



ORIGINAL ARTICLE

EFFECT OF WI-FI ELECTROMAGNETIC RADIATION ON SELECTED MORPHOLOGICAL PARAMETERS OF CHICKEN EMBRYO

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

The aim of this study was to observe the effect of Wi-Fi radiation on certain morphological parameters of chicken embryos. The chicken eggs were divided into two groups: a control group (CO 9) and an experimental group (WiFi 9). The WiFi 9 group was exposed to Wi-Fi radiation at a frequency of 2.4 GHz (300 $\mu\text{W}/\text{m}^2$) for a period of 9 days. A statistically significant reduction in body weight ($P < 0.001$) was observed in the WiFi 9 group (1.561 ± 0.343 g) compared to the CO 9 group (1.724 ± 0.087 g). The WiFi 9 group exhibited an insignificant decrease in heart weight (0.017 ± 0.004 g) compared to the CO 9 group (0.019 ± 0.002 g). Liver weight remained unchanged in both the WiFi 9 and CO 9 groups. Petechial haemorrhages of varying extent were also observed on the head, trunk, and developing limbs. However, Wi-Fi exposure had no effect on embryo viability or on the occurrence of malformations. Although not all of the observed changes in the developing embryos were statistically significant, the results indicated a potential risk associated with continuous exposure of the organism to non-ionising radiation.

Key words: body weight; chicken embryo; heart weight; liver weight; Wi-Fi

INTRODUCTION

The rapid advancements in wireless technology have provided users with numerous advantages; however, these developments have also given rise to a range of questions and legitimate concerns regarding their potential adverse effects on the health of the population. Wireless devices function by emitting a Non-ionising Radiation (NR) at

various frequencies. The impact of this radiation on the organism is generally described as thermal, depending on the Specific Absorption Rate (SAR), and as non-thermal, otherwise termed biological [1]. The exposure limits used globally are based exclusively on the heating effect caused by the intensity of radiofrequency radiation received. This is known as the thermal effect, and exposure is regulated so that it does not exceed 10 W/m^2 [2]. According to the

available literature, the non-thermal effect is predominantly manifested when the energy emitted by radio waves does not result in an increase in the temperature of a cell, tissue, or organ. Instead, changes in cell kinetics and their proliferation have been observed. This phenomenon has been shown to result in DNA damage [3], impact the functions of the cytoplasmic membrane and the signal transduction [4, 5], influence the function of the central nervous system [6], and enhance free radical production [7, 8]. In accordance with medical ethics, studies of the effects of NR are not conducted directly on humans [9]. However, epidemiological studies aimed at monitoring the effects of long-term NR exposure on people living near mobile phone base stations have shown an increasing number of cases with headaches, memory impairment, dizziness, and sleep disorders [10, 11, 12, 13, 14, 15, 16, 17, 18].

A number of studies, where animal models (rats) were used, have revealed significant morphological changes in various organs. In the nervous system, NR exposure has been demonstrated to impact the neurotransmitter production [19] and reduce the number of neurons [20]. Severe changes have also been documented in the cardiovascular system, where the cardiomyocytes were irregularly shaped and the spaces between them were wider [10]. Their mitochondria were altered, and the rough endoplasmic reticulum was dilated. Furthermore, a reduction in the number of myofibrils was observed [21]. Exposure to NR has also been demonstrated to result in a significant decrease in immunoglobulin levels (IgA, IgE, IgM, and IgG), as well as in total leukocyte, lymphocyte, eosinophil, and basophil counts. On the other hand, a significant increase in neutrophil and monocyte counts was observed [22]. In the digestive system, hepatic steatosis, necrosis, and vacuolisation of hepatocytes were observed [10, 23]. In the kidneys, the structure of nephrons was damaged [24], and in the testes, distinct morphological changes occurred in the seminiferous epithelium [25, 26, 27]. Slight degenerative changes, such as cytoplasmic vacuolation, chromatin condensation and necrosis, were observed in some endocrine and exocrine cells in the pancreas [28].

The majority of experimental studies focused on the effects of NR on adults and juveniles. However, growing evidence has suggested that this environmental stress can also significantly affect the embryonic development. During this critical phase of development, the processes of intensive cell division and differentiation occur. Con-

sequently, the developing organism is particularly vulnerable to the influence of various environmental factors. In the study of the effects of NR on the developing organism, chick embryos are frequently used as an animal model. A number of similarities have been identified between avian and mammalian embryos with regard to their developmental stages [9]. Another advantage of using this model is that its use does not require the approval of protocols for the use of experimental animals, as chick and quail embryos *in ovo* are exempted from the legislation on the protection of animals used for scientific purposes.

The aim of this study was to observe the impact of Wi-Fi on chicken embryos. The experiment involved the continuous exposure of chicken embryos to Wi-Fi (2.4 GHz; 300 $\mu\text{W}/\text{m}^2$) from the embryonic day (ED) 1 to the ED 9. The primary focus of this study was the monitoring of embryonic viability, the occurrence of malformations, and the evaluation of the morphological parameters.

MATERIALS AND METHODS

Chicken embryos and the exposure system

The fertilized chicken eggs (*Gallus gallus domesticus*) of the Lohmann Brown breed were obtained from a poultry farm (LP Nitra A.S., Parovske Haje, Slovakia). The cracked eggs were excluded from the study. A total of 95 eggs was randomly divided into two groups: a control group (CO 9, $n = 45$) and a group exposed to Wi-Fi radiation (WiFi 9, $n = 50$). Automatic incubators (River System ET49) were used to incubate the eggs, which were placed blunt end up in a forced draft with a temperature of 37.5 °C and a humidity level of 60%. The incubation rack ensured the eggs were rotated periodically at 3-hourly intervals. The WiFi 9 group was continuously exposed (24 hours per day) to NR at a frequency of 2.4 GHz and an average power density of 300 $\mu\text{W}/\text{m}^2$ during 9 embryonic days. The eggs of the CO 9 group were incubated in the same room but in a specially shaded incubator, under the same conditions [29]. The average power density and uniformity of signal distribution were monitored using a TriField® device (USA).

Chicken embryo sampling and morphometry

On the ED 9, the whole embryos from both groups (CO 9, WiFi 9) were extracted from the eggs and weighed



Fig. 1. Chicken embryo of the experimental WiFi 9 group on the embryonic day 9 – macroscopic evaluation (representative photographs).

A: hemorrhages on the head (lateral side); B: hemorrhages on the head (dorsal side);
C: hemorrhages in the trunk and developing wings and hind limbs. Scale bar = 2000 µm.

(analytical balance, KERN ABJ-NM/ABS-N) individually after the removal of the yolk sac. Subsequently, a comprehensive examination was conducted under a dissecting microscope (Olympus SZ61, Tokyo, Japan) equipped with a digital camera (Promicra, Prague, Czech Republic), with the aim of detecting external anomalies of the eye, beak, palate, body wall, and limbs, as well as internal anomalies, including heart and liver anomalies. The Hamburger and Hamilton developmental stages [30] were utilised to evaluate the normal body development as well as for identifying various morphological malformations. The organs, including the heart and liver, were collected and weighed.

Statistical analysis

The data obtained were evaluated by Student's t-test in Microsoft Excel 2010 and reported as means and standard deviations (Mean $\pm$ SD). Statistical significance was set at the level of $p < 0.05$ and the p-value of $p < 0.001$ indicated high significance.

Ethical statement

In accordance with the Directive 2010/63/EU, the use of the chicken embryo as an animal model does not necessitate ethical committee approval for animal experimentation.

RESULTS

Of the 45 hatching eggs assigned to the CO 9 group, six were found to be unfertilised. In the WiFi9 group, fertilisation failure was observed in four out of 50 hatching eggs. The presence of dead embryos was not detected in either group. During the macroscopic evaluation, no significant malformations of the head (eye, beak), body wall, or developing pelvic limbs and wings were observed.

However, in WiFi 9 we observed petechial haemorrhages on the head around the eyes, more extensive subcutaneous haemorrhages in the area of the crown of the head, and petechial haemorrhages on the body walls and on the forming wings and pelvic limbs (Fig. 1). At the macroscopic level, no alterations were observed in the heart and liver. The size, shape, and topography of these organs were unchanged (Fig. 2).



Fig. 2. Chicken embryo of the experimental WiFi 9 group on the embryonic day 9 with open body cavity – macroscopic evaluation (representative photograph)

Black arrow – heart, white arrow – liver. Scale bar = 1000 µm.

The total embryo weight in the CO 9 and WiFi 9 groups, as well as the organ weight (liver, heart) at ED 9, are shown in Fig. 3. In the WiFi 9 group, the average weight of the chick embryo was significantly reduced (1.561 ± 0.343 g, $P < 0.001$) in comparison to the CO 9 group (1.724 ± 0.087 g). Furthermore, the WiFi 9 group exhibited an insignificant decrease in heart weight (0.017 ± 0.004 g) in comparison to the CO 9 group (0.019 ± 0.002 g). The liver weight in both the WiFi 9 (0.026 ± 0.004 g) and CO 9 (0.027 ± 0.006 g) groups was unchanged.

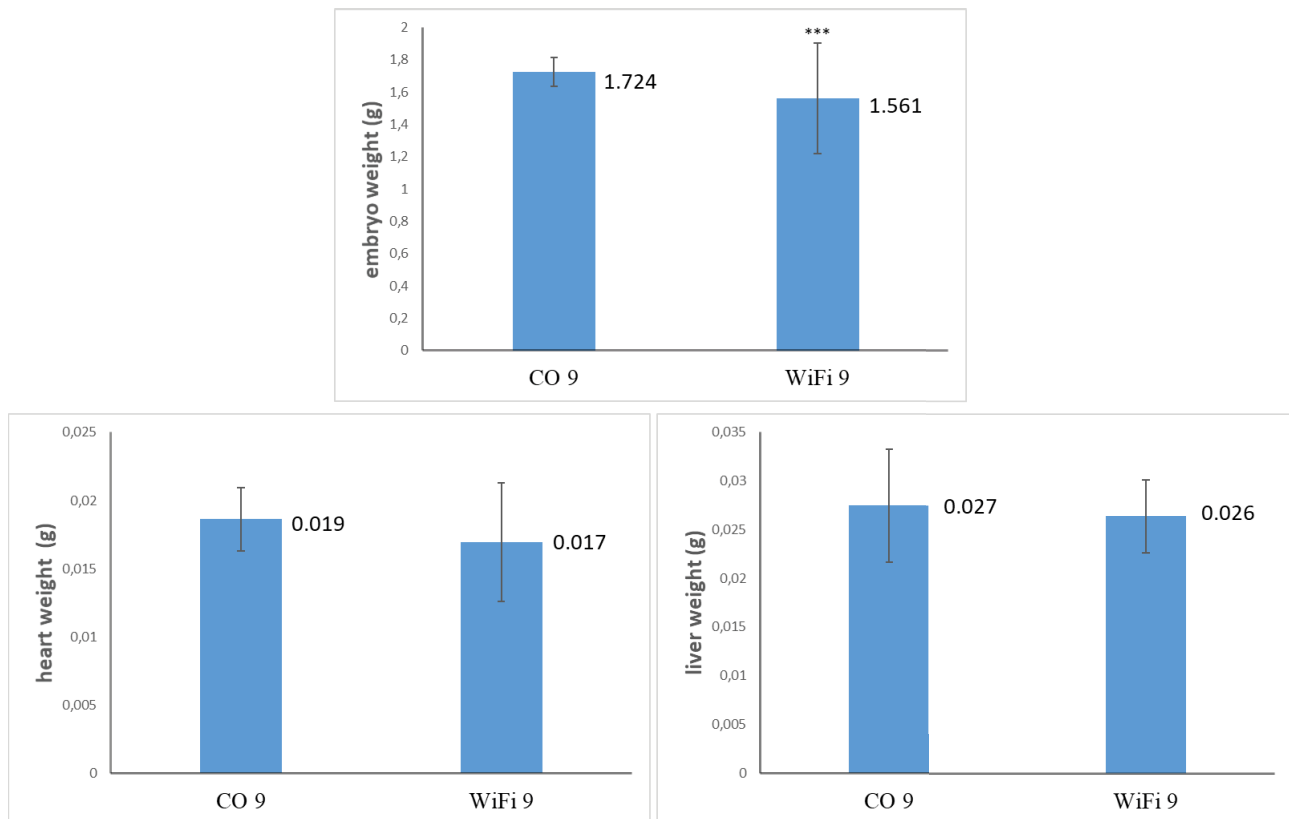


Fig. 3. Morphological parameters of the chicken embryo on the embryonic day 9
CO 9 – control group, WiFi 9 – experimental group, *** $P < 0.001$ – highly significant

DISCUSSION

All living organisms are continuously exposed to natural NR, the source of which is the planet itself (magnetic field 0.02–0.07 mT). However, it should be noted that in the 20th century, new sources of artificial NR began to increase rapidly, resulting in a constant rise in the intensity of non-ionizing electromagnetic radiation [31]. The present study aims to monitor the effect of Wi-Fi on some morphological parameters of the chick embryo. Chicken embryos represent a widely available model organism that is utilised in the studies involved in developmental biology, embryology, immunology, genetics, virology, and other scientific fields [32]. However, the available literature revealed significant differences in the response of the developing organism to the NR effect. It is possible that these variations may be associated with the divergent experimental conditions under which the chick embryos were incubated. The factors that may be of particular relevance in this regard include the duration and intensity of exposure, the distance from the source of NR, and, on the other hand, the stage of embryo development.

The experimental studies addressing this issue can be divided into several groups. A number of studies were conducted to monitor the effect of NR using low frequencies of 50 Hz [9]. In this area of research, both positive and negative effects of NR on the body have been documented. The sources of these frequencies are various electronic devices, such as the television, electric oven, washing machine, hair dryer, shaver, and many other electrical devices used in industry as well as in medicine [9]. As demonstrated in the available literature, NR at a frequency of 50 Hz (2 h) had a stimulatory effect on cells obtained from mouse embryos at ED 11 (chondrocytes, mesenchymal cells, and red blood cells) and promoted their proliferation and differentiation [33]. However, Lahijani et al. [34] reported alterations in brain structure, monophthalmia, microphthalmia, anophthalmia, and growth retardation in chick embryos exposed to 50 Hz for a period of four days. In a subsequent study, the same authors observed an increase in the amount of lymphatic tissue, subcutaneous hemorrhages that occurred throughout the body, and changes in the liver with an increased incidence of apoptotic cells in chick embryos exposed to

NR (24 hours before their incubation) on the ED 13, ED 14, ED 15, and ED 19 [34].

Further experimental studies focused on the monitoring of NR at frequencies of 900–1800 MHz. This electromagnetic radiation is primarily generated by telecommunication towers, radio transmitters, and other wireless devices such as mobile phones [9]. It has been found that NR with a frequency ranging from 900 MHz to 1800 MHz (15 min/4 times daily) can induce subcutaneous haemorrhage in chicken embryos on the ED 7 and ED 10, and anophthalmia or monophthalmia, beak malformation, abdominal hernia, brain malformation, and retinal thickening on the ED 14 [35]. As demonstrated by the findings of other researchers in the field, analogous results have been reported in chick embryos. For instance, in the study by Siddiqi et al. [36], an increased incidence of embryo mortality was observed in the experimental group (1800 MHz, 50 min/day) at ED 15. Dead embryos showed significant limb and beak deformities; their skin was not covered with feathers as in the control group, and subcutaneous hemorrhages were also identifiable. Furthermore, defects of the ventral chest and ventral abdominal walls, as well as the occurrence of umbilical hernia, were observed. The average body weight of the chick embryo also exhibited variation, being lower in the experimental group (7.13 g) compared to the control group (8.84 g). However, these differences were only insignificant. Batellier et al. [11] also observed elevated levels of chick embryo mortality at 900 MHz, the source of which was an active mobile phone (repeated calls at 3-min intervals for 2 min). The mortality was found to be approximately 4.5% on the ED 4, 1% from the ED 5 to ED 7, less than 1% from the ED 7 to the ED 14, and 6.1% from the ED 18 to the ED 21. In the related study, Zareen et al. [37] investigated the impact of NR on the ED 10 and ED 15. Fertilized eggs were exposed to different doses of electromagnetic radiation from mobile phones. The authors found that long-term NR exposure (15 and 20 min/2x daily), both at lower doses and at higher doses, caused a significant increase in the weight and body length of embryos compared to the control group.

The aim of the present study was to monitor the effect of Wi-Fi (2.4 GHz, 300 $\mu\text{W}/\text{m}^2$) on chicken embryos that were exposed continuously from the ED 1 to the ED 9. In contrast to the above mentioned studies, no embryo deaths were observed in either the CO 9 or Wi-Fi 9 group. Furthermore, no significant alterations in the shape and size of

the eyes, beak, or malformations of the developing limbs were observed in the Wi-Fi 9 group. However, in contrast to the findings of other authors, a statistically significant decrease in embryo weight compared to the control group was observed. Heart weight was also reduced, though not significantly, while the weight of the liver was unchanged by Wi-Fi. It is hypothesised that the reduction in embryo weight and mortality may be related to apoptosis. It is known that, particularly during ontogenesis, the embryo undergoes a natural process of cell loss in which damaged and redundant cells are eliminated. Concurrently, this provides more favourable conditions for the development of cells with correctly programmed receptors. The process of apoptosis is further stimulated by free oxygen radicals, which are increased by the effect of NR [38, 39, 40].

Although the macroscopic and morphometric parameters were not significantly altered by Wi-Fi, more detailed histological studies revealed changes in the tissues and organs of developing chick embryos. Our earlier research revealed that Wi-Fi did not have a significant effect on organogenesis at ED 9. However, we observed a significant vascular congestion of blood vessels of different calibre in organs such as the liver, spleen, lungs, kidneys, and gonads, as well as in the developing mesenchyme. An increase in collagen and glycosaminoglycan production was also noted in the cartilage matrix and perichondrium of the future bone skeleton of the embryo [29]. In addition to these changes, a significant decrease in elastic fibres and an increase in the amount of collagen in the wall of large vessels in the chorioallantoic membrane was found using optical density measurement [41]. Based on our observations, we can conclude that the continuous exposure of chicken embryos to Wi-Fi at an intensity of 300 $\mu\text{W}/\text{m}^2$, which is well below the value that induces a thermal effect in tissues (10 mW/m^2) [2], from the 1st to the 9th embryonic days may possibly have an effect on regulatory mechanisms involved in embryogenesis. Therefore, it may be beneficial to consider reevaluating the protective limits, particularly in the context of developing organisms.

CONCLUSIONS

The available literature demonstrated that NR can have a significant impact on the developing organism, although the degree of damage to the organism depends on many

factors. The results of this study indicated that Wi-Fi radiation with a frequency of 2.4 GHz (300 $\mu\text{W}/\text{m}^2$), to which chicken embryos were continuously exposed from the ED 1 to the ED 9 did not affect embryo survival. No malformations were observed in the head, body wall, or developing limbs; however, petechial haemorrhages of varying extent were observed in these areas. In addition to these changes, a significant decrease in the body weight of the embryo was observed in the WiFi 9 group. Although most of the observed changes were insignificant, they point to the potential risk associated with constant exposure of the organism to NR.

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Conflict of Interest

We declare that all listed authors are without a Conflict of Interest.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

This study did not require any Ethical approval (Directive 2010/63/EU).

Authors' Contributions

All authors contributed to the study revision. Material preparation, data collection and analysis were performed by K. H., S. A., P. H., J. M. and V. A. All authors read and approved the final manuscript.

Generative AI Statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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