1. Anna KOZIOROWSKA[1,2], 2. Weronika WILCZAK[3], 3. Katarzyna KOZIOŁ[2,4], 4. Robert KRASOWSKI[5] 5. Marek KOZIOROWSKI[2,4]

[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 BiotechnologyPoland (student of biotechnology)
[4]Rzeszow University, College of Natural Sciences, Institute of Biology and Biotechnology, Poland
[5]Non-public Education Center in Limanowa, Poland
ORCID 1. 0000-0003-1344-4033, 3. 0000-0002-2212-5176, 5. 0000-0002-7468-6063

doi:10.15199/48.2023.01.64

The electromagnetic field influence on the steroidogenesis process in the sexually immature lambs uterus

Abstract: *This paper presents the results of studies on the influence of the electromagnetic field on the steroidogenesis process of immature lambs. The aim of the research was to determine how the electromagnetic field influences the synthesis of aromatase proteins in the tissues of the uterus of lambs. Tissue cultures were performed and subjected to an electromagnetic field with a frequency of 50 or 120 Hz. An immunihistochemical reaction was performed which showed the presence of aromatase in the sheep's myometrial and endometrial tissues. It has been shown that the extremely low-frequency electromagnetic field affects the activity of steroidogenesis, one of the basic processes in the reproductive period*

Streszczenie: *W niniejszej pracy zostały przedstawione wyniki badań oddziaływania pola elektromagnetycznego na process steroidogenezy niedojrzałych owiec. Celem badan było określenie w jaki sposób pole elektromagnetyczne wpływa na syntezę białek aromatazy w tkankach macicy jagniąt. Przeprowadzono hodowle tkankowe i poddano je oddziaływaniu pola elektromagnetycznego o częstotliwości 50 lub 120 Hz. Przeprowadzono reakcję immunihistochemiczną, która wykazała obecność aromatazy w tkankach myometrium i endometrium owcy. Wykazano, iż pole elektromagnetyczne ekstremanie niskiej częstotliwości oddziałuje na aktywność steroidogenezy, jednego z podstawowych procesów w okresie rozrodczym. (**Wpływ pola elektromagnetycznego na proces steroidogenezy w macicy niedojrzałych płciowo jagniąt**).*

Keywords: electromagnetic field, steroidogenesis, tissue culture, immunohistochemical reaction.
Słowa kluczowe: pole elektromagnetyczne, steroidogeneza, hodowle tkankowe, reakcja immunohistochemiczna

Introduction

The extremely low frequency electromagnetic field (ELF-EMF) belongs to the group of non-ionizing fields. It covers frequencies ranging from 0 to 300 Hz [1]. People have been exposed to its influence for many years. This is due to the development of technology and the increasing number of artificial field sources in the environment. The effects of the electromagnetic fields impact on organisms depend on field's physical parameters. Different time of exposure, frequency, value of the magnetic induction and the nature of the field cause different biological effects. The energy of the electromagnetic fields absorbed directly in the body causes the generation of electrically induced currents and the heating of tissues. This can cause undesirable effects. For many years, there have been conducted the studies on the effects of electromagnetic fields on cell and tissue cultures *in vitro*. Analyzes of cell or tissue cultures under certain physical parameters of electromagnetic fields outside the human body exclude the influence of other factors and do not fully reproduce the *in vivo* conditions. [2]. However, they are very important stage of research allowing for applying of results for humans. A very important element of the research is also the selection of the correct electromagnetic field generation system and ensuring its appropriate parameters and environmental conditions for *in vitro* tests. [3]. The electromagnetic field generation system used for the presented studies provided the appropriate field parameters.

Lambs were chosen as the research model in the presented studies. A significant problem in sheep breeding is the reproductive control. From an economic point of view, the most desired by breeders are the breeding and sheep deliveries at a similar time. This is why there are sought the artificial methods of inducing heat and fertilization at the same time. One of such methods is the use of hormones that allows to synchronize oestrus during the reproductive season, but also allows for fertile mating outside this period, what's more, it enables an increase in the number of ovulations and the induction of early sexual maturation. In lambs, the uterus is not fully developed. Its maturation begins with an increased secretion of estrogens, which leads to the first surge of gonadotrophins and ovulation. Lambs reach sexual maturity between the ages of 5 and 12 months. Therefore, it is important to understand the mechanism responsible for regulating the process of steroidogenesis in the uterus. Its knowledge would allow both to accelerate the maturation of the lamb's uterus, but also could be used to synchronize oestrus, which allows, for example, to coordinate sheep littering with the current demand for raw materials - meat, milk or fibers.

The estrogens and the progesterone are the main hormones that modulate the functions of the female reproductive system. Their source in mature specimens are mostly ovaries. They work by binding to appropriate intracellular receptors, the estrogen receptor (ER) and the progesterone receptor (PR), respectively. The estrogens are important for the proper growth and differentiation of the reproductive system and they are involved in the monthly preparation of the body for the possible pregnancy [4]. They are steroid hormones responsible for the development of, among others, mammary glands, central nervous and skeletal systems or an increase in adipose tissue [5]. In the case of the not yet matured sheep, high levels of the estrogen receptor ER and the progesterone receptor PR in the uterus are observed at the time when secondary sexual characteristics begin to develop and reproductive ability is achieved [6].

One of the factors that may influence the acceleration of sexual maturation of lambs, through the action of estrogens, is the electromagnetic field. The aim of the study was to determine how the electromagnetic field with the frequencies of 50 or 120 Hz and the magnetic induction of 8 mT, generated by a generator with innovative flat applicators, affects changes in the synthesis of aromatase protein in the endometrium and myometrium tissues collected from the sexually immature lambs. Aromatase converts testosterone into estradiol 17-beta, increasing the pool of estrogens - hormones strongly involved in

reproductive processes. Successful synchronization of sheep maturation would greatly facilitate the breeding of these animals.

The influence of the electromagnetic field on the uterine tissue cultures

The research material consisted of the uterine tissues of six sexually immature lambs, collected in a sheep slaughterhouse. The collected organ fragments were transported to the University's laboratory at 4 ° C. The lambs were between 7 and 10 months old. Samples of myometrium and endometrium of 100 ± 5 mg were prepared from the uterus of each individual. The tissues were divided into the test groups and the control groups, which were not subjected to the electromagnetic field, but cultured at the same time and under the same environmental conditions. First, the tissues were placed in a 24-well plate with 1 ml of the prepared medium and pre-incubated at 37°C and an atmosphere of 5% CO_2 and 95% O_2 in a shaking water bath for 2 hours. Then, the culture medium was replaced with fresh ones and the tissues were subjected to the electromagnetic field generated by a Magneris generator (Astar, Poland) with a magnetic induction of 8 mT and a frequency of 50 or 120 Hz for 2 or 4 hours.

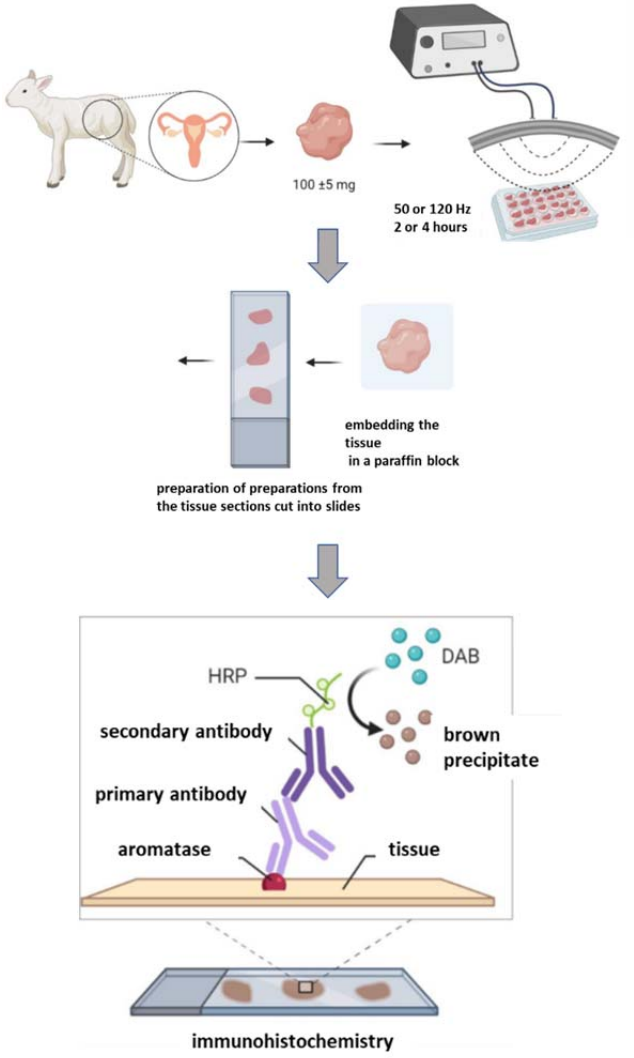

Fig. 1. The diagram of the subsequent stages of the experiment - collection of the material, the tissue culture subjected to the electromagnetic field, the immunohistochemical reaction.

The Magneris generator has flat applicators for which the distribution of the electromagnetic field under its surface is given. The applicators are placed above the plates, which are rocked throughout the cultivation process, which allows for the proper washing of the tissues with the medium, ensuring proper diffusion of gases and fluids into the tissue fragments.

Conducting an immunohistochemical reaction

After the collection has been carried out, the tissues were placed in the reagent for fixation and histological preparations. The dehydrated tissues were embedded in the paraplast blocks, then cut on a rotary microtome (Leica RM2265). The sections were applied to slides, followed by an immunohistochemical reaction. The sequence of the procedures performed is shown in Fig. 1.

The photographs of the stained uterine sections were taken with a camera connected to a microscope (Moticam 3.0) in 2-3 replications for each preparation at 40x magnification. Separate pictures were taken for endometrial and myometrial tissues.

The semi-quantitative analysis of the immunohistochemical reaction

The intensity of the immunohistochemical reaction was assessed using the ImageJ program (National Institutes of Health, Bethesda, MD, USA). Relative optical density (ROD) was calculated on the basis of the formula (1) [7]:

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

where: GL_{blank} is 255 which is the gray level measured after removing the glass from the path of the light, $GL_{produkt}$ this is the gray level of the diaminobenzidine bronze reaction products; GL_{tlo} it is the gray level of unstained areas of tissue.

The statistical significance analysis was performed in GraphPad Prism 9. The two-way ANOVA and Dunnett's post-hoc test were used to show the differences between the test and control samples. The p value <0.05 was used, and the following values were found to be statistically significant:
* p<0,05; ** p<0,01; *** p<0,001.

The research results

The performed histological analysis of the uterine tissues showed the presence of aromatase both in the uterine fragments exposed to electromagnetic field and in the control samples. The aromatase was found in the myometrium (Figures 2A-F) and the endometrium (Figures 3A-F). The obtained results are given in the form of the arithmetic mean and standard deviation (SD).

After the myometrium tissues exposure to electromagnetic field of 50 Hz, the relative optical density (ROD) is higher (p = 0.0216) at 2 hours (2.39 ± SD) than the control (2.005 ± SD).

There is also a trend showing a decrease in the presence of aromatase after 4 hours of exposure compared to both the control and 2 hours of exposure. Electromagnetic field with a frequency of 120 Hz, regardless of the incubation time, did not change the level of aromatase (Figure 3).

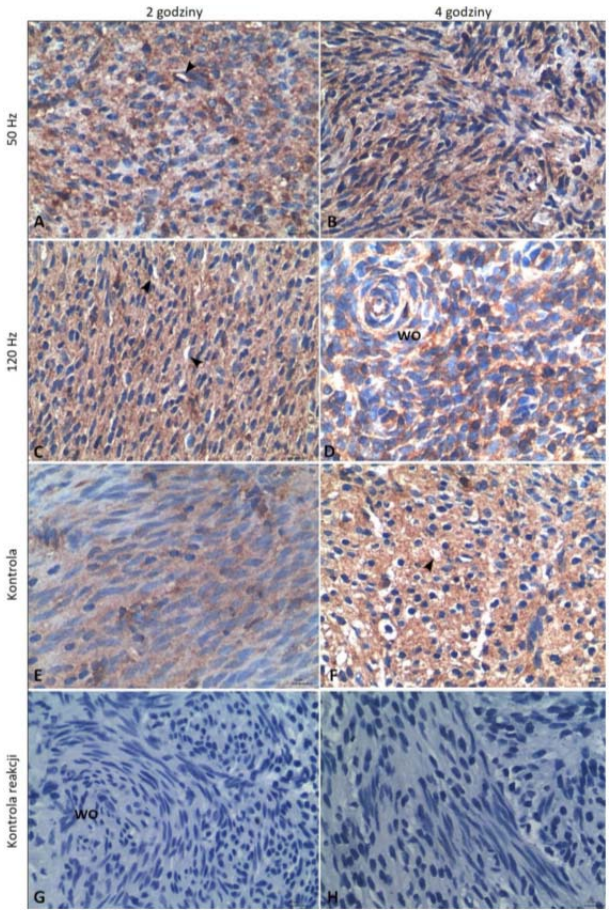

Fig. 2. Histological pictures of the myometrium of sexually immature lambs exposed to the electromagnetic field with a frequency of 50 Hz (A and B) and 120 Hz (C and D). Controls (E, F) are tissues incubated under the same conditions but without the influence of the electromagnetic field. The myometrium consists of i.a. from the inner layer with a circular arrangement of muscle cells (WO) and cells arranged in longitudinal fragments (WP). Pictures taken at 40x magnification.

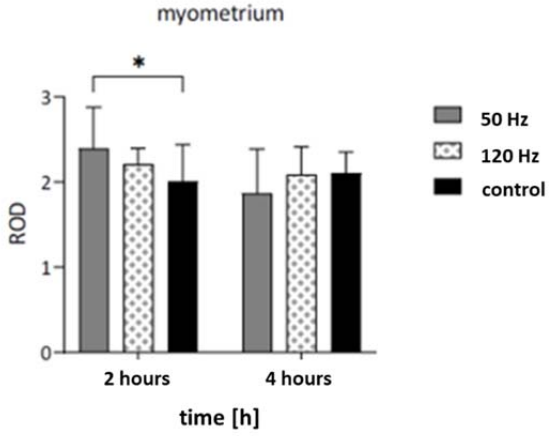

Fig. 3. Comparison of the the aromatase ROD means for myometrium tissues subjected to electromagnetic field at 50 or 120 Hz for 2 or 4 hours and control tissues.

The aromatase ROD differed depending on the frequency studied and the time the tissues were exposed to the electromagnetic field. The endometrium tissues exposed to 50 Hz for 2 hours has a higher ROD (2.765 ± SD) than the control (1.63 ± SD; p = 0.002). Similarly, during the 4-hour exposure, the presence of aromatase was higher (2.838 ± SD) compared to the control sample (1.797 ± SD; p <0.001).

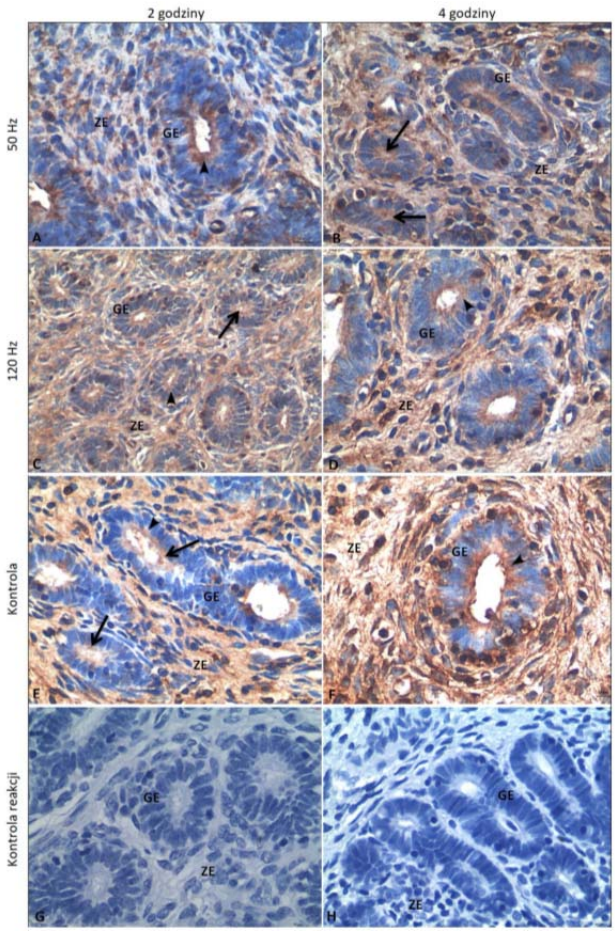

Fig. 4. Histological pictures of the endometrium of a sexually immature lamb exposed to an electromagnetic field for 2 or 4 hours at a frequency of 50 Hz (A and B) and 120 Hz (C and D). Controls (E, F) are tissues incubated under the same conditions but without the influence of the electromagnetic field. Endometrial stromal (ZE) cells are visible between the endometrial glands (GE).

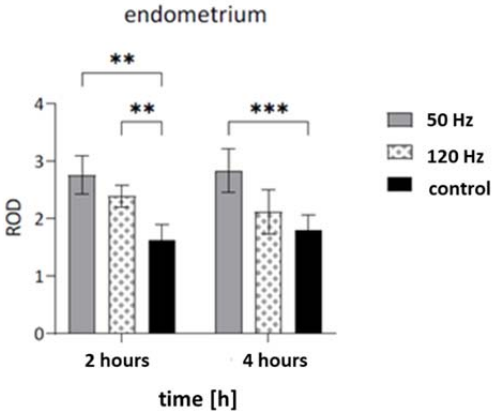

Fig. 5. Comparison of the the aromatase ROD means for endometrium tissues subjected to electromagnetic field at 50 or 120 Hz for 2 or 4 hours and control tissues..

Treatment of the endometrium tissues with electromagnetic field at a frequency of 120 Hz induced a significant increase (p = 0.0069) in the level of aromatase after 2 hours of incubation (2.39 ± SD) compared to a 2-hour incubation of the control samples (1.63 ± SD). In contrast, 4-hour exposure to electromagnetic field at a frequency of 120 Hz (2.123 ± SD) showed no significant statistical differences between the controls (1.797 ± SD; p = 0.1556) (Figure 5).

Conclusions

The electromagnetic field is a factor influencing the reproductive processes in females [8]. It may also change the synthesis and release of androgens and estrogens from pig fetuses in the peri-implantation period. The influence of the electromagnetic field on the steroidogenic pathway, also in embryos, may cause disturbances in their proper development and implantation [9].

In large sheep or other livestock farms, unsynchronized ester cycles require an increased workload of the breeder. The ability to control sexual maturation in farm animals would allow, for example, to coordinate sheep lambing with the current demand for raw materials - food or fibers. Moreover, it is possible that the period between reaching sexual maturity and giving birth to new offspring would be shortened as the maturation of lambs is relatively long - it lasts from 6 to 7 months [10]. Due to the fact that the steroidogenic activity of the uterus may also depend on external environmental factors, they were studied using photoperiod, food availability or temperature. Another factor that may affect uterine tissue is the electromagnetic field [11].

Studies on sheep uterine tissues have shown that the extremely low-frequency electromagnetic field affects the activity of steroidogenesis, one of the basic processes in the reproductive period. The endometrium of sexually immature lambs is significantly sensitive to the effects of electromagnetic field of extremely low frequency. The myometrium also responds to electromagnetic field exposure, albeit to a significantly lower degree. The sensitivity effect can be measured by higher aromatase activity, which results in higher steroidogenesis activity and better regulation of reproductive processes.

However, further *in vivo* studies are needed to reach the final conclusions which would allow the assessment of the field-induced changes not only in the uterine tissues, but also in the regulation of the hypothalamic-pituitary-uterine axis. They would also allow the participation of e.g. the ovaries to be taken into account. On the basis of the obtained results, it can be concluded that the electromagnetic field of extremely low frequencies may be a promising alternative to the methods used so far.

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

The part of the research was supported by the project Interdisciplinary Center for Preclinical and Clinical Research (the project number RPPK.01.01.00-18-0001/18).

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,

Weronika Wilczak, Eng, Rzeszow University, College of Natural Sciences, Institute of Biology and Biotechnology, ul. Pigonia 1, 35-310 Rzeszów (student)

Katarzyna Kozioł, PhD., Rzeszow University, College of Natural Sciences, Institute of Biology and Biotechnology, ul. Pigonia 1, 35-310 Rzeszów, Poland. e-mail: kkoziol@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, Professor, 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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