**1. Anna KOZIOROWSKA[1,2], 2. Jakub SIUTA[3], 3. Ewelina BATOR[2], 4. Robert KRASOWSKI[4]
5. Marek KOZIOROWSKI[2,5]**

[1]Rzeszow University, College of Natural Sciences, Institute of Materials Engineering, Poland
[2]Rzeszow University, Interdisciplinary Center for Preclinical and Clinical Research, Werynia 2, 36-100 Kolbuszowa, Poland
[3]Rzeszow University, College of Natural Sciences, Institute of Biology and Biotechnology, Poland (student of biotechnology)
[4]Non-public Education Center in Limanowa, Poland
[5]Rzeszow University, College of Natural Sciences, Institute of Biology and Biotechnology, Poland
ORCID 1. 0000-0003-1344-4033; 3. 0000-0001-5510-7938; 5. 0000-0002-7468-6063

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Electromagnetic field as a factor affecting the activity of the synthesis of the enzyme 3 beta-hydroxysteroid dehydrogenase (3β-HSD) in the cells of the adrenal cortex of lambs

***Abstract:** The article presents the results of studies on the impact of extremely low-frequency electromagnetic field on the level of 3β-steroid dehydrogenase (3β-HSD) in the adrenal cells of sexually immature lambs. Adrenal tissue cultures were performed and exposed to 50 or 120 Hz electromagnetic fields. Immunohistological analysis showed the presence of 3βHSD receptors in adrenal tissues. There were no significant differences in the amount of 3-beta-hydroxysteroid dehydrogenase receptor present in the cells of the adrenal cortex at different times of exposure (2 or 4 hours) for both tested frequencies compared to tissues from the control group. The obtained results show that the influence of the electromagnetic field on the level of steroid dehydrogenase in the adrenal glands is insignificant, regardless of the frequency of the electromagnetic field (in the extremely low range) and the duration of exposure.*

Streszczenie:** Artykuł prezentuje wynikii badań oddziaływania pola elektromagnetycznego ekstremalnie niskiej częstotliwości na na poziom 3β dehydrogenazy steroidowej (3β-HSD) w komórkach naddnerczy niedojrzałych płciowo jagniąt. Przeprowadzono hodowle tkankowe nadnerczy i poddano je oddziaływaniu pola elektromagnetycznego o częstotliwości 50 lub 120 Hz. Przeprowadzona analiza immunohistologiczna wykazała obecność receptorów 3βHSD w tkankach nadnerczy, Nie stwierdzono istotnych różnic w ilości receptora dehydrogenazy 3-beta-hydroksysteroidowej obecnej w komórkach kory nadnerczy przy różnych czasach oddziałuwania (2 lub 4 godziny), dla obu badanych częstotliwości w porównaniu do tkanek z grupy kontrolnej. Uzyskane wyniki świadczą, że wpływ pola elektromagnetycznego na poziom dehydrogenazy steroidowej w nadnerczach jest nieistotny bez względu na częstotliwość pola elektromagnetycznego (w zakresie ekstremalnie niskich) i czas trwania ekspozycji. **(Pole elektromagnetyczne jako czynnik oddziałujący na aktywność syntezy enzymu dehydrogenazy beta-3-hydroksysteroidowej (HSD) w komórkach kory nadnerczy jagniąt)

Keywords: electromagnetic field of extremely low frequency, enzyme 3β-HSD; adrenal cortex; lamb
Słowa kluczowe: pole elektromagnetyczne ekstremalnie niskich częstotliwości, enzym dehydrogenaza 3 beta-hydroksysteroidowa (3β-HSD); kora nadnerczy; jagnię.

Introduction

The electromagnetic field is the result of the presence of magnetic and electric fields in the environment. All living organisms, including humans, are the source of the electromagnetic field. The environment may contain natural electromagnetic fields (Earth, Sun, atmospheric phenomena) and man-made artificial ones. Wherever electricity flows, an electromagnetic field is created.

The spectrum of electromagnetic radiation includes all lengths and frequencies of electromagnetic waves. The longest waves and those with the lowest frequency are included in the spectrum of non-ionizing waves, as the frequency increases, the energy of the waves increases and the radiation becomes ionizing. The standards of the electromagnetic field that are in force in Poland are consistent with current knowledge supported by scientific research and correspond to the values of EU regulations. They protect people against too high values of fields emitted by devices.

Non-ionizing radiation that has no thermal effect on humans includes:

• extremely low-frequency field, mainly from electrical devices and transmission lines,

• radio waves, commonly used in broadcasting,

• microwaves, carrying so much energy that they are used in microwave ovens to heat food; they also include waves emitted from telecommunications devices,

• infrared, widely used, among others, in night vision cameras and in medicine.

Exposure to extremely low-frequency electromagnetic fields does not show the effect of increasing temperature, because the emitted waves have too low energy, which is too weak to damage DNA directly leading to genotoxic effects [1]. The genotoxic effects of fields can occur indirectly by generating oxygen radicals or by impairing the radical scavenging mechanism [2].

A biological effect is the reaction of an organism or cell to stimulation by an external factor. The biological effect does not mean a disease state, but is a sign of the activation of adaptation processes [3]. The electromagnetic field acts as a regulator of biological functions, including reduction/induction of the inflammatory process, regulation of cell differentiation and gene expression [4]. It was also found that the electromagnetic field has a modulating effect on the removal of pesticides from the body of the honey bee [5]. Due to its therapeutic properties, extremely low frequencies are used during the physiotherapy process. The therapeutic effect of such fields is associated, among others, with the improvement of neurotransmission. By increasing blood flow, it contributes to an increase in the metabolism of nervous tissue and has a positive effect on its regeneration [6]. Devices using fields in the range of 1-120 Hz have a beneficial effect on chronic and acute pain and stimulate muscles [7].

Our previous research has shown that an extremely low-frequency field may be a factor interacting with the endocrine system. One of the most important endocrine glands are the adrenal glands. The adrenal glands are paired endocrine glands located above the upper poles of the kidneys. They consist of two main layers: medullary and cortical. The latter is divided into three layers, arranged in turn towards the medulla: zona glomerulosa, zona fasciculata and zona reticularis.

In mammals, the fetal adrenal glands play a key role in late pregnancy as the steroid hormones produced by the fetal adrenal glands are involved in fetal maturation and neonatal adaptation to the extrauterine life [8]. The adrenal glands are the source of many bioactive substances, including steroid hormones, catecholamines, cytokines, neurotransmitters and neuropeptides. These substances interact with various types of cells in the adrenal gland itself, which translates into its function in conditions of stress and many diseases [9].

In the adrenal cortex, the process of steroidogenesis takes place and glucocorticoids, mineralocorticoids and steroid hormones (estrogens, gestagens and androgens) are produced. The starting substrate for the synthesis of the steroid chain is cholesterol. The steroid hormones produced by the adrenal cortex differ in each layer due to the specific enzymes they contain. The zona glomerulosa produces mineralocorticoids, the zona fasciculata glucocorticoids, and the zona reticularis produces androgen precursors, mainly dehydroepiandrosterone (DHEA) and androstenedione. The amount of steroid hormones in the body depends on the presence of their subsequent precursors (substrates) and the enzymes involved in their conversion (synthesis). One of the key enzymes involved in the synthesis is 3β-hydroxysteroid dehydrogenase (3β-HSD), which is involved in the synthesis at the beginning of the steroidogenesis pathway. The effects of steroid hormones also involve molecular receptors for 3β-HSD present in the target tissue, which make the tissue reactive to the hormone. The diagram of the synthesis of steroid hormones with an indication of the location of 3β-HSD action is shown in Figure 1.

The aim of the research presented in this paper is to determine the effect of the electromagnetic field of extremely low frequencies of 50 and 120 Hz and the value of magnetic induction of 8 mT on the synthesis of 3-beta-hydroxysteroid dehydrogenase (3βHSD) in all adrenal cortex layers of sexually immature lambs.

Materials and methods

The research material consisted of adrenal tissues of sexually immature lambs collected immediately after slaughter in a specialized slaughterhouse in Lesko. The research group consisted of tissues from 6 animals, divided into a group exposed to the electromagnetic field and a control group.

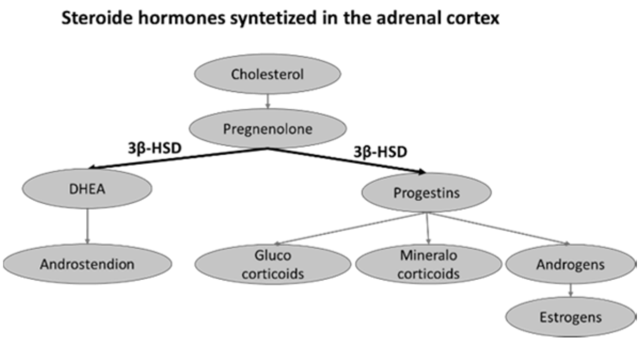

Fig. 1 Simplified diagram of the synthesis of steroid hormones with the participation of the 3β-HSD enzyme

Carrying out tissue culture and the influence of the electromagnetic field on tissues

The prepared tissue sections were placed on a culture plate in a water bath with shaking to ensure optimal environmental conditions for the tissues. After a 2-hour pre-incubation and culture medium exchange, cultures were performed and the tissues were exposed to an electromagnetic field for 2 or 4 hours. The electromagnetic field with a frequency of 50 or 120 Hz was selected for the tests. Control group samples were obtained by incubation under the same conditions without treatment with an electromagnetic field. After incubation, tissues from both groups were fixed for histological preparations.

Conducting an immunohistochemical reaction

In order to dehydrate the tissues, they were placed in alcohol, changing its concentration, and then exposed to xylene. Dehydrated and overexposed in xylene tissues were transferred to paraffin. After infusing the tissues with paraffin, histological blocks were made. The successive stages of the procedure are shown in Figure 2.

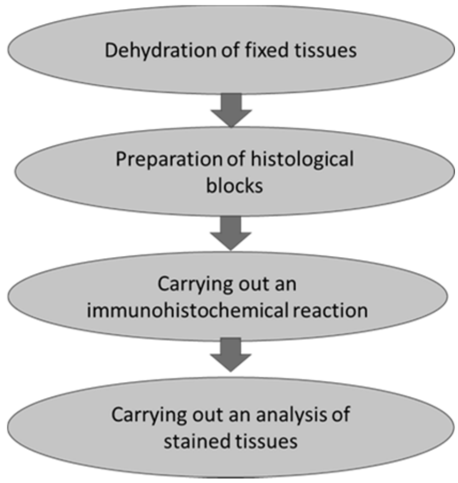

Fig. 2 Steps in the procedure of the immunohistochemistry method

Paraffin-embedded tissues were cut into 5 μm sections using a microtome (Leica). The resulting paraffin sections were transferred to a water bath with distilled water at about 45°C to stretch the tissues. After straightening, the adrenal sections were placed on glass slides and allowed to dry. For each test sample (50 Hz 2 hours, 50 Hz 4 hours, 120 Hz 2 hours, 120 Hz 4 hours, control 2 hours and control 4 hours), two microscope slides were prepared for sections.

Analysis of the intensity of the immunohistochemical reaction

In order to determine the influence of the extremely low-frequency electromagnetic field on the adrenal tissues, photographs of the examined fragments of the organ were taken using an Olympus CX41 microscope coupled with a Moticam 3.0MP camera and a computer equipped with the Motic Images Plus 3.0 image analysis system. The analysis consisted in examining the gray level of brown precipitate in the adrenal layers, indicating the presence of the enzyme 3-beta-hydroxysteroid dehydrogenase, using the ImageJ program. The obtained results were used to calculate the value of relative optical density (ROD) using formula (1) [10]:

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

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

Analyzes were made on the basis of photographic documentation of all three layers of the adrenal cortex using an optical microscope at 40-fold magnification for ROD calculations and 4-fold magnification to observe the course of reactions in all adrenal layers.

A brown precipitate indicates the presence of 3β-HSD. There is a noticeable difference between zona fasciculata and zona reticularis (brown colouration) and zona glomerulosa (no or slight brown color).

The photos were taken under a microscope at 40x magnification. All three layers of the adrenal cortex were photographed in several replicates for each sample (2 or 4 hours 50 Hz and 2 or 4 hours 120 Hz) and control (2 and 4 hour control).

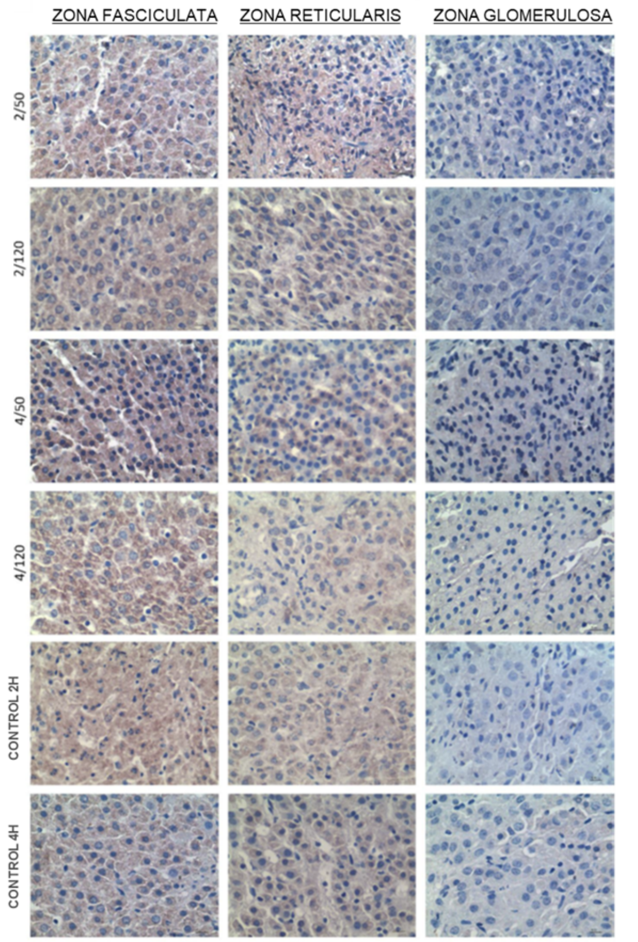

Fig. 3. Sample photos of the examined adrenal layers taken after the immunohistochemical reaction - the tissue of the layers of the adrenal glands: zona fasciculata, zona reticularis and zona glomerulosa

Photographic documentation was used to calculate the relative optical density of 3-beta-hydroxysteroid dehydrogenase. Using Microsoft Excel, the ROD was calculated for samples from the same layers of the adrenal cortex and treated with an extremely low-frequency electromagnetic field for the same period of time. ROD values calculated in four replicates were used to plot the graphs, taking the average of their results. In order to assess statistical significance, ANOVA analysis of variance ($p<0.05$) was used along with the Shapiro-Wilk test for the assessment of the normality of data distribution using the GraphPad Prism 9 program.

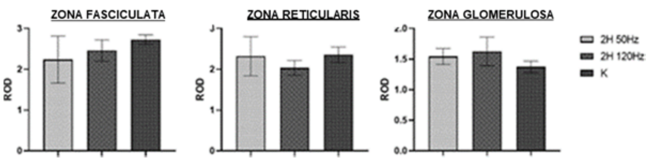

Fig. 4. Comparison of relative optical density (ROD) values for three layers of the lamb's adrenal cortex when exposed to an electromagnetic field for 2 hours.

Samples from the same incubation period (2 and 4 hours) and from the same layer of the adrenal cortex were analyzed. No statistically significant differences were observed between samples treated with extremely low-frequency electromagnetic field and control samples ($p>0.05$), as well as between samples originating from the same layer of the adrenal cortex, differing in incubation time and field frequency ($p>0.05$) (Figures 4, 5 and 6).

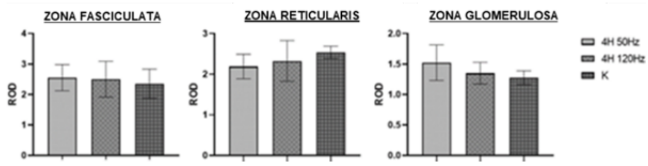

Fig. 5. Comparison of relative optical density (ROD) values for 3 layers of lamb adrenal cortex. The samples obtained after 4 hours of exposure to the electromagnetic field were analyzed.

Based on the obtained results, there were no significant differences in the amount of 3-beta-hydroxysteroid dehydrogenase present in the cells of the adrenal cortex exposed to the extremely low-frequency electromagnetic field for 2 or 4 hours, for both tested frequencies, compared to cells from the control group.

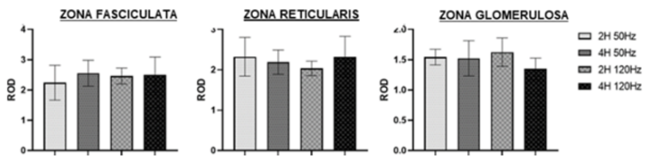

Fig. 6. Comparison of relative optical density (ROD) values for 3 layers of lamb adrenal cortex. The impact of 2 hours was compared to 4 hours.

Conclusions

The exposure of humans to electromagnetic fields can cause significant changes or harmful effects in biological systems. The adrenal dysfunction may result in a deficiency of adrenal steroidogenic enzymes. It is associated with disorders of the cardiovascular system [11], deregulation of mineral and glucose metabolism, impaired masculinization, or the body's response to stress.

The results of our previous studies showed a stimulating or inhibiting effect of extremely low frequency fields (≤120 Hz) on animal cells and tissues in in vitro cultures [12]. We have confirmed its effect on the reproductive system of both female and male. The field was a factor influencing the synthesis and secretion of the steroid sex hormone - estradiol E_2, which is one of the most important hormones involved in all stages of reproduction. In the domestic pig model, we confirmed that, depending on the frequency and duration of exposure, there are significant changes in the synthesis and release of estradiol E2 in the presence or absence of progesterone (the second important steroid hormone involved in the regulation of reproduction). This proves that progesterone may act as a potentially protective factor against the effects of electromagnetic fields [13]. In the European roe deer model, we demonstrated the influence of the electromagnetic field on the number of steroid hormone receptors, leading to their increase or decrease, depending on the physical parameters of the field and the reproductive period from which the testicular tissue was collected (unpublished data being prepared for publication).

Based on the results presented in this paper, we can conclude that the electromagnetic field of extremely low frequencies of 50 Hz and 120 Hz has no significant effect on the synthesis of the beta-3-hydroxysteroid dehydrogenase enzyme in the adrenal cortex cells of sexually immature lambs. It is probably related to the low activity of reproductive functions and too short a period of life for stress adaptation. It can also be assumed that the adrenal glands, which are involved in the period of neurohumoral stress adaptation, respond to external factors relatively late only after the reaction of the nervous system.

Based on the results of these studies, it can be hypothesized that the presence of lambs in the vicinity of objects generating extremely low-frequency electromagnetic fields should not cause changes in the steroidogenesis process in these animals during the period of entering sexual maturity. However, further studies in sheep models are needed to determine whether 50 and 120 Hz fields may have an effect on other components of lamb steroidogenesis.

Conflicts of Interest: The authors declare no conflict of interest.

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Authors:
Anna Koziorowska, PhD, DSc. Eng., Rzeszow University, College of Natural Sciences, Institute of Material Engineering, ul. Pigonia 1, 35-310 Rzeszów, Poland. e-mail: akozioro@ur.edu.pl,
Jakub Siuta, Eng, Rzeszow University, College of Natural Sciences, Institute of Biology and Biotechnology, ul. Pigonia 1, 35-310 Rzeszów (student)
Ewelina Bator, PhD, Rzeszow University, Interdisciplinary Center for Preclinical and Clinical Research,Werynia 2a, 36-100 Kolbuszowa, Poland. e-mail: ebator@ur.edu.pl
Robert Krasowski, PhD, Non-public Education Center in Limanowa, ul. Bronisława Czecha 5G, 34-600 Limanowa Poland. e-mail:robertk2@onet.eu
Marek Koziorowski, Titular Prof. MVD, Rzeszow University, College of Natural Sciences, Institute of Biology and Biotechnology, ul. Pigonia 1, 35-310 Rzeszów, Poland. e-mail: *mkoziorowski@ur.edu.pl*

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