**1. Anna KOZIOROWSKA[1,2], 2. Wiktor CZAJKA[3], 3. Bartosz PIECHOWICZ[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. 0000-0002-2056-2399; 5. 0000-0002-7468-6063

doi:10.15199/48.2023.02.37

The electromagnetic field and thyroid reactivity to thyroid stimulating hormone (TSH)

***Abstract:** This paper presents the results of studies on the influence of extremely low frequency electromagnetic field on TSH receptor synthesis in thyroid cells from sexually immature lambs. Thyroid tissue cultures were performed and subjected to an electromagnetic field with a frequency of 50 or 120 Hz. The performed immunohistological analysis showed the presence of TSH receptors in the thyroid tissues, both in the study groups exposed to the electromagnetic field and in the control groups. Based on the conducted research, it can be concluded that the electromagnetic field may prove to be a good tool for changing the activity of the thyroid gland and potentially other endocrine glands, which is applicable both in medicine and physiotherapy.*

Streszczenie:** W niniejszej pracy zostały przedstawione wyniki badań oddziaływania pola elektromagnetycznego ekstremalnie niskiej częstotliwości na na syntezę receptora TSH w komórkach tarczycy niedojrzałych płciowo jagniąt. Przeprowadzono hodowle tkankowe tarczycy i poddano je oddziaływaniu pola elektromagnetycznego o częstotliwości 50 lub 120 Hz. Przeprowadzona analiza immunohistologiczna wykazała obecność receptorów TSH w tkankach tarczycy, zarówno w grupach badanych poddanych oddziaływaniu pola elektromagnetycznego, jak i w kontrolnych. Na podstawie przeprowadzonych badan można wnioskować, iż pole elektromagnetyczne może okazać się dobrym narzędziem do zmieniania aktywności gruczoły tarczycy jak i potencjalnie innych gruczołów wewnątrzwydzielniczych, co ma zarówno zastosowanie w medycynie i fizjoterapii.* ***(Pole elektromagnetyczne, a reaktywność tarczycy na hormon tyreotropowy (TSH))

Keywords: electromagnetic field of extremely low frequency, thyrotropic receptor; thyroid; lamb
Słowa kluczowe: pole elektromagnetyczne ekstremalnie niskich częstotliwości, receptor tyreotropowy; tarczyca; jagnię.

Introduction

An electromagnetic field is a state in which an electrically charged object is subjected to an electromagnetic force, as a result of which energy flows through this space. The forces that occur in this space can be described using two vectors - E the electrical component and H the magnetic component. All household appliances that are powered by electricity generate an electromagnetic field. This field is intentionally generated by some devices, e.g. by radio devices, devices used in medicine [1]. On a daily basis, however, we most often encounter electromagnetic fields generated by devices as an additional effect. Such devices include household appliances (electric kettle, fridge, TV, computer, lamps) and electrical power equipment such as transformer stations or high voltage transmission lines. All these devices powered from the network generate in Poland an electromagnetic field with a frequency of 50 Hz. In medical applications, the electromagnetic field is generated intentionally and the electromagnetic wave is generated in a controlled manner using specially designed generators that provide specific parameters for a specific application.

Living organisms have always been exposed to the electromagnetic field, but since the twentieth century this exposure has been constantly increasing, as the demand for electricity and new technologies is increasing. [2]. Low-frequency fields affect all systems with charged particles, including the human body. Electric currents exist in the human body due to chemical reactions that occur as part of the body's normal functions, even in the absence of external fields. For example, nerves transmit signals through electrical impulses. Most biochemical reactions, from digestion to brain activity, are tuned to the alignment of charged molecules (voltage differences, membrane channels) [2].

During exposure to electromagnetic fields, both the intracellular and extracellular mechanisms are exposed to its effects. The mechanisms by which information is exchanged between cells and biomechanical signaling is transformed under the influence of an electromagnetic field have also been studied for many years. It has been shown that the field can penetrate both cellular and nuclear membranes, stimulating or inhibiting various types of cells and tissues [3]. The electromagnetic field affecting cells in *in vitro* cultures disturbs cell proliferation, the cell cycle and cell viability (Fig. 1) [4] and change the biochemical profile [5].

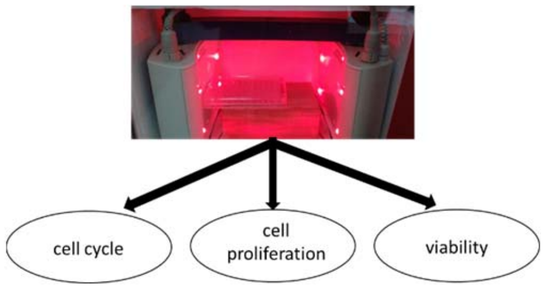

Fig. 1. The effects of electromagnetic fields on cells in *in vitro* cultures

The impact of electromagnetic fields on humans can also cause unprecedented visual effects called magnetosphenes. These are light sensations in the eye generated by an electromagnetic field with a dominant magnetic component. The explanation of this phenomenon is the subject of research by doctors, physicists and bioelectromagnetism researchers, and the resulting hypotheses do not explain the real nature of the phenomenon, i.e. they do not indicate which retinal cells and why are stimulated by the electromagnetic field to light effects [6].

The thyroid gland is a small organ at the base of the neck that is classified as an endocrine gland. It is responsible for the production and release of two hormones: triiodothyronine (T3) and thyroxine (T4), which regulate the function of most body tissues, and influence the body's metabolism and thermogenesis (heat production). The level of thyroid hormones in newborns is especially important. Hypothyroidism causes impaired postnatal development of the central nervous system, which results in incurable cretinism. Thyroid function is controlled by the pituitary gland, which releases thyroid stimulating hormone (TSH), which stimulates the thyroid gland to produce T3 and T4 [7, 8].

The function of the thyroid gland and pituitary gland are closely related (negative feedback): increased levels of thyroid hormones reduce the release of TSH by the pituitary gland, and hormone deficiency stimulates the production of TSH. The hormones that are produced by the thyroid glands regulate key physiological processes. The energy used by cells is largely regulated by the availability of thyroid hormones [9]. Thyroid hormones regulate important metabolic processes for optimal development and growth, but also regulate metabolism. After birth, thyroid hormones determine the proper development of the structures of the cerebral cortex, and their deficiency at this time causes permanent impairment that cannot be removed.

From the presence of TSH receptors in thyroid cells, it can be concluded to what extent the body is adapted to respond to the TSH hormone produced by the pituitary gland. The number of receptors on the thyroid cells is important in regulating the activity of the thyroid gland. This makes it possible to change the activity of the thyroid gland after applying external factors. One such factor may be the electromagnetic field.

The aim of the research was to determine the influence of the electromagnetic field of extremely low frequencies with the frequencies of 50 Hz and 120 Hz and the magnetic induction value of 8mT on the synthesis of the TSH receptor in the thyroid cells of sexually immature lambs.

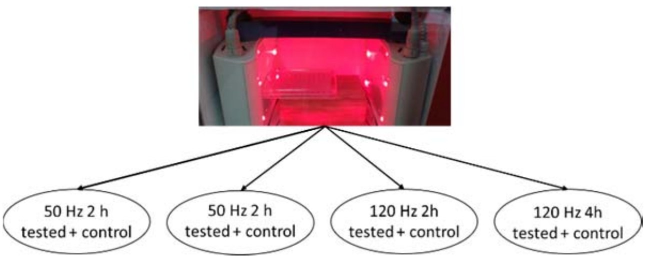

Fig. 2 The division of research groups in the experiment

The influence of electromagnetic fields on the cultures of thyroid tissues

Thyroid tissues collected post-mortem from sexually immature lambs constituted the research material. Fragments of thyroid tissue were placed on culture plates and divided into 8 research groups (Fig. 2):

- 50 Hz, 2 hours - test group and control group,
- 50 Hz, 4 hours - test group and control group
- 120 Hz, 2 hours - test group and control group,
- 120 Hz, 4 hours - test group and control group.

After a 2-hour pre-incubation and an exhange of the medium, the tissues were placed in a water bath at 37 °C and an atmosphere of 5% CO_2 and 95% O_2. A flat applicator of the Magneris generator (Astar, Poland), which emitted an electromagnetic field, was placed over the examined tissues. with a magnetic induction of 8 mT and a frequency of 50 or 120 Hz for 2 or 4 hours. The plates with tissue fragments were shaken in the bath throughout the cultivation process, which allowed for proper washing of the tissues with the medium, ensuring proper diffusion of gases and fluids into the tissue fragments. Tissues of the control groups were incubated at the same time under the same conditions without treatment with the electromagnetic field. After the electromagnetic field was exposed to the thyroid tissue, the samples were transferred to formalin for 24 hours for fixation and immunohistochemical reaction.

Conducting an immunohistochemical reaction

After fixation and dehydration, the tissues were placed in paraplast - paraffin for embedding dehydrated tissues. The prepeared blocks were cut on a Leica RM2265 microtome into sections 0.5 μm thick. The sections were applied to slides followed by immunohistochemical reaction. Primary and secondary antibodies were applied and then binding of the antibodies to the antigen was detected.

Analysis of the intensity of the immunohistochemical reaction

In order to analyze the conducted reaction, photos of the preparations were taken using the Olympus CX41 light microscope, to which the Moticam 3.0 M camera was connected. The photos were taken at a magnification of 200 times. The resulting photos were entered into the ImageJ program (National Institutes of Health, Bethesda, MD, USA). The areas of thyroid cells where the immunopositive signal appeared were marked and the gray level (GL) was measured for each of the areas.

Based on the formula (1), the ROD - relative optical density was calculated [10]:

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

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 calculated RODs were collected and then the arithmetic mean ± standard error was calculated from them. To determine the differences between individual ROD values (in the control and research samples), two-way ANOVA was used, followed by Bonferroni's post hoc comparative test. Statistical significance between groups was found. The calculations were performed in Microsoft Office Excel and GraphPad Prism 9. The p value <0.05 was used and the following values were considered statistically significant: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The results of the research

The immunohistochemical methods are used to detect antigens in cells found in tissue slides through the use of the principle of specific binding of antibodies to antigens. Due to the secondary antibodies that bind to the primary antibody, a color reaction takes place, thanks to which we can determine the location of TSH receptors.

The performed immunohistological analysis showed the presence of TSH receptors in the thyroid tissues, both in the study groups exposed to the electromagnetic field and in the control groups. A positive response was observed in both the thyroid slides exposed to the electromagnetic field for 2 hours or 4 hours, and at both tested frequencies (50 Hz or 120 Hz).

Based on the analysis of the photos, it can be concluded that the greatest number of TSH receptors is found on the epithelial cells of the thyroid follicles, which is consistent with the studies of other authors [8, 9].

Figure 3 shows the photos of the tested and control tissues subjected to the electromagnetic field with a frequency of 50 Hz for 2 and 4 hours, and Figure 4 - 120 Hz for 2 and 4 hours.

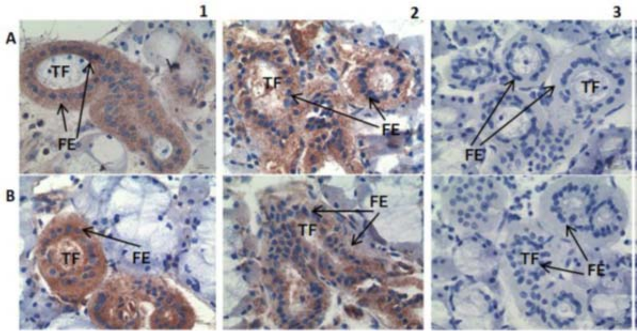

Fig. 3 The presence of the TSH receptor in the thyroid gland of the sexually immature lamb under the influence of the electromagnetic field (column 1) at the frequency and for the time of: A - 50 Hz, 2 hours; B - 50 Hz, 4 hours. The columns marked with the numbers mean: 1 – a research sample; 2 – a control sample; 3 – a reaction control. The control test took place in the same conditions as the test test, but the tissue was not under the influence of the electromagnetic field. The reaction control took place without the formation of non-specific bonds, as evidenced by the absence of a brown precipitate in the photos. The photos show a thyroid follicular (TF) and a follicular epithelium (FE).

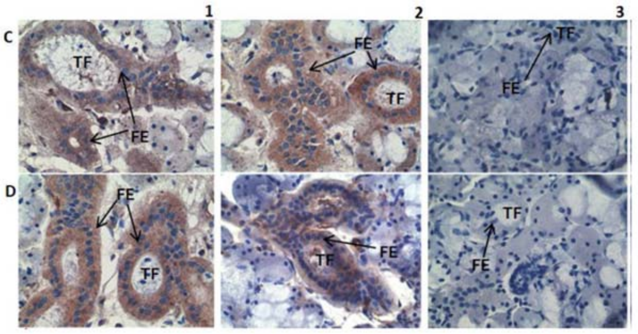

Fig. 4 The presence of the TSH receptor in the thyroid gland of sexually immature lamb, after exposure to the electromagnetic field (column 1) at the frequency and for the time of: 4 hours; C - 120 Hz, 2 hours, D - 120 Hz, 4 hours. Other markings as in Figure 3.

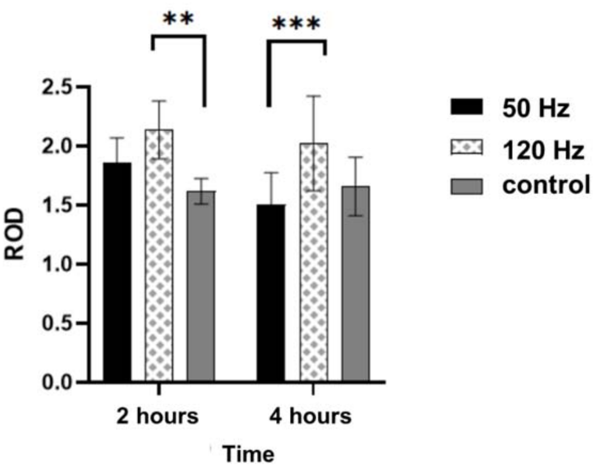

Fig. 5 Comparison of the ROD value of the TSH receptor in thyroid tissue that has been treated with 50 Hz (black bar) and 120 Hz (white-gray bar) and control tissues (gray bar). Number of individuals n = 6. Graph made in GraphPad.

The number of TSH receptors on the surface of thyroid cells varied depending on the duration of exposure and the frequency of the electromagnetic field (Fig. 5). Differences in the number of receptors were found between the tissue exposed to the electromagnetic field with a frequency of 120 Hz compared to the control sample (K). Differences between the 2-hour exposure (ROD K = 1.617 to 2.136 p <0.05) and an insignificant increase were found for 4-hour exposure to the electromagnetic field, where the differences were respectively (ROD K = 1.658 to 2.022, p <0.0638). Differences between the 2-hour exposure (ROD K = 1.617 to 2.136 p <0.05) and an insignificant increase were found for 4-hour exposure to the electromagnetic field, where the differences were respectively (ROD K = 1.658 to 2.022, p <0.0638).

Conclusions

The proper functioning of the thyroid gland and the activity of the hormones it produces are crucial for maintaining the proper productive performance of pets. This affects, among other things, growth, milk production and hair fiber production. Thanks to thyroid hormones, animals can adapt their metabolism to different environmental conditions, changes in nutrient requirements and availability, and to homeostatic changes in different physiological phases. This is especially important in free-living and grazing animals, where the main physiological functions (food consumption, hair growth, reproduction) are clearly seasonal. [11].

The thyroid hormones T3 and T4 are regulated by the hormone secreted by the pituitary gland - TSH. When TSH binds to the TSH receptor, the cell is stimulated to produce thyroid hormones. Hormone production is therefore dependent not only on the presence of TSH in the thyroid gland, it is also dependent on the presence of TSHR receptors on the surface of follicular cells in the thyroid gland. When cells have more TSH receptors, tissue becomes more reactive [9, 12]. Thyroid function has crucial meaning to the metabolism of almost all tissues and is critical to the development of the central nervous system [13].

Interest in the diagnosis of thyroid hormones increased after the Chernobyl nuclear power plant accident, when enormous amounts of radioactive iodine^{131} were released into the environment, building up into the thyroid cells as well as non-radioactive iodine. In order to prevent the incorporation of radioactive iodine into the thyroid cells as well as other tissues of the body, the only method is to administer an increased amount of potassium iodide beforehand. Both of these elements (iodine and iodine^{131}) compete to the same degree for binding to the thyroid gland and body tissues. This is especially important nowadays, when there is a risk of damage to nuclear power plants in Ukraine.

In sheep farms located in areas endemic to iodine deficiency (e.g. in Podkarpacie region), thyroid stimulation with the use of an electromagnetic field can be used along with iodine supplementation in the diet. This may allow an influence on the metabolic activity of farmed animals. At the same time, it should be remembered that while hyperthyroidism increases metabolism and simultaneously increases circulating blood pressure with the acceleration of the heart rate, lowering the level of thyroid hormones increases the accumulation of adipose tissue in the body. Very often the cause of the decrease in the amount of thyroid hormones is the endemic lack of iodine in southern Poland. This is counteracted by adding an appropriate amount of iodine to table salt. Also for obese people with a diagnosed decreased activity of the thyroid gland, it is preferable to use teas with bladder wrack (*Fucus vesiculosus*), sea algae with the ability to uptake and store iodine from seawater.

The results of the research presented in the article indicate the stimulation of the synthesis of TSH receptors in the follicular cells of the thyroid gland after exposure to an electromagnetic field of extremely low frequencies. Increasing the number of TSH receptors in the thyroid gland increases the reactivity of the glandular tissue. The obtained results suggest that the electromagnetic field should not be influenced in physiotherapeutic procedures in people with hyperthyroidism. However, in people with hypothyroidism, the use of electromagnetic fields for thyroid stimulation may be considered. The electromagnetic field may prove to be a good tool for modifying the activity of the thyroid gland and potentially other endocrine glands, which has applications in both medicine and physiotherapy.

More research is needed to better understand the effects of electromagnetic fields on thyroid tissue.

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,
Wiktor Czajka, Eng, Rzeszow University, College of Natural Sciences, Institute of Biology and Biotechnology, ul. Pigonia 1, 35-310 Rzeszów (student)
Bartosz Piechowicz, PhD, DSc., Rzeszow University, College of Natural Sciences, Institute of Biology and Biotechnology, ul. Pigonia 1, 35-310 Rzeszów, Poland. e-mail: bpiechowicz@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, Prof., Rzeszow University, College of Natural Sciences, Institute of Biology and Biotechnology, ul. Pigonia 1, 35-310 Rzeszów, Poland. e-mail: *mkoziorowski@ur.edu.pl*

REFERENCES

1. Mika T, Kasprzak W. Fizykoterapia. Wydawnictwo Lekarskie PZWL, 2015.
2. D'Angelo C, Costantini E, Kamal MA, Reale M. Experimental model for ELF-EMF exposure: Concern for human health. Saudi Journal of Biological Sciences, 2015, 22(1), 75–84.
3. Koziorowska A. Biological effects of the EMF influence on animal cells and tissues in *in vitro* cultures – a summary of own research, Przegląd Elektrotechniczny, 2018, 94(12), 206-209.
4. Koziorowska A, Romerowicz-Misielak M, Filipek A, Koziorowski M. Electromagnetic fields with frequencies of 5, 60 and 120 Hz affect the cell cycle and viability of human fibroblast bj *in vitro*, Journal of Biological Regulators and Homeostatic Agents, 2017, 31(3), 725-730.
5. Koziorowska A, Kozioł K, Gniady S, Romerowicz-Misielak M. An electromagnetic field with a frequency of 50 Hz and a magnetic induction of 2.5 mT affects spermatogonia mouse cells (GC-1spg line), Przegląd Elektrotechniczny, 2018, 96(6), 132-135.
6. Krawczyk A, Korzeniewska E, Łada-Tondyra E. Magnetofosfeny – historia i współczesne implikacje, Przegląd Elektrotechniczny, 2018, 94(1), 61-64.
7. Sawicki W, Malejczyk JT. Histologia. PZWL Wydawnictwo Lekarskie, 2020.
8. Shahid MA, Ashraf MA, Sharma S. Physiology, Thyroid Hormone. In StatPearls Publishing, 2021.
9. Mullur R, Liu YY, Brent GA. Thyroid Hormone Regulation of Metabolism. Physiological Reviews, 2014, 94(2), 355–382.
10. Smolen AJ. Image Analytic Techniques for Quantification of Immunohistochemical Staining in the Nervous System. In Methods in Neurosciences, 1990, 3, 208–229.
11. Todini, L. Thyroid hormones in small ruminants: Effects of endogenous, environmental and nutritional factors. Animal, 2007, 1(7), 997–1008.
12. Hoermann, R., Midgley, J. E. M., Larisch, R., & Dietrich, J. W. Recent Advances in Thyroid Hormone Regulation: Toward a New Paradigm for Optimal Diagnosis and Treatment. Frontiers in Endocrinology, 2017, 8, 364.
13. de Escobar, G. M., Obregón, M. J., & del Rey, F. E. Iodine deficiency and brain development in the first half of pregnancy. Public Health Nutrition, 2007, 10(12A), 1554–1570.