

Effect of an 1800 MHz electromagnetic field emitted during embryogenesis on the blood picture of one-day-old domestic hen chicks (*Gallus gallus domesticus*)

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Abstract

Exposure to artificial electromagnetic fields emitted mainly by mobile telephony has been steadily increasing with the development of modern technology. Haematological indices are among the most common indicators of the body's physiological status. The aim of the study was to determine the effect of an 1800 MHz electromagnetic field emission on the blood picture of one-day-old domestic hen chicks. During the experiment, chick embryos were exposed to artificial electromagnetic fields throughout incubation for 13 ´ 2 min/day, 4 ´ 10 min/day and 1 ´ 40 min/day. After hatching, blood was collected from 10 one-day-old chicks from each group to determine: red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell count, and leukocyte differential count. In addition, the heterophil/lymphocyte ratio was calculated. The present study is probably the first to show an increase in the red blood cell count, haemoglobin concentration, haematocrit, white blood cell count, segmented heterophils and the heterophil/lymphocyte ratio, and a decrease in lymphocyte percentage of embryos exposed to an 1800 MHz electromagnetic field. The observed changes may be indicative of the stress-inducing effect of EMF on living organisms.

Electromagnetic fields, haematology, embryo, chicken, mobile phone

Humans in the modern society are exposed to an ever-increasing number of electromagnetic fields (EMFs) generated by the production and supply of electricity, television (TV) sets, personal computers (PC), radio communication and mobile phone networks (Gye and Park 2012). Fields generated by mobile telephony deserve a special attention due to their prevalence. The International Telecommunication Union reports that global mobile cellular subscriptions exceeded 7 billion (The State of Broadband 2016). Research carried out for many years does not provide a conclusive answer as to the effect of an electromagnetic field on living organisms (Platano et al. 2007; Batellier et al. 2008; Geoffrey et al. 2009; Augner et al. 2012). One of the reasons is that determining the way in which EMFs affect living organisms involves taking into account a large number of factors. The very fact that the organism behaves like a conductor when exposed to 100 MHz waves, has intermediate properties at 1000 MHz and acts like a dielectric at 10000 MHz, shows how important the change of just one field parameter may be. To evaluate the effect of the EMFs on the processes occurring in the body empirically, it is necessary to conduct research on living organisms (Brent 1999). For many decades, the developing chicken embryo has been considered an ideal model for investigating a large number of vital processes

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in different areas of biological sciences, in particular, studies of the interactions between growing tissues and organs of the embryo as well as the influence of chemical compounds or physical factors (Veicsteinas et al. 1996; Pawlak et al. 2013). Blood cells contribute to maintaining the body's homeostasis by taking part, directly or indirectly, in multiple physiological and pathological processes. Their metabolic activity can be influenced by both internal and external factors, including the electromagnetic field.

The aim of the study was to investigate the effect of the 1800 MHz electromagnetic field emission on the blood picture of one-day-old domestic hen chicks. The following experimental indices were studied: red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (Hct), white blood cell count (WBC) and leukocyte differential count. For the purpose of the study, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and the heterophil/lymphocyte ratio (H:L) were also determined.

Materials and Methods

Biological material

The experiments were approved by the First Local Ethics Committee for Experimentation on Animals in Krakow (Resolution No. 113/2013). The biological material was represented by normally shaped hatching eggs weighing about 62 g (± 5 g) obtained from a Ross 308 broiler breeder flock at the peak of lay.

Three hundred sixty eggs used in the experiment were incubated using standard procedures in Masalles type 65 DIGIT incubators (1–18 days of incubation: temperature 37.8 ± 0.1 °C, relative humidity 55%, 19–21 days of incubation: temperature 37.2 ± 0.1 °C, relative humidity 65%).

Experimental design

The eggs were divided into 2 equal groups:

Group 1 (control) – development of embryos in a standard incubator;

Group 2 (experimental) – development of embryos in an incubator equipped with a switched-on electromagnetic field generator.

The experiment was conducted in three sequential test series (60 eggs in the control group and 60 eggs in the experimental group in each series). In each series, embryos from experimental incubators were exposed daily to an electromagnetic field from the first to the last day of incubation. The EMF exposure time was as follows:

Series 1 – 26 min per day (EMF emitted 13 times per day between 6:00 h and 23:00 h, at equal time intervals, 2-minute emissions);

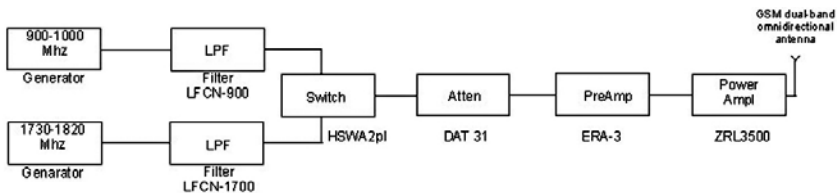
Series 2 – 40 min per day (EMF emitted 10 times per day between 6:00 h and 23:00 h, at equal time intervals, 4-minute emissions);

Series 3 – one 40-minute emission from 16:00 h.

The EMF exposure time was selected based on Ericsson Consumer Lab (2016) data on duration of calls made by mobile phone users.

EMF generator

The electromagnetic field source consisted of a specially designed generator emitting radio frequency electromagnetic waves (Global System for Mobile Communications: 900/1800 MHz) (Fig. 1).



GSM - Global System for Mobile Communications, LPF - Low Pass Filter, Atten - Digital Step Attenuator, PreAmp - Wideband preamplifier, Power Ampl - Low Noise Amplifier

Fig. 1. Block of generator

The maximum power output of the generator that could be emitted amounted to 330 mW. The output power was delivered to a Yagi Global System for Mobile Communications (GSM) ceiling antenna with omnidirectional characteristics. Antenna parameters: indoor 8dBi, omni ceiling antenna bands: GSM-1800 MHz – a linear polarized antenna produced by IAPT. The generator was computer-controlled via USB interface. The software allowed to select the strength of generated signal and generator operation time. During the experiment, the antenna remained at a fixed distance from the egg tray (24 cm above the eggs). Embryos developing in eggs were situated in the area where electric and magnetic fields ranged from 4.23 V/m to 6.25 V/m (± 0.01 V/m) and from 0.010 A/m to 0.014 A/m (± 0.001 A/m), respectively. Power density fluctuated between 0.090 W/m² and 0.110 W/m² (± 0.001 W/m²), while frequency reached the value of 1800 MHz. Power density of the electromagnetic wave was measured on the surface of the egg using a Tenmars TM-195 3-axis meter. This device allows, among others, performing isotropic measurement of intensity of electric fields (0.01–20.0 V/m) and intensity of magnetic fields (0.1–532.6 mA/m) as well as power density (10.0–106.94 mW/m²) in the range of frequency from 50 MHz to 3.5 GHz. The specific absorption rate (SAR) for an egg in the array was calculated based on a model of an egg using a commercial software package CST Microwave Studio, version 2008 (CST, Darmstadt, Germany). The SAR value calculated for the experimental group amounted to 4.2×10^{-4} W/kg.

Haematological analysis

Blood samples were collected from newly hatched chicks (day 21). In each series the samples were obtained from 10 exposed and 10 control individuals. The total number of erythrocytes (RBC), the total number of white cells (WBC), haematocrit (Hct) value, and the total haemoglobin concentration (Hb) were determined at once. An aliquot of the blood was transferred to microcapillary tubes, which were centrifuged at $2000 \times g$ for 10 min, using an MPW-351R centrifuge (MPW Med. Instruments Poland). The haematocrit value was calculated as the percentage of red blood cell pellet in the total blood column. Haemoglobin content was determined in total blood according to the cyanmethaemoglobin method using a BioTek Eon spectrophotometer (BioTek® Instruments, USA) at 540 nm (following the Biochemtest® kit instruction). Blood cell counts (RBC and WBC) were manually obtained using a Bürker haemocytometer according to the method provided by Natt and Herrick (1952). Additionally, smears were prepared and percentages of different kinds of white blood cells (lymphocytes, monocytes, heterophils, and eosinophils) were determined. Next, erythrocyte indices (MCH, MCV, and MCHC) were calculated using standard formulas (Bomski 1995).

Analysis of the number and the quality of hatched chicks as well as unhatched eggs was conducted at the end of incubation to assess whether embryos developed properly.

Statistical analysis

Statistical analyses were carried out using a two-way ANOVA, after testing for homogeneity of variance (Levene test) followed by *post hoc* Tukey test. The level of significance was set at $\alpha = 0.05$. The data were presented as means \pm standard deviation (SD). Results were analysed using the STATISTICA 12 program.

Results

Red blood cell count in the chicks exposed during embryogenesis to EMF 10 times/day for 4 min ($3.69 \times 10^6/\mu\text{l}$) was significantly higher than the value observed in control chicks ($2.89 \times 10^6/\mu\text{l}$). This indicator also increased significantly in chicks exposed continuously for 40 min/day to EMF ($3.73 \times 10^6/\mu\text{l}$) in relation to the control value ($2.82 \times 10^6/\mu\text{l}$) (Table 1).

Haemoglobin (Hb) concentration in chicks from the experimental group (series 1) amounted to 13.40 g/dl, which was significantly higher than the concentration in chicks from the control group (10.45 g/dl). In chicks exposed during incubation to EMF 10 times/day for 4 min, Hb concentration (14.18 g/dl) increased in relation to the control value (10.52 g/dl). Also the exposure to EMF once a day for 40 min caused a significant increase of this indicator (14.25 g/dl) compared to the control value (10.38 g/dl) (Table 1).

The Hct value in chicks from the experimental group (series 1) was 30.9% which is significantly higher compared to the value obtained in the control birds (25.2%). Haematocrit also increased significantly in chicks exposed to EMF 10 times/day for 4 min (30.0%) in relation to the control value (26.0%). Likewise, the exposure to EMF once a day for 40 min increased this indicator significantly (31.6%) compared to the control value (25.3%) (Table 1).

Table 1. Red blood cell indicators of control and experimental chicks in three experimental series, presented as mean \pm SD (n = 10).

| Series | Group | RBC [$10^6/\mu\text{l}$] | | Hb [g/dl] | | Hct [%] | | MCV [fl] | | MCH [pg] | | MCHC [g/dl] | |
|--------|--------------|----------------------------|------|-----------|------|---------|------|----------|-------|----------|-------|-------------|-------|
| | | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD |
| I | Control | 3.00 | 0.51 | 10.45 | 0.96 | 25.20 | 3.39 | 85.79 | 15.14 | 35.74 | 6.70 | 41.76 | 3.30 |
| | Experimental | 3.54 | 0.84 | 13.40* | 1.24 | 30.90* | 4.36 | 92.19 | 25.42 | 40.09 | 10.78 | 43.75 | 4.27 |
| II | Control | 2.89 | 0.56 | 10.52 | 1.15 | 26.00 | 4.08 | 89.84 | 27.27 | 36.34 | 8.45 | 40.45 | 7.30 |
| | Experimental | 3.69* | 0.51 | 14.18* | 1.90 | 30.00* | 3.16 | 81.24 | 11.90 | 38.39 | 8.64 | 47.25 | 7.79 |
| III | Control | 2.82 | 0.62 | 10.38 | 1.26 | 25.30 | 4.42 | 89.70 | 22.30 | 36.82 | 9.92 | 41.04 | 10.78 |
| | Experimental | 3.75* | 0.55 | 14.25* | 0.81 | 31.60* | 3.06 | 84.63 | 19.07 | 38.15 | 5.55 | 45.09 | 4.85 |

Significant differences ($\alpha = 0.05$) in relation to control group are marked with **.

SD - standard deviation, RBC - red blood cell, Hb- haemoglobin, Hct- haematocrit, MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin, MCHC - mean corpuscular haemoglobin concentration

White blood cell count in chicks exposed during embryogenesis to EMF 13 times/day for 2 min ($27.0 \times 10^3/\mu\text{l}$) was significantly higher than in the control chicks ($19.20 \times 10^3/\mu\text{l}$). White blood cell count also increased significantly in birds exposed to EMF 10 times a day for 4 min ($27.1 \times 10^3/\mu\text{l}$) in relation to the control value ($19.2 \times 10^3/\mu\text{l}$). Also the exposure to EMF once a day for 40 min led to a significant increase in WBC compared to the control value ($27.80 \times 10^3/\mu\text{l}$ vs. $18.90 \times 10^3/\mu\text{l}$) (Table 2).

In both the second and third test series, the percentage of segmented heterophils in the blood of control chicks was significantly higher in the experimental group compared to control chicks – series 2: 13.2% vs. 10.1%, series 3: 14.0% vs. 10.2% (Table 2).

The percentage of lymphocytes in chicks exposed during incubation to EMF 13 times/day for 2 min (78.3%) was significantly lower than in the control chicks (82.6%). Also in birds exposed to EMF 10 times a day for 4 min, this indicator (79.3%) was significantly lower compared to the value observed in the control birds (83.2%). Similarly, single daily 40-minute exposure caused a significant decrease in lymphocyte percentage in relation to the control value (77.8% vs. 82.9%) (Table 2).

Regardless of the duration of EMF emission, the heterophil to lymphocyte (H:L) ratio was higher in chicks exposed to an additional EMF during incubation (Table 2).

The other changes in red and white blood cell indices (Tables 1 and 2) were not significant.

The analysis of variance showed no significant effect of the EMF exposure length (series factor) on the analysed haematological indicators.

The analysis of the number and quality of hatched chicks as well as unhatched eggs did not reveal any disturbances in embryogenesis.

Discussion

Haematological indices are among the most frequently analysed indicators of the body's physiological status. Haematological tests are used to evaluate the effect of abiotic factors, such as the electromagnetic radiation, on living organisms. The most commonly analysed erythrocyte indicators include RBC, Hb, Hct, MCV, MCH, and MCHC. Although blood is essential to both human and animal organisms, there are only a few studies investigating the impact of electromagnetic fields emitted by mobile phones on the haematological indices. In our study we found that the Hct value and Hb concentration increase significantly in the blood exposed to EMF during embryogenesis (all test series). An increase in the Hct value was also observed by

Table 2. White blood cell indicators of control and experimental chicks in three experimental series, presented as mean \pm SD (n = 10).

| Series | Group | WBC [10 ³ / μ l] | | Segmented heterophils [%] | | Band heterophils [%] | | Eosinophils [%] | | Monocytes [%] | | Lymphocytes [%] | | H:L ratio | |
|--------|--------------|------------------------------------|------|------------------------------|------|-------------------------|------|--------------------|------|------------------|------|--------------------|------|-----------|------|
| | | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD |
| I | Control | 19.20 | 4.20 | 10.50 | 1.43 | 0.00 | 0.00 | 0.40 | 0.97 | 6.50 | 2.01 | 82.60 | 2.41 | 0.13 | 0.02 |
| | Experimental | 27.00* | 5.70 | 13.50 | 4.58 | 0.10 | 0.32 | 1.10 | 0.99 | 7.00 | 1.49 | 78.30* | 4.27 | 0.17* | 0.07 |
| II | Control | 19.20 | 2.80 | 10.10 | 1.60 | 0.00 | 0.00 | 0.50 | 0.85 | 6.20 | 2.10 | 83.20 | 2.57 | 0.12 | 0.02 |
| | Experimental | 27.10* | 6.30 | 13.20* | 3.79 | 0.00 | 0.00 | 0.90 | 0.88 | 6.60 | 2.12 | 79.30* | 3.50 | 0.17* | 0.05 |
| III | Control | 18.90 | 1.90 | 10.20 | 1.99 | 0.00 | 0.00 | 0.60 | 0.84 | 6.30 | 1.70 | 82.90 | 2.56 | 0.12 | 0.03 |
| | Experimental | 27.90* | 5.90 | 14.00* | 3.50 | 0.10 | 0.32 | 1.00 | 1.15 | 7.10 | 1.60 | 77.80* | 3.77 | 0.18* | 0.05 |

Significant differences ($\alpha = 0.05$) in relation to control group are marked with ***. SD - standard deviation, WBC - white blood cells, H:L heterophil:lymphocyte

Forgác et al. (2005), who studied the effect of an 1800 MHz EMF on the haematological indices of male mice. Similar results were obtained by Dasdag et al. (2002), who used a much lower frequency field (50 Hz) in their study. Similar to the studies presented above, increases in haemoglobin concentration were reported by Hashem and El-Sharkawy (2009) as well as Hanafy et al. (2013). No effect of EMF on this erythrocyte indicator was observed by Forgác et al. (2005), Shi et al. (2005), and Dasdag et al. (2002). However, it should be noted that except for Forgác et al. (2005), all other investigators used a field with completely different characteristics than in our study.

The erythrocyte number calculated in the blood of embryos showed a significant effect of the field emission (series 2 and 3) on RBC. Similar results were obtained by Forgác et al. (2005) and Sarookhani et al. (2012b), who used a 950 MHz field and showed no effect of this field on the red blood cell count. No effect was also reported by Picazo et al. (1995) and Shi et al. (2005), who studied the effect of a much lower electromagnetic field (50 Hz) on RBC in mice, and by Dasdag et al. (2002) in humans. A decrease in the erythrocyte count in personnel exposed to radar frequencies of 1250–1350 MHz was observed by Goldoni (1990).

Our analyses did not show a significant effect of an 1800 MHz electromagnetic field on the calculated values of MCV, MCH, and MCHC. No effect of an electromagnetic field on these indicators was reported by Cakir et al. (2009), either. In contrast, in a study with mice exposed to a much lower radiation frequency (50 Hz), Sarookhani et al. (2012a) found an increase in MCV.

Previous studies conducted by Pawlak et al. (2014) on chick embryos demonstrated that the radio frequency electromagnetic field causes a significant increase in corticosteroid concentration, especially during the last days of incubation. It is known that corticosteroids are one of the factors influencing red blood cell indicators. It can therefore be presumed that the increases in haematocrit, haemoglobin

concentration (series 1, 2 and 3), and erythrocyte count (series 2 and 3), observed in embryos exposed to an electromagnetic field, result from the increased corticosteroid concentration due to the stress induced by EMF.

In all the experimental series, a significant increase in the leukocyte count (WBC) was accompanied by a decrease in the lymphocyte percentage. Increased WBC as a result of EMF was also observed in personnel exposed to radar frequencies of 1250–1350 MHz (Goldoni 1990) and in female mice exposed to EL-EMF (50 Hz) (Hashem and El-Sharkawy 2009). However, a study by Shi et al. (2005) showed no effect of EMF

(50 Hz) on WBC in mice. In our study, we also observed a significant increase in the percentage of segmented heterophils in chicks exposed during embryogenesis to EMF for 40 min/day (series 2 and 3). Unfortunately, there are no studies available regarding the effect of EMF on the leukogram. As reported by Davis et al. (2008), changes in the white blood cell profile could suggest the body's stress response. This conjecture was also confirmed in our study as the heterophil/lymphocyte ratio in chicks exposed to an additional EMF during incubation increased. As it is known, in birds the level of stress is manifested by an increase in the heterophil count and a decrease in the lymphocyte count, which increases the H:L ratio (Skwarska 2012).

In summary, an 1800 MHz electromagnetic field alters the blood picture of one-day-old domestic hen chicks. Increases in RBC, Hb concentration, Hct value, WBC, segmented heterophils and the H:L ratio, which were paralleled by a decrease in the lymphocyte percentage in the blood of chicks exposed to an electromagnetic field during incubation, may be indicative of the stress-inducing effect of EMF on living organisms. The study revealed no significant effect of the duration of field emission on the blood picture of one-day-old hen chicks.

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