



EFFECT OF ELECTROMAGNETIC WI-FI RADIATION ON THE DEVELOPMENT OF CHICKEN EMBRYO

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ABSTRACT

Significant technological progress in the field of wireless devices that were primarily intended for military purposes, has resulted in their common manipulation by the general population. Wi-Fi, mobile phones, and other modern devices offer many advantages to their users. On the other hand, their excessive usage creates an environmental burden, also known as electromog. The objective of our current study was the observation of the Wi-Fi radiation effect on the histological structure of the organs in the 9-day-old chicken embryo. On day 9 of incubation, the embryological material was routinely processed for preparation of hematoxylin-eosin, picosirius red and periodic acid Schiff stained histological sections. Radiation with a frequency of 2.4 GHz and average power density of $300 \mu\text{W}\cdot\text{m}^{-2}$ applied during the entire development up to the 9th embryonic day did not fundamentally affect general organogenesis. However, in the parenchyma of organs such as the liver, spleen, lungs, kidneys, and gonads, as well as in the developing mesenchyme, obvi-

ous vascular congestion of the blood vessels of different caliber was observed. Also, an increase in collagen and glycosaminoglycans production in the cartilaginous matrix and perichondrium of the future bone skeleton as well as developing connective tissue was noted. Although these morphological changes were just subtle, they point to the Wi-Fi radiation's ability to influence the histogenesis of the individual.

Key words: chicken embryo; organogenesis; Wi-Fi

INTRODUCTION

Wi-Fi (Wireless Fidelity) is a set of standards allowing devices to connect to a wireless local area network (WLAN). This network functions on the principle of spreading non-ionizing radiation (NIR) at different frequencies for wireless communication. Wi-Fi has become an integral part of everyday functioning, as it allows people to connect to the internet in various places without the use of cables. It provides easy data transfer, control

of household appliances, and other benefits that facilitate the functioning of modern society. Wi-Fi hot spots that allow wireless connection to the users in the covered area are becoming increasingly common everywhere around the world. They are found in homes, schools, restaurants, airports as well as many means of transport. Based on this, a massive cumulation of this artificial NIR in the environment can be expected. There are several important characteristics of NIR, which are significantly different from the natural electromagnetic radiation such as the light and heat from the sun, the Earth's natural electric and magnetic fields. Many wireless devices operate via pulsations, which are usually much more dangerous for biological objects than are non-pulsed/continuous waves of the natural origin [20, 28]. Artificial waves are also polarized and represent stronger forces of electrically charged chemical groups than non-polarized fields [21]. After all, the windows of exposure, where the specific intensities of radiation cause maximal biological effects are prominent [4, 20]. Even though typical Wi-Fi exposures in the environment are several orders of magnitude below guideline values according to the International Commission on Non-ionizing Radiation Protection – ICNIRP [8], a better understanding of their interaction with living systems will be inevitably required, if we take into account, all of the above-mentioned facts.

The developing organism, in which an extensive division and differentiation of cells and tissues take place, is extremely sensitive to various environmental influences [19, 23, 25, 26, 31, 33], which also include exposure to a man-made NIR. Due to the ever-increasing trend in the use of Wi-Fi and other wireless technologies, and the general lack of information in the developmental field, further professional research is considerably needed. Therefore, we decided to observe the effect of Wi-Fi radiation with a frequency of 2.4 GHz and a power density of $300 \mu\text{W}\cdot\text{m}^{-2}$ on the developing chick, which was applied continuously during a nine-day incubation period.

MATERIALS AND METHODS

Chicken embryos and the exposure system

Fertilized chicken eggs were randomly divided into the control group (CO, $n = 6$) and the group exposed to Wi-Fi radiation (Wi-Fi, $n = 6$). A River system ET49 automatic

incubator was used for egg incubation, in which the temperature was set to 37.5°C and the air humidity was 60 %. The Wi-Fi group was continuously exposed to NIR with a frequency of 2.4 GHz with an average power density of $300 \mu\text{W}\cdot\text{m}^{-2}$ for 9 days. The eggs of the CO group were incubated in the same room, but in a specially shaded incubator under the same incubation conditions (Fig. 1).

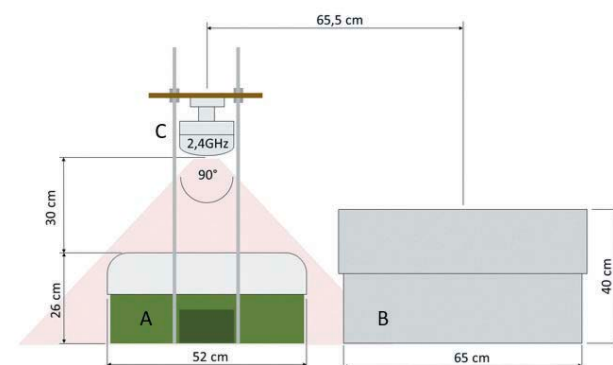


Fig. 1. Wi-Fi exposure system

For the purposes of the study, two egg incubators were used. One incubator was directly exposed to Wi-Fi radiation (A), and another one was housed within a shielding box (B). The source of the Wi-Fi signal was Mikrotik Metal 52 ac 15s antenna (C). The distance of eggs from the source of radiation was 45 cm.

Tissue sampling and processing for histological analysis

On the 9th embryonic day (ED 9), whole embryos from both groups were fixed in a solution of modified Davidson's fluid (mDF) and embedded into paraplast. Longitudinal sections through embryos were stained with a common hematoxylin-eosin method (H-E, $n = 10$) as well as special histochemical methods of picosirius red (P-R, $n = 10$) to detect collagen (Col) and periodic acid Schiff (PAS, $n = 10$) to detect glycosaminoglycans (Gag). The preparations were observed with a Zeiss Axio Lab A1 light microscope and photographed with an Axio Cam ERc 5 camera.

RESULTS

In all 9-day-old chicken embryos from both groups, the presence of well-developed organs without any significant morphological changes was noted. The shape, size, and topography of all studied organs and tissues of chicken embryos appeared to be generally unaffected. The head area showed well-organized brain hemispheres such as *myelencephalon*, *metencephalon*, *cerebellum*, and optic lobe as well as an almost definitively developed eye with its

basic tunics – *tunica fibrosa*, *tunica vasculosa*, and *tunica interna bulbi* (Fig. 2). The heart located in the body cavity owned very well developed walls of both atria and ventricles. The large vessels such as the aorta and pulmonary artery were also well formed. The lungs had distinguishable parabronchi, and the liver was composed of regular cords of hepatocytes. Tubular and sacculated organs such as the trachea, oesophagus, stomachs, and intestines were clearly defined and contained four basic tunics (*tunica mucosa*, *tela submucosa*, *tunica muscularis*, and *serosa*). The Lumen of the glandular stomach was covered with a simple columnar epithelium, which produced rich secretion at this stage. The underlying *lamina propria* connective tissue possessed short precursors of tubular gastric glands. The muscular stomach had a very well-developed smooth muscle (*tunica muscularis*), but the cuticle-producing glands were not yet present. Other organs located in the body cavity were gonads with the typical genital cords. The primordial germ cells were relatively large, round in shape with pale cytoplasm and a dark nucleus. The genital cords communicated with each other and were surrounded by embryonic mesenchyme. In the mesenchyme, cells gradually differentiated into the cells of the definitive connective tissue stroma. A coelom epithelium was present on the surface of the indifferent gonad. The indifferent gonad was attached to the mesonephros with its long surface. Spleen with red and white pulp, and the kidneys in mesonephros stage with typical large mesonephric corpuscles, mesonephric tubules as well as Wolffian and Mülle-

rian ducts (*ductus mesonephricus et paramesonephricus*) were also characteristic. Caudal to the mesonephros, the rudiments of the future definitive kidney, the metanephros were present. In the vertebral canal, composed at this stage of hyaline cartilage precursor, was a spinal cord with well established grey and white matter (Fig. 3).

Despite the well-progressed organogenesis that was observed at the level of light microscopy in both investigated groups, several delicate structural changes in the Wi-Fi group were observed. Within the cartilaginous model of the future bone skeleton, when stained with the P-R, a strengthened formation of Col in the perichondrium and transforming mesenchymal connective tissue were found. With the use of PAS staining, an increased Gag production in the mesenchymal connective tissue close to the perichondrium was noted (Fig. 4). In the parenchymatous organs, mainly the liver, spleen, lungs, kidneys, and gonads, as well as in the developing connective tissue of the skin corium and subcutis, an increased congestion of blood vessels of various caliber (Figures 4 and 5) was noted.

DISCUSSION

The interaction of radiation with biological objects depends on various factors. As for a living matter, it is e.g. water content, specificity of the organ structure such as the presence of a prominent fibrous capsule on its surface that eliminates the effective removal of accumulated heat,

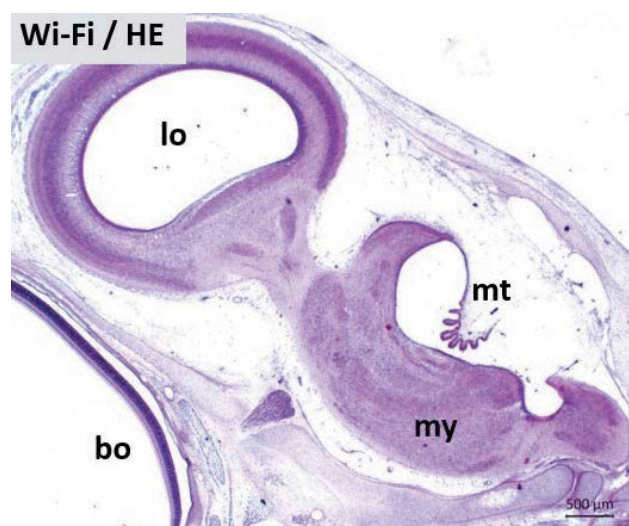


Fig. 2. Longitudinal section throughout the head region of chicken embryo (ED 9) from Wi-Fi group with normal general organogenesis (H-E staining, orig. magn. x 125, scale 500 µm); bo – *bulbus oculi*; lo – *lobus opticus*; mt – *metencephalon*; my – *myelencephalon*

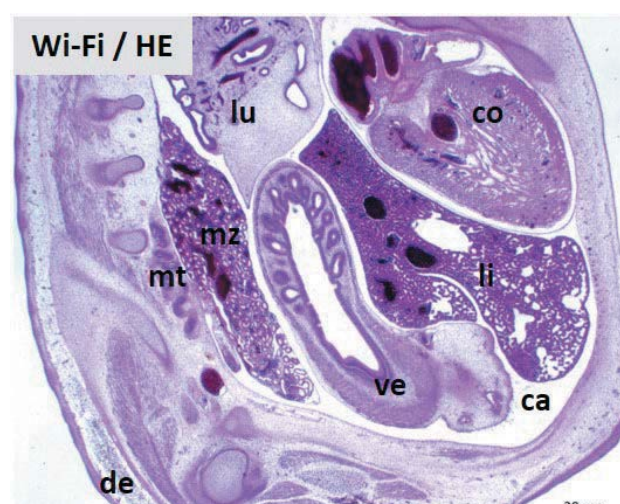


Fig. 3. Longitudinal section throughout the body cavity of chicken embryo (ED 9) from Wi-Fi group with normal general organogenesis (H-E staining, orig. magn. x 125, scale 20 µm) ca – *body cavity*; co – *heart*; lu – *lungs*; li – *liver*; ve – *stomach*; mz – *mesonephros*; mt – *metanephros*; de – *dermis*

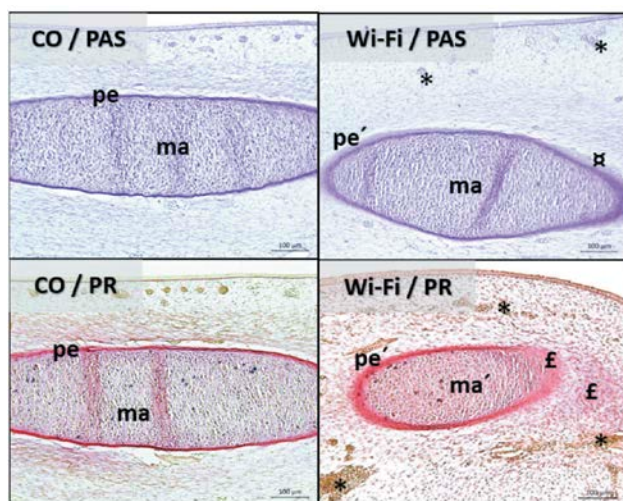


Fig. 4. Developing cartilaginous model of bone skeleton and connective tissue of the chicken embryos (ED 9) from the control (CO) and Wi-Fi group (P-R and PAS staining, orig. magn. x 200, scale 100 μ m)

pe – perichondrium; pe' – thickened perichondrium; ma – cartilaginous matrix; ma' – cartilaginous matrix with increased collagen production; □ – overproduction of glycosaminoglycans; £ – overproduction of collagen fibres; * – congested blood vessels

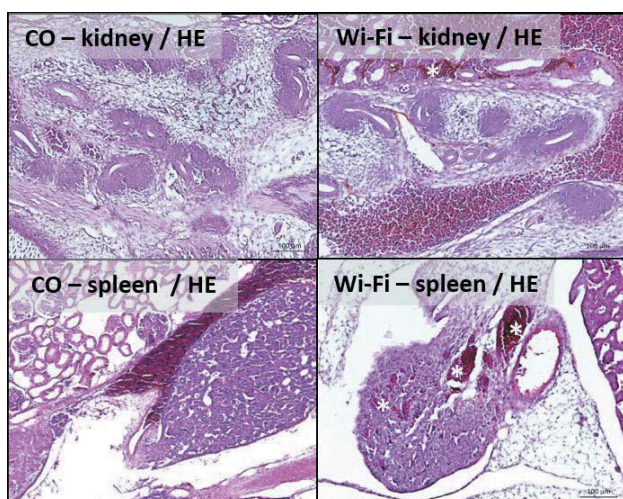


Fig. 5. Representative microphotographs of developing organs of the chicken embryos (ED 9) from the control (CO) and Wi-Fi group (H-E staining, orig. magn. x 200, scale 100 μ m)

* – congestion of blood vessels

superficial or deeper organ position in the body, and other characteristics [1, 30]. The radiation intensity and the distance of the object from the radiation source are very important factors as well [1, 18]. Currently, it is proven that NIR can influence biological systems in a variety of ways. The most debated is the thermal effect, which occurs at relatively high radiation intensities as well as at a short distance of a biological object from the radiation source (Wi-Fi antenna) with a frequency of 2.45 GHz it is up to 16 cm and the frequency of 5 GHz it is up to 33 cm [23, 29,

32]. The thermal effect is based on the oscillation of tissue molecules with a subsequent increase in temperature in the organ [1, 3]. The depth of penetration of the NIR depends on the frequency of the microwaves, as well as the type of tissue that is affected. In general, lower frequencies of microwave radiation reach deeper into the tissues [10, 11, 15, 17]. On the other hand, there is a non-thermal/biological effect which includes e.g. oxidative stress in tissues, calcium ions dysregulation, genetic and epigenetic changes, and many others that occur already at much lower radiation intensities, and which the organism commonly encounters in the environmental conditions [6, 13, 19, 26, 34].

Due to very limited information about the relationship of NIR with the prenatal development of the individual, we were interested in how exposure to Wi-Fi radiation with a frequency of 2.4 GHz and at low intensity of 300 μ W.m⁻² that is far below the permitted limit for both occupational and general public exposures according to ICNIRP [8] will affect the organogenesis of the chicken embryo during a nine-day incubation period. In general, any severe morphological changes between the studied groups at the common histological staining method by H-E was not observed. This greatly agrees with normal morphology found in 7 and 18 days-old embryos either via macroscopical inspection (shape, size, weight of embryonic bodies as well as skull dimensions) or through the microscopical observation (structure of the brain, heart, liver, spleen, bursa of Fabricius, and residual yolk sac) during exposure to various frequencies of radiation (1.8–5.6 GHz) at a field strength representing also common environmental conditions [31]. On the other hand, when special stainings of P-R and PAS were used, there was a thickening of the perichondrium around the cartilaginous model of the future bones due to the increase of Col production (P-R), as well as elevated production of Gag (PAS) in the amorphous matrix in the connective tissues in the Wi-Fi group compared to the controls. It can be assumed that Wi-Fi radiation enhanced the production of the fibrous and amorphous component by fibroblasts, which is in accordance with our previous study focused on collagen production in the allantochorionic membrane of the chicken embryo under the influence of the same Wi-Fi setup [7]. A very similar phenomenon has been found during IR, UV light, laser, or magnetic stimulation that is used for therapeutic purposes and acceleration of regeneration processes in the organism through driving the ionic currents and influencing the excitable cell mem-

branes [12, 16]. But therapeutic radiation, however stronger in character is applied for a much shorter time compared to almost permanent exposure to some amount of Wi-Fi radiation. So the consequences of long-term exposure to Wi-Fi and similar radiations, which continuously affect living systems, will need to be further monitored, especially in their sensitive periods of development. Another structural changes that were detected at the level of light microscopy was significant vascular congestion mainly in the connective tissue of the skin and subcutaneous tissue, as well as in all parenchymatous organs. Even though radiation with a power density not exceed the currently valid protective limits for the controlled exposures [8] was used, that there was most probably an induced thermal effect in the developing embryonic tissues which are very watery in nature. As a **result**, subsequent obvious vascular congestion as a manifestation of the activation of thermoregulatory mechanisms in the tissues was seen. It is still a challenge, how to measure a possible temperature increase in the tissue of the chicken embryo, the development of which takes place inside the egg. The thermal effect was registered also in our previous work focused on the testicles of adult rats, which were affected by a similar type of NIR (2.45 GHz) permanently during 3 weeks [1]. This phenomenon was described mostly in the organs, which are particularly susceptible to the thermal effect due to their specific structure, such as high water content, thick fibrous sheath, and superficial position of organs in the organism such as the brain, eye, salivary glands, and testes [2]. It also should not be forgotten that the entire embryonic development of a bird individual takes place in the egg, the egg-white as well as the shell, which may prevent effective removal of the accumulated heat from the tissues.

Many authors point to changes that are caused by a non-thermal effect, the consequences of which are oxidative stress, increase in expression of stress proteins, enhancement of apoptosis, intracellular calcium increase, cell proliferation deviation, DNA damage, and many others [2, 5, 9, 22, 24, 27, 35]. Non-ionizing radiation also had an especially adverse effect on embryonic stem cells and the developing organisms [14, 25, 30, 33].

Determining whether the interaction of radiation with the biological system is thermal or non-thermal is still very challenging and establishing sufficient measurement methods to evaluate the rise in temperature of tissues will be needed. In any case, even non-thermal influences can cause

adverse effects on the organism. Current standards for the protection of human health from NIR vary greatly around the world, but they are mostly based on the inaccurate assumption that there are no adverse health effects other than those that are caused by tissue heating [2]. Therefore, it will be necessary to call on the relevant authorities to consider the situation and make corrections in this regard.

CONCLUSIONS

In our study, the effect of Wi-Fi radiation on the developing organs of the chicken embryo was investigated. Histological sections were stained using multiple staining methods to visualize different tissue components. Using the common H-E method, the overall structure of organs and tissues of chicken embryos was not generally affected by Wi-Fi. By applying the histochemical methods P-R and PAS, stimulation of fibroblasts into the production of glycosaminoglycans and collagen fibres in the Wi-Fi group was observed. In some parenchymatous organs and connective tissue of skin and subcutis, significant vessel congestion was noted, which may indicate the thermal effect of NIR produced by Wi-Fi.

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