embryonic diapause (Ptak i in., 2012). The development of the embryo is stoped from August to December to minimize the risk of stressful conditions for childbirth and development of young individuals, such as large temperature fluctuations or lack of food. The implantation occurs after about 5 months - at the turn of December and January (delay implantation). There is a

rapid growth and implantation of the cell, while the young childbirth itself occurs in May, when it is warm and a lot of food in the environment.

The main part of the female deer reproductive system is the uterus consisting of two corners connected to connective tissue. The endometrium lists the uterine cavity, allowing the embryo to implant (Kurek i in., 2021). It consists of two layers - the basal and functional (functional). The first of them combines an endometrium from a myometrium (outer layer), while the functional layer, located closer to the light of the uterus, changes under the influence of hormones during the reproductive cycle. Another name of this tissue is the uterus mucosa, and its main tasks include the preparation of the reproductive system for pregnancy, and as a result of maintaining it, or in the absence of fertilization - peeling to repeat the entire process in the next reproduction cycle. In the functional layer, the embryo implant or it is removed after each cycle (Paulson and Comizzoli, 2021). The height and spontaneous exfoliation of the endometrium depends on the sex hormones of females - progesterone and estradiol (E2) (Critchley i in. 2020).

Aromatase is an enzyme extremely important for the proper development and operation of the reproductive system in both female and male individuals. Its participation is crucial in regulating synthesis of estrogens from testosterone (Hong et al., 2009) in many tissues - including in endometrium or ovaries (Molehin et al. 2021). This enzyme is extremely important in reproductive cycles in females, but also has a big role in the course of spermatogenesis, or in the growth of prostate in males. It also takes part in sexual differentiation in the brain and in defense against neurodegeneration (Stocco, 2012). Too much aromatase can lead to increased estrogen production, which in consequence may affect the occurrence of endometriosis. The substrates of the aromatase enzyme are, for example, testosterone and androstendion. Estrogens affect the correct formation of functional endometrial tissue when preparing the system for pregnancy, as well as its removal in the absence of fertilization.

Estradiol (E2) - one of the main estrogens - participates in many key mechanisms of the female reproductive system, among others: modulates the functions and structure of the uterus. This hormone has a participation in the cyclicality of sexual behavior and its regulated expression in uterine and ovarian cells has a great impact on the correctness of the rut cycle (Stocco, 2008) It can be synthesized from testosterone with the participation of aromatase, or from estrone through 17β-hydroxytroid dehydrogenase (17β-HSD) (Ryan, 1982, Meinhardt i Mullis, 2002). The scheme showing the formation of estradiol (E2) is shown in Figure 1.

The main products of aromatase are:

- Estron, which synthesis substrate is androstenodion;
- 17β-Estradiol, which precursor is testosterone;
- Estriol, formed by transforming 16α-hydroxytestosterone;
- 16α-hydroxyestron from 16α-hydroxyandrostenedionu (Ryan, 1982).

Estrogens combine with their specific eceptors in many tissues, and they are divided into two types: α and β estrogen receptors. The former are mainly in the uterus, kidneys, hearts or liver, while the second in the ovaries, lungs or nervous system (Amenyogbe i in., 2020). Estrogens, in addition to their role in the growth and functioning of the mammary gland and uterus, can also lead to the formation of hormone -dependent cancers in them. This is due to their ability to activate cell proliferation. In the case of the mammary gland, healthy cells have low aromatase content, but the sick cells are the opposite - high aromatase content, which leads to increased estrogen production. Similar results were obtained during endometriosis research (Stocco, 2012). Aromatase inhibitors have been shown to be used to treat estrogen - dependent diseases by reducing their concentration in cells (Pavone i Bulun, 2012).

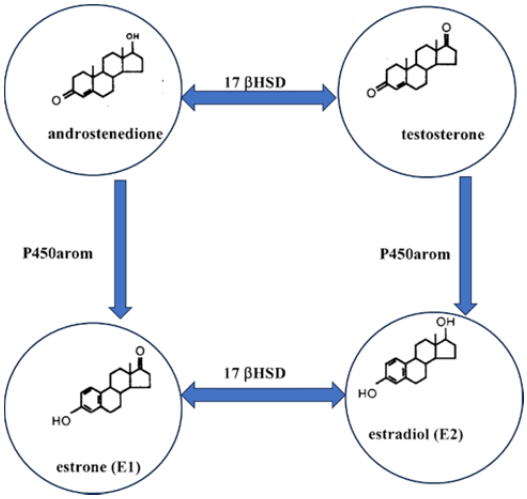


Fig. 1 Diagram showing the formation of estradiol (E2) (modified on the basis of Meinhardt and Mullis, 2002).

Purpose of research

The purpose of the conducted research was to assess the impact of an extremely low frequency electromagnetic field on the production of aromatase, which affects the production of estrogens in the endometrium of European roe-deer (*Capreolus capreolus*).

Materials and methods

The experimental material (uterus tissues) was taken during the selection hunting in the forests of the Świerczów Forest District (Podkarpackie Voivodeship, Poland). After placing in PBS (physiological salt buffered with phosphate), the samples were delivered on ice (4°C) to the laboratory. Three parts of tissue from each roe-deer were taken for the study.

Conducting the tissue culture and influence of the electromagnetic field on them

In order to provide slides of tissues the optimal environmental conditions, the prepared samples were placed on a culture plate in a water bath. After 2 hours of pre-incubation and exchange of culture medium, the tissue culture was carried out. The tissues have been exposed to an electromagnetic field with a frequency of 50 Hz for 2 or 4 hours, generated by Astar Magneris generator with the flat, innovative applicators. The slides were divided into 3 groups: from each individual: one piece was a control - it was not treated with an electromagnetic field, one was treated for 2 hours and one for 4 hours. The samples of the control group were incubated in the same conditions and the same time, without treating them with an electromagnetic field. After incubation the tissue from all groups were fixed to perform histological preparations.

Conducting an immunohistochemical reaction

The immunohistochemical technique used in the research uses antibodies to visualize a given antigen produced by the tissue. It can be used regardless of whether the tissue is

healthy or sick, but its biopsy is required and then sinking the tissue in paraffin. After preparing such a slide and incubation, it is possible to see under a fluorescent or optical microscope whether the antibody has been attached. The choice of equipment depends on the marker chosen for visualization. It can be a fluorescent dye, an enzyme like horseradish peroxidase or colloidal gold. Such a marker can be attached to the primary or secondary antibody. This technique is used in laboratory studies and in the detection of many diseases, such as cancer or neurodegenerative disorders. The stages of the immunohistochemical method of the procedure were presented at Koziorowska paper (Koziorowska et al. 2023).
In order to dehydrate the tissues, they were placed in alcohol, changing its concentration, then they were subject to xylene. Dehydrated and x -rayed in xylene tissues were moved to the paraffin. Histological blocks were made. Tissues embedded in paraffin were cut into scraps with a thickness of 5 μm using a microtome (Leica). The slide with applied scraps is shown in Fig. 2.

Figure 2. The basic slide with applied slides of uterine tissues after cutting paraffin blocks; Created with BioRender.com.

For each sample (50 Hz 2 hours, 50 Hz and 4 hours, control) two microscopic slides were prepared. In order to determine the impact of the electromagnetic field of extremely low frequencies on uterine tissues, 5 photos of all samples that have been subjected to immunohistochemical reaction were taken, using a camera connected to a light microscope (Olympus CX41). Then, using the ImageJ programme, the relative optical density (ROD) in gray (8 bit) was calculated according to the given formula:

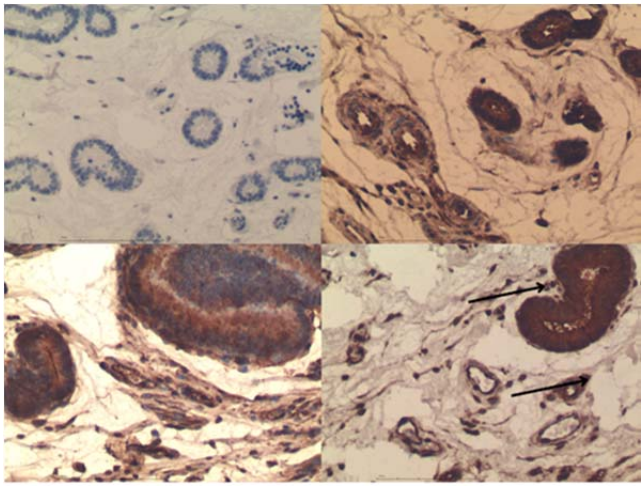
$$(1) \quad ROD = log\frac{GL_{blank}}{GL_{product}} / log\frac{GL_{blank}}{GL_{background}}$$

where: GL_{blank} is 255 - the gray level measured after removing the glass from the path of light, $GL_{product}$ - the gray level of the diaminobenzidine bronze reaction products; $GL_{background}$ - the gray level of unstained tissue areas.

The results of the research

The photos from the optical microscope showing the effect of dyeing the uterine tissue using an immunohistochemical reaction are presented in the photo 1.
Based on the photos taken, it can be seen that the substrate for horseradish peroxidase combined properly (control photos), so the immunohistochemical reaction was performed correctly and the antibodies connected to the tested enzyme. For photos taken in repetitions for each slide, the relative optical density (ROD) was calculated. Pictures for calculations were taken at 40x enlargement.

On the pictures there is noticeable an increase in the number of points dark brown colored with the increase in time through which tissues were subjected to the impact of the electromagnetic field. These changes are associated with changes in the concentration of aromatase in slides. In photographs with incubation time in stress conditions was 4 hours, it is very difficult to notice cell nuclei colored with hematoxylin. There can also be noticed the difference between control tissues and tissues influenced by the field with the frequency of 50 Hz for 2 hours. To check the difference between control and the tested tissues, the relative optical density was calculated (Chart 1).



Phot. 1. The photos from the optical microscope showing the effect of dyeing the uterine tissue using an immunohistochemical reaction, at 20x enlargement. The arrows are marked with the reaction products of horseradish peroxidase with DAB. Upper row: from the left: tissue stained only with hematoxylin, right: control tissue, without the operation of the electromagnetic field; Lower row: from the left: tissue after oparation of the electromagnetic field with a frequency of 50 Hz for 2 hours, right: tissue after operation of the electromagnetic field with a frequency of 50 Hz for 4 hours).

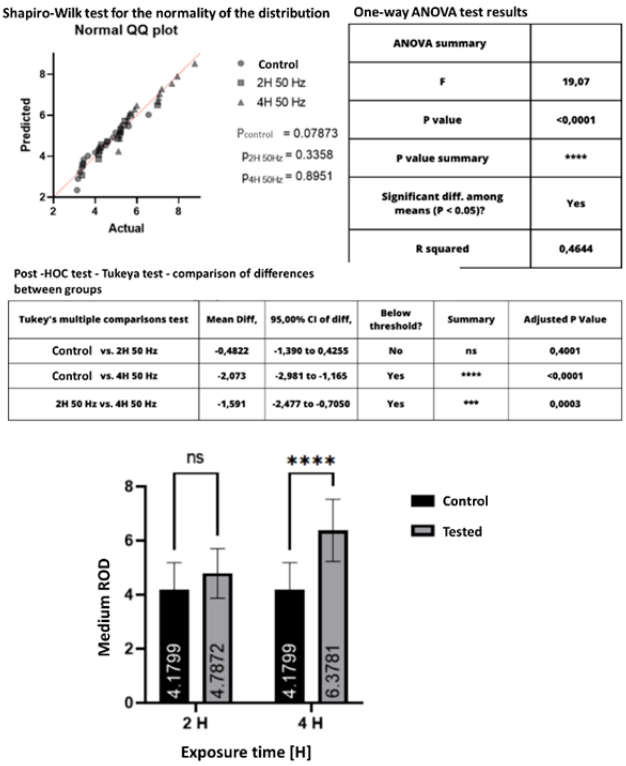


Chart 1: The average ROD chart for each of the analyzed groups with standard deviation

The results of the conducted research indicate the operation of the electromagnetic field with a frequency of 50 Hz as a factor causing an increase in the production of aromatase enzyme by the tissue of the roe-deer endometrium. The high amount of aromatase can lead to inflated estrogen production.

Conclusions

The available literature did not find the results of research on the effect of an electromagnetic field with an extremely low frequency on a change in the concentration of aromatase in the deer endometrial. The purpose of the research presented in this work was to check the impact of

an electromagnetic field with an extremely low frequency of 50 Hz on the production of aromatase by the tissue of the uterus of the European roe-deer (*Capreolus capreolus* L.). The high amount of aromatase can lead to inflated estrogen production. Such hormonal disorders can have serious health effects. Estradiol's ability to activate proliferation can cause disease changes, such as endometriosis. The results of the conducted tests confirm the operation of the electromagnetic field with a frequency of 50 Hz as a factor causing an increase in the production of this enzyme by the tissue of the roe-deer endometrium tissues.

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