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The effects of exposure to a 900 MHz electromagnetic field on the hatchability of domestic chicken embryos (*Gallus gallus domesticus*)*

Krzysztof Pawlak[1#], Zenon Nieckarz[2], Marcin Lis[1], Bartosz Bojarski[1], Barbara Tombarkiewicz[1], Magdalena Swadźba[1], Jerzy Niedziółka[1]

[1]University of Agriculture in Kraków, Faculty of Animal Breeding and Biology,
Institute of Veterinary Sciences, Department of Veterinary Science,
Animal Reproduction and Animal Welfare,
al. Mickiewicza 24/28, 30-059 Kraków; [#]e-mail: rzpawlak@cyfronet.pl
[2]Jagiellonian University in Kraków, Faculty of Physics, Astronomy and Applied Computer Science,
Department of Experimental Computer Physics,
ul. Łojasiewicza 11, 30-348 Kraków

From the second half of the 20th century there has been a rapid increase in electromagnetic radiation (EMR), generated in part by mobile phone networks. The aim of the study was to examine the effects of an electromagnetic field (EMF) with a frequency of 900 MHz on the hatchability of chicken embryos. The experimental groups were exposed to the electromagnetic field during the entire incubation period, for 10x4 minutes per day or 1x40 minutes per day. The results obtained indicate that an electromagnetic field with a frequency of 900 MHz, irrespective of the duration of a single exposure, significantly accelerates the process of pipping and hatching. In the experimental groups subjected to electromagnetic radiation the time between pipping and hatching was reduced. No influence of the 900 MHz EMF was observed on the hatching rate, number of developmental defects in the embryos, body weight, or the number of culled chicks.

KEY WORDS: electromagnetic field / hatchability / mobile telephony / chicken embryo

From the second half of the 20th century there has been a rapid increase in electromagnetic radiation (EMR), generated by power grids, electrical devices and installations, induction units, radio and television transmitters, radio communication transmitters, wireless Wi-Fi routers, and most importantly, mobile phone base stations. The scale of the problem has been presented in a report published by the We Are Social agency [20], according to

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which the number of mobile phone users worldwide increased by 141 million just in the last year.

Living organisms are known to be capable of generating bioelectric fields, and thus humans and animals are vulnerable to exposure to the EMF emitted by mobile telephony [15]. Although research on the influence of electromagnetic fields, including those emitted by mobile phone base stations, has been conducted for many years, it has not provided any conclusive evidence regarding its impact on living things [1, 10, 14, 22].

Embryogenesis is one of the most important developmental stages, and the biological value of adult organisms and breeding results are largely dependent on proper embryonic development. The embryo is especially sensitive to external factors that may have a detrimental effect on its development [6, 21].

The specific characteristics of the chicken embryo (its well-known embryogenesis, development outside the mother's body and wide availability) make it a popular model for studying the influence of environmental factors on living organisms [6, 12, 18, 19]. One of the most important indicators of normal chicken embryogenesis is hatchability.

The study was designed to determine the effect of exposure to an electromagnetic field with a frequency of 900 MHz, which corresponds to the EMF generated by mobile phones, on development, hatchability and the occurrence of developmental abnormalities in chicken embryos.

Materials and methods

The study was conducted on 800 chicken eggs of normal shape and weight of 62 g, (±5 g), obtained from hens of the Ross 308 line. The eggs were incubated under standard conditions in two Masalles type 65 DIGIT incubators (days 1-18 of incubation: temperature 37.8 ±0.1ºC, relative humidity 55%; days 19-21 of incubation: temperature 37.2 ±0.1ºC, relative humidity 65%). One of the incubators used in the experiment was equipped with an aerial emitting an electromagnetic field at a frequency of 900 MHz. An EMF generator emitting radio-frequency electromagnetic waves (800-1,200 MHz) typical for GSM networks was specially designed for the purposes of this experiment. The mean output power of the generator was 330 mW and the maximum power was 2W. The output power was delivered to a Yagi GSM omnidirectional ceiling antenna. During the experiment the antenna was placed at a fixed distance of 24 cm above the eggs. The eggs remained in an area where the magnitude of the electric field ranged from 4.23 V/m to 6.25 V/m (±0.01 V/m) and the *strength* of the magnetic field ranged from 0.010 A/m to 0.014 A/m (±0.001 A/m), while power density varied from 0.090 W/m^2 to 0.110 W/m^2 (±0.001 W/m^2) and the frequency was 900 MHz. Measurements of the power density of the electromagnetic field were taken using a Tenmars TM-195 3-axis meter.

The experiment was divided into two consecutive stages. In each stage the eggs were assigned to two *groups* of 200 each: two control groups – incubation without additional EMF (stage I – group K1, stage II – group K2) and two experimental groups – incubation

under exposure to additional EMF at a frequency of 900 MHz (stage I – group D1, stage II – group D2). Embryos from the experimental groups were subjected to the electromagnetic field on each day of the incubation period. The total time of EMF exposure in each stage of the experiment was 40 minutes per day:

- stage I – repeated exposure – emission 10 times per day between 6:00 and 23:00 at regular intervals; 4-minute duration of a single emission
- stage II – single exposure beginning at 16:00

The duration of exposure for the experiment was chosen on the basis of information provided by Ericsson Consumer Lab regarding the duration of phone conversations by their users [7].

Unfertilized eggs and dead embryos were eliminated on the 7th and 19th days of incubation following assessment using Ovolux lamps (Masalles). In the case of eggs without visible embryos, the contents of the embryonic disc were evaluated according to a method devised by Darmos and Borzemska [5].

Hatching was monitored according to a procedure proposed by Borzemska and Malec [3], beginning after 440 hours of incubation. Each time the incubator was opened, the eggs were examined and the times of pipping and hatching were recorded. Charts were constructed on the basis of these observations (Fig. 1 and 2).

After hatching the nestlings were weighed using lab scales with accuracy to 0.1 grams.

The age of all embryos that died between the 3rd and 20th day of incubation, from culled or unhatched eggs, was determined following a key developed by Borzemska [2]. After incubation pathological analysis of the unhatched embryos was performed. The examination included assessment of overall development, the morphology of internal organs, the position of the embryo inside the egg, yolk sac retraction and developmental abnormalities.

The experimental data were analysed by Student's t-test using Sigma Stat 2.03 software (Systat Software GmbH, Germany). Prior to this all data were initially checked for normality by the Shapiro-Wilk test, which revealed that all the data had a normal distribution.

Results and discussion

Analysis of the hatching process revealed that pipping began earlier in the experimental groups exposed to the electromagnetic field during incubation (D1 and D2) than in the control groups (K1 and K2). The difference in time between observation of the first signs of pipping in the control and experimental group was 16 hours in series I (K1 vs D1) and 20 hours in series II (K2 vs D2) (Fig. 1). The differences were significant at $P<0.05$.

As in the case of pipping, hatching was earlier in the groups subjected to the electromagnetic field (D1 and D2). Acceleration of the hatching process was statistically significant ($P<0.05$), and was 24 hours in series I and 28 hours in series II (Fig. 2).

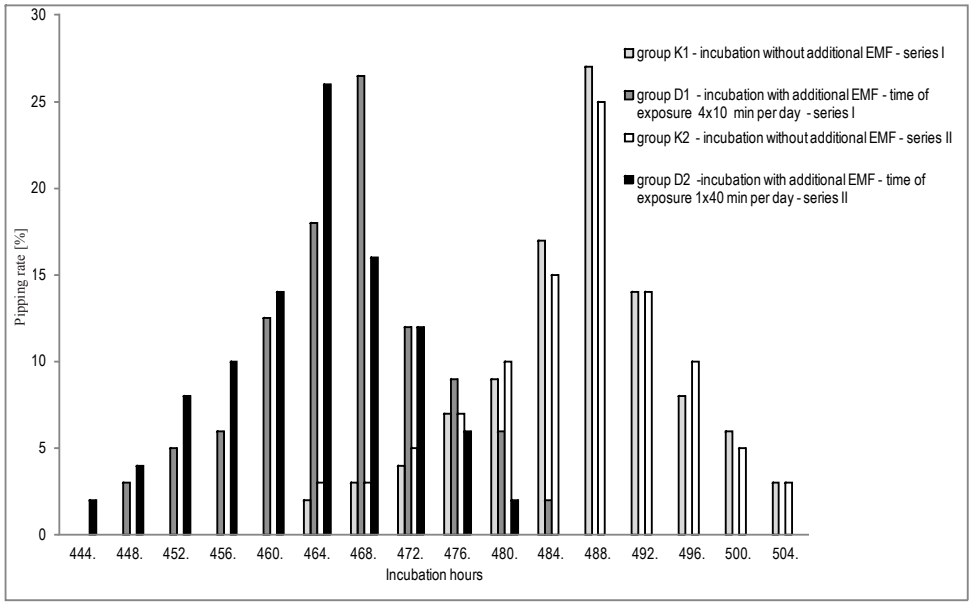

Fig. 1. Effect of exposure to a 900 MHz electromagnetic field during incubation on the pipping rate of chicken embryos

The calculations performed also revealed that the average time between initiation of pipping and hatching was statistically significantly shorter in the groups stimulated with EMF at a frequency of 900 MHz ($P<0.05$).

The available literature provides no studies on the effect of a radio-frequency electromagnetic field on pipping and hatching time. However, Sechman et al. [17] also observed an acceleration of the hatching process in chicks following application of an EMF at a much lower frequency (50 Hz) than the one used in our experiment. Stimulation of the pipping and hatching processes may be linked to the stress response observed in animals exposed to electromagnetic fields at frequencies similar to those emitted by mobile phones [4, 13, 16].

Measurements of nestling body weight taken directly after hatching showed no statistically significant differences between embryos incubated under standard conditions and those exposed to the additional electromagnetic field at a frequency of 900 MHz (Fig. 3). A similar lack of influence of an EMF on chicken embryo weight was reported by Veicsteinas et al. [19].

Analysis of the embryonic death rate expressed as a percentage, conducted after the 6th, 18th and 21st day of incubation, revealed no statistically significant differences

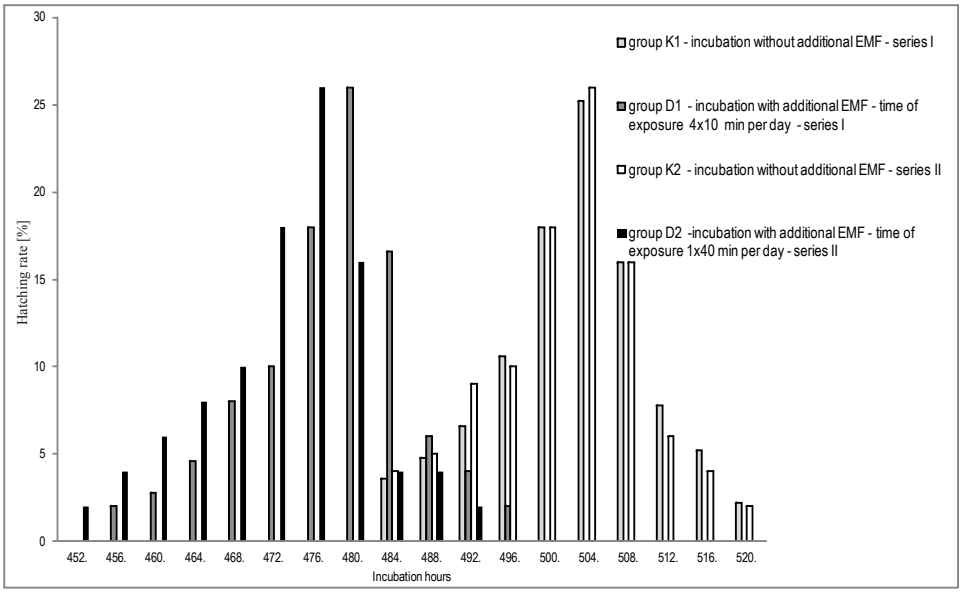

Fig. 2. Effect of exposure to a 900 MHz electromagnetic field during incubation on the hatching rate of chicken embryos

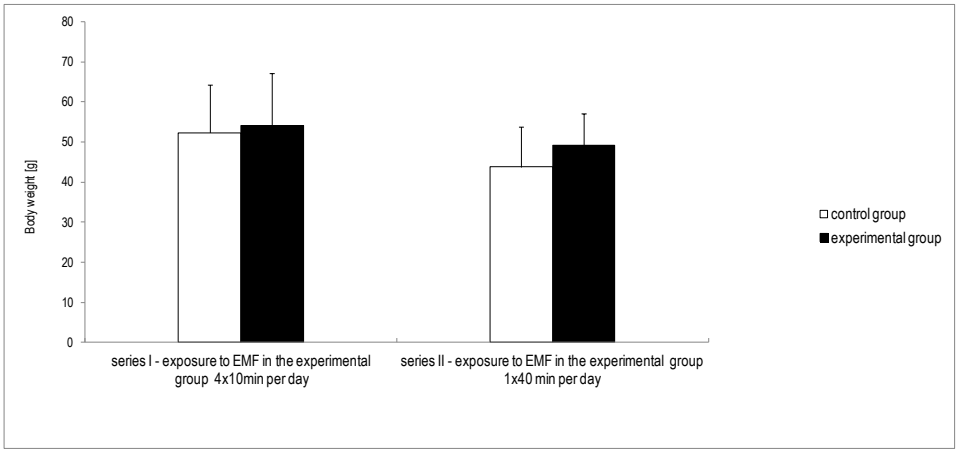

Fig. 3. Effect of exposure to a 900 MHz electromagnetic field during incubation on the body weight of 1-day old chicken nestlings (mean values; n=200)

between the control and experimental groups in either series (I and II). The percentage of dead embryos after 6 days of incubation ranged from 2.5% to 3.0%. This figure was considerably lower in the middle of the incubation period (days 7-18): no dead embryos in K1 and D1 and 0.5% in K2 and D2. The highest mortality rate was observed between days 19 and 21 of incubation: 4.5% in K1, 4% in D1 and K2 and 3.5% in D2 (Fig. 4). These results indicate that a 900 MHz electromagnetic field does not influence mortality in chicken embryos. The percentage of dead embryos in each incubation stage was within the range of natural mortality rates. A lack of impact of EMF on the mortality rate of chicken embryos in the initial stage of incubation has also been reported by Nishimura et al. [11]. In contrast, Batellier et al. [1] observed a sharp increase in the mortality rate of chicken embryos exposed to electromagnetic fields between the 9th and 12th day of incubation. However, these results may have been influenced by the fact that the experimental design did not include turning of the eggs during incubation, which is known to be necessary for proper embryonic development.

Hatchability from fertilized eggs calculated for each group was 92.5% in K1, 93% in K2 and D2 and 93.5% in D1 (no statistically significant differences were detected). Hatchability results obtained for the control and experimental groups were comparable

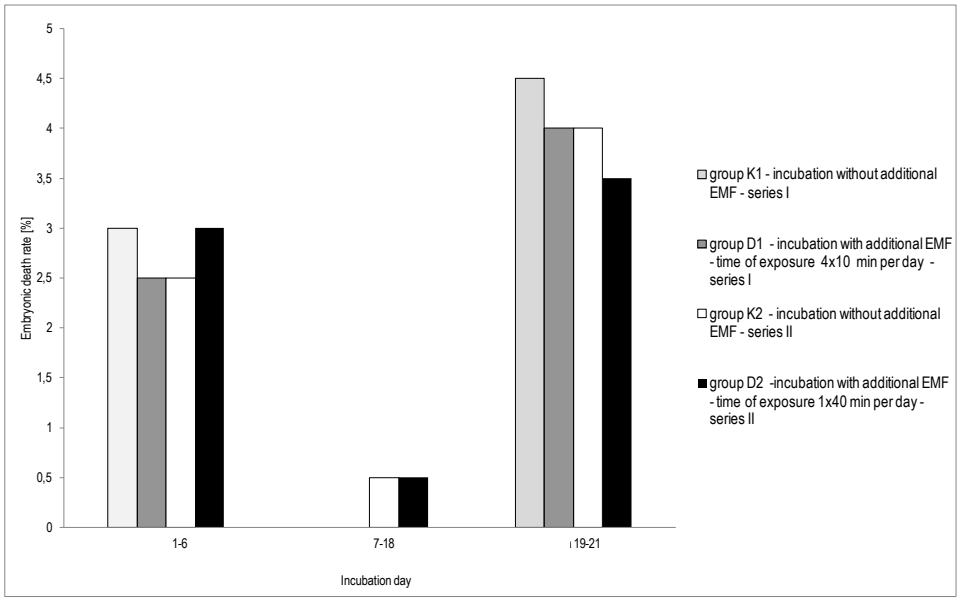

Fig. 4. Effect of exposure to a 900 MHz electromagnetic field during incubation on the mortality rate of chicken embryos

with rates expected for this line of chickens. A study by Sechman et al. [17] also demonstrated that an electromagnetic field did not affect hatchability, but at a frequency of 50 Hz. Other research teams (Ingole and Ghosh; Jyoti et al.) studying the response to mobile phone radiation have reported a decrease in the number of nestlings [8, 9]; however, the size of these experimental groups was very small (n=6).

The pathological examination of the unhatched embryos revealed no statistically significant influence of the 900 MHz EMF on the number of developmental abnormalities in any of the groups. In groups K1 and K2 a lack of complete yolk sac retraction was observed (3 embryos in each group), and in group D1 incorrect embryo position inside the egg (1 observation) and one underdeveloped embryo.

During the culling process only one nestling had a defect requiring culling (deformation in group K1).

This experiment demonstrated that the means of EMF exposure does not affect developing chicken embryos. All parameters studied in both experimental groups, i.e. D1 (repeated exposure to EMF – 10x4 minutes per day) and D2 (single exposure to EMF – 1x40 minutes per day), were comparable and did not differ statistically.

To sum up, stimulation of the chicken embryos with a 900 MHz electromagnetic field, irrespective of the duration of a single exposure, significantly accelerates the process of pipping and hatching. The time between pipping and hatching in the groups subjected to EMF was statistically significantly shorter. The experiment did not confirm an impact of the 900 MHz EMF on the hatchability rate, the number of developmental abnormalities, body weight, or the number of culled nestlings.

REFERENCES

1. BATELLIER F., COUTY I., PICARD D., BRILLARD J.P., 2008 – Effects of exposing chicken eggs to a cell phone in "call" position over the entire incubation period. ***Theriogenology*** 69, 737-745.
2. BORZEMSKA W.B., 1984 – Vademecum chorób drobiu. PWRiL, Warszawa.
3. BORZEMSKA W.B., MALEC H., 1986 – Biologiczna i patomorfologiczna ocena lęgu kur przy zaburzeniach synchronizacji klucia. ***Medycyna Weterynaryjna*** 43, 409-412.
4. DANIELS W.M.U., PITOUT I.L., AFULLO T.J.O., MABANDLA M.V., 2009 – The effect of electromagnetic radiation in the mobile phone range on the behaviour of the rat. ***Metabolic Brain Disease*** 24, 629-641.
5. DARMOS K., BORZEMSKA W., 1979 – Routine method of differentiating eggs NF (nonfertile), POD (pre-ovipositional death) and FND (fertile, no development) from the PD (positive development) eggs in incubation refuse. ***Zeszyty Naukowe SGGW*** 9, 41-48.
6. DŻUGAN M., LIS M., DROBA M., NIEDZIÓŁKA W.J., 2011 – Effect of cadmium injected in ovo on hatching results and the activity of plasma hydrolytic enzymes in newly hatched chicks. ***Acta Veterinaria Hungarica*** 59, 337-347.
7. Ericsson Consumer Lab, 2014 – https://www.ericsson.com/networked-society/consumerlab (z 20 września 2014 roku)

8. INGOLE I.V., GHOSH S.K., 2006 – Cell Phone Radiation and Developing Tissues in Chick Embryo – A Light microsopic Study of Kidneys. ***Journal of the Anatomical Society of India*** 55, 19-23.
9. JYOTI KOHLI R.K., BAGAI U., 2014 – Effect of mobile phone frequency radiation on early development of chick embryo. ***International Journal of Science, Environment and Technology*** 3, 1273-1280.
10. LUIIS G.N., NEWRY R.J., KING B.V., JOHN A.R., 2009 – Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. ***PLoS One*** 47, 6440-6446.
11. NISHIMURA I., IMAI S., NEGISHI T., 2009 – Lack of chick embryotoxicity after 20 kHz, 1.1 mT magnetic field exposure. ***Bioelectromagnetics*** 30, 573-582.
12. PAWLAK K., SECHMAN A., NIECKARZ Z., WOJTYSIAK D., 2013 – Effect of weak electromagnetic field on cardiac work, concentration of thyroid hormones and blood aminotransferase level in the chick embryo. ***Acta Veterinaria Hungarica*** 61, 383-392.
13. PAWLAK K., SECHMAN A., NIECKARZ Z., 2014 – Plasma thyroid hormones and corticosterone levels in blood of chicken embryos and post hatch chickens exposed during incubation to 1800 MHz electromagnetic field. ***International Journal of Occupational Medicine and Environmental Health*** 27, 114-122.
14. PLATANO D., MESIRCA P., PAFFI A., PELLEGRINO M., LIBERTI M., APOLLONIO F., BERSANI F., AICARDI G., 2007 – Acute exposure to low-level CW and GSM-modulated 900 MHz radiofrequency does not affect Ba 2+ currents through voltage-gated calcium channels in rat cortical neurons. ***Bioelectromagnetics*** 28, 599-607.
15. POLITAŃSKI P., BORTKIEWICZ A., ZMYŚLONY M., 2016 – Wpływ pól elektromagnetycznych zakresu radiowego i mikrofalowego, emitowanych przez urządzenia łączności bezprzewodowej, na funkcjonowanie wybranych elementów układu nerwowego. ***Medycyna Pracy*** 67, 411-421.
16. PROCHNOW N., GEBING T., LADAGE K., KRAUSE-FINKELDEY D., EL OUARDI A., BITZ A., STRECKERT J., HANSEN V., DERMIETZEL R., 2011 – Electromagnetic field effect or simply stress? Effects of UMTS exposure on hippocampal longterm plasticity in the context of procedure related hormone release. ***PLoS One***. May 5: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0019437
17. SECHMAN A., NIEDZIÓLKA J., LIS M., RZASA J., 2006 – Changes in thyroid hormone levels in chicken embryos exposed to extremely low frequency electromagnetic field. ***Archiv Fur Geflugelkunde*** 70, 41-47.
18. SU G., LETCHER R.J., CRUMP D., FARMAHIN R., GIESY J.P., KENNEDY S.W., 2016 – Sunlight irradiation of highly brominated polyphenyl ethers generates polybenzofuran products that alter dioxin-responsive mRNA expression in chicken hepatocytes. ***Environmental Science & Technology*** 50, 2318-2327.
19. VEICSTEINAS A., BELLERI M., CINQUETTI A., PAROLINI S., BARBATO G., MOLINARI TOSATTI M.P., 1996 – Development of chicken embryos exposed to an intermittent horizontal sinusoidal 50 Hz magnetic field. ***Bioelectromagnetics*** 17**,** 411-424.
20. We Are Social, 2016 – http://wearesocial.com/uk/special-reports/digital-in-2016.

21. YAHAV S., COLLIN A., SHINDER D., PICARD M., 2004 – Thermal manipulations during broiler chick embryogenesis: effects of timing and temperature. ***Poultry Science*** 83, 1959-1963.
22. ZENI O., CHIAVONI A.S., SANNINO A., ANTOLINI A., FORIGO D., BERSANI F., SCARFÌ M.R., 2003 – Lack of genotoxic effects (micronucleus induction) in human lymphocytes exposed in vitro to 900 MHz electromagnetic fields. ***Radiation Research*** 160, 152-158.