Effect of incubating egg exposure to magnetic field on the biophysical blood properties of newly-hatched chicks

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Abstract: Due to widespread of human exposure to electromagnetic fields, there has been increasing public concern about the potential health risks from low-frequency electromagnetic fields; ELF-EMF. The magnetic fields (MFs) affects functions of the living organisms, such as DNA synthesis and ion transportation through the cell membranes. In the present work, the effects of short-term exposure to magnetic fields (MFs) prior to incubation were investigated on the biophysical blood properties of chicks hatched from layer-type breeder eggs. The eggs were exposed to a MF of 0.75mT at 50 Hz for 20, 40 and 60min before incubation. This study was performed by measuring the dielectric relaxation of hemoglobin (Hb) molecules and the membrane solubility of red blood cells (RBCs) using the non-ionic detergent octylglucoside. Exposure of the eggs to a MF increased the conductivity of the Hb molecules. The pronounced increase in the conductivity of the exposed eggs might be attributed to an increase in the surface charge of the Hb macromolecules, resulted from the formation of highly active molecular species. This speculation can be supported bythe increase in the relaxation time of the exposed groups. The solubilization process of the RBC membrane indicates a loss in the mobility of RBCs in the blood of hatching chicks.

Keywords: Incubating egg; magnetic field; biophysical blood properties; newly-hatched chicks.

INTRODUCTION

The extremely low-frequency as well as low-intensity electromagnetic fields onthe biological systems is a matter of interest to researchers and is of public concern due to the nearly constant human exposure to time-varying magnetic fields (MFs) arising from, for instance, electric appliances, power lines, and home wiring. The most prevalent and unavoidable exposure source is Earth's magnetic field (flux densities of 30 to 70μT). In houses, offices and laboratories, the flux densities of 0.1 to 0.2μT at power-line frequencies are commonly encountered and stronger fields of up to several hundred μT can occur near some electrical appliances.

Debate about the toxic effect of EMF has arisen, and no definite conclusion has been reached regarding the potential mechanisms of the phenomenon until recently. Many reported reviewson animal and cells have shownthat the relatively weak magnetic fields can interact and produce biological effects (Liburdy, 1992; Luben, 1991; Tenforde, 1991), such as promoting or co-promoting tumors (Ubeda *et al*., 1995; Mevissen M *et al*. 1995; L'oscher. *et al*., 1993) and reducing melatonin production (Reiter *et al*., 1993; Stuchly *et al*, 1992). The body of a living bird is composed of many cells that communicate with their environment through information transfer carried out by electrical impulses or chemical substances. Body EMFs are characterized by certain specific frequencies that can be interfered with via external EMF radiation and this interference can modify the cells' biological responses (Shafey *et al*. 2007; Liboff and Jenrow, 2000; Hyland, 2000). Animals exposed to EMFs can suffer a deterioration of health, changes in behavior and alterations in reproductive success (Marc *et al*. 2000; Fernie *et al*., 2000; L'oscher and K'as 1998; Farrel *et al*., 1997; Doherty and Grubb 1996; Cox *et al*., 1993; Delgado *et al*., 1982). Chicken embryos exposed to pulsed MFs have been shown to suffer increased mortality and morphological abnormalities (Grigoriew, 2003; Youbicier and Bastide, 1999; Farrel *et al*., 1997; Ubeda, 1994). Moreover, studies about the early stage of embryonic development stage is responsive to the fluctuating magnetic fields (Cameron *et al*. 1993), accompanied by a decrease in the embryos' mortality and acceleration of external popping and hatching (Sechman *et al*., 2006), causes abnormal development for chick embryos exposed to MF higher than1 A/m (Juutilainen *et al*., 1987). Incubating eggs were used to determine the effect of ELF-EMF on blood plasma levels of thyroid hormones. Results of this study demonstrate that ELF-EMF stimulates the activity of the thyroid gland, affects the peripheral iodothyronine metabolism and influences the hatching parameters (Sechman *et al*., 2006). Moreover, Vesna *et al*. (2005) in their work found a significant increased number of NPY-containing nerve fibres in the rats' thyroid exposed to ELF-EMF compared to the controls. Since the dielectric measurement is a pure

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physical technique depends on the charge distribution and dipole moment in the system, so it expected that this technique will provide new information regarding the EMF Effects. So, the previously measured dielectric properties of various biological materials indicated useful results about the structural changes under any internal or external changes (Ghannam and Mady, 2012; Ghannam *et al*. 2002; Polk and Postow, 1996). Moreover, Fakhry *et al*. 2012 conclude that it can be used with the combination of such molecular techniques to facilitate and improve early cancer diagnosis. Turbidity is an indirect indicator of microstructure changes in lipid aggregates (Marks *et al*., 1995; Paternostre *et al*., 1995; Jürgen, 1995). The rate of change in optical density is expected to be increased with increasing the surfactant concentration as a result of the transformation of vesicle structures (Chern *et al*., 2006). The solubilization of pure phospholipid membranes by detergents have been the subject of several studies (Paternostre *et al*., 1995 and 1997; Bielawski, 1990; Annela, 1966). While the non-ionic detergents are assumed to solubilize the proteins of the membrane proteins without affecting the structural features (Marc le Maire, 2000). This study was aimed to examine the effects of short-term exposure to MFs prior to incubation on the biophysical blood properties of chicks hatched from layer-type breeder eggs. This aim was achieved by measuring the dielectric relaxation of hemoglobin (Hb) molecules and the membrane solubility of red blood cells (RBCs) using the non-ionic detergent octylglucoside.

MATERIALS AND METHODS

Ethical note

All experiments in this study were conducted in accordance with the approved guidelines of King Saud University Local Animal Care and Use Committee.

Egg preparation and incubation

This study employed a total of 384 freshly laid eggs produced by a layer-type breeder flock (Leghorn, King Saud University) at 50 weeks of age. The birds were fed a standard breeder ration (16% CP, 12MJ of ME per kg, 3.4% calcium and 0.45% available phosphorus) and were reared under standard husbandry conditions. The eggs were exposed to a MF of 0.75mT at 50 Hz for 20, 40 and 60min (MF20, MF40 and MF60) before incubation. The eggs were transferred to separate compartments in the hatching tray on day 19 of incubation to allow for chick identification at hatching. A total of nine birds from each treatment, in addition to the unexposed group, were selected at hatching and blood samples were collected via heart puncture into tubes containing EDTA. Blood samples from each treatment type were pooled together into three samples, and the biophysical blood properties of the chicks were determined.

Magnetic field exposure facilities

A homogeneous MF generator in which eggs can be housed for MF exposure was designed and constructed. Four solenoids were constructed with 270 turns each of electrically insulated, 2.2-mm-diameter copper wire, which was wound around a parallel double-walled cylindrical chamber, constructed of 2-mm-thick copper and it had internal and external diameters of 45 and 55 cm, respectively (fig. 1). The four coils were connected in parallel to minimize the total impedance of the wire and to produce a homogeneous MF within the chamber volume. The coils were connected to a varistor fed from the main power outlet (220Vpp at 50 Hz). The strength of the MF was controlled and varied by the power source. The MF inside the chamber was calibrated at different locations to determine the most homogenous zone inside the chamber. Hand-held Gauss/Tesla meter, model 4048 with probe T-4048-001 (USA) and accuracy ±2%, was used to calibrate the MF. The homogeneity of the MF under different field intensities is shown in fig. 2. It was found that the mid region of the chamber is the most homogenous MF. The exposure facility was temperature-controlled and free from alternating electric fields to minimize any external fields that may interfere with the MF and affect the measured phenomena. The temperature inside the irradiation chamber was constantly measured using a thermocouple thermometer, which can give temperature readings within ±0.1^{o}C. There was no difference in temperature between the room and the chamber.

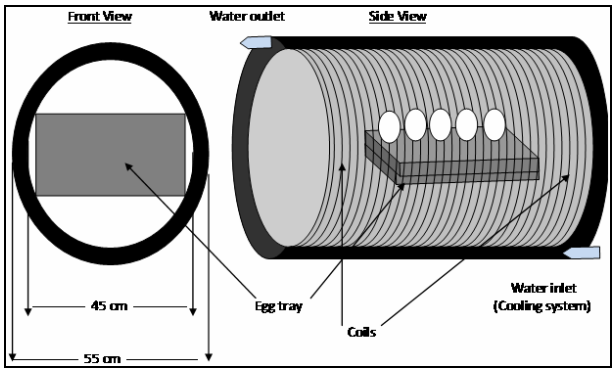

Fig. 1: Magnetic field (MF) exposure facilities

Hemoglobin (Hb) dielectric relaxation

Erythrocytes were isolated by centrifuging blood suspended in EDTA at 3500 rpm and 4°C for 10min, and the plasma and the top third of the cell volume were removed to eliminate leukocyte contamination. The collected blood was hemolyzed to produce hemoglobin, and its concentration was adjusted and controlled spectro-photometry using distilled water. Hemoglobin dielectric measurements were taken in the frequency range of 20 Hz to 100kHz using a Wayne Kare precision component analyzer, model 6440B (UK), connected to a conductivity cell of type 19250-60, manufactured by Cole Palmer Co. The sample cell has two square, black, platinum

electrodes with a cell constant of k=1cm^{-1}. The measurements were performed at 20°C. The measured values of capacitance C and resistance R as functions of the frequency (f) were used to calculate the real (ε') and imaginary (ε") parts of the complex permittivity ($\varepsilon^* = \varepsilon' - i\varepsilon''$), the conductivity (σ) and the relaxation time (τ) using the following equations:

$$\varepsilon' = \frac{ck}{\varepsilon_o} \quad \varepsilon'' = \varepsilon' \tan\delta = \varepsilon' \frac{1}{2\pi fRC}$$

$$\sigma = \frac{k}{R}\left(\Omega^{-1}m^{-1}\right), \tau = \frac{1}{2\pi f_c} \qquad (S)$$

Where $\tan\delta$ is the phase lag between the displacement vector D and the electric field E, ε_o is the permittivity of free space, and f_c is the critical frequency corresponding to the midpoint of the dispersion curve.

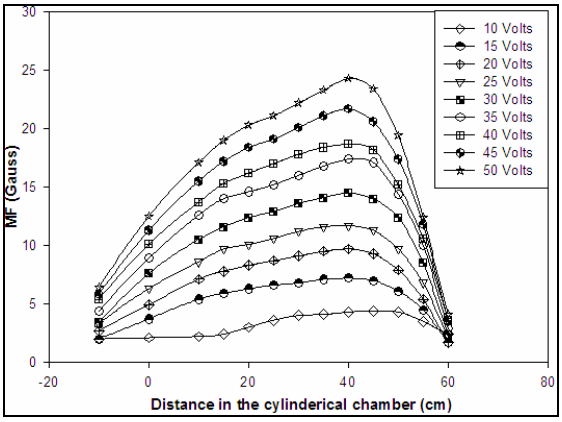

Fig. 2: MF strength calibration under different current intensities and location. Egg tray is located in the region of 10-50 cm inside chamber

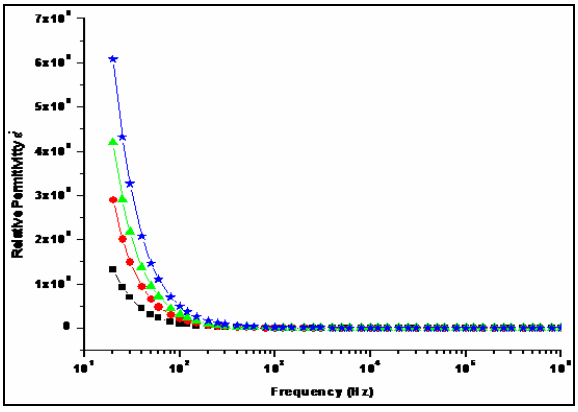

Fig. 3: Relative permittivity ε' as functions of the applied frequency in the range of 20 Hz to 100 kHz for eggs that were not exposed (control) and those that were exposed to a magnetic field of 7.5 G for different durations in minutes; (control, ■), (20 ●), (40 ▲) and (60*)

RBC membrane solubilization

A detailed description of the method and devices used in this study has been given previously (Paternostre *et al*., 1995; Jurgen, 1995). Erythrocyte concentrations were adjusted and controlled by spectrophotometry using a 0.9% NaCl isotonic solution. Solubilization of the RBC membranes was performed using the non-ionic detergent octylglucoside OG (Sigma). The optical density (OD) of the RBC membranes as a function of the detergent concentration was measured using a UV/Visible spectrophotometer (Shimatzu PC6101) at 620nm. The absorbance wavelength was chosen in a region in which the change in the size of the RBC membranes would have a large effect on the OD due to the light scattering, and in which there is no absorption band for the proteins, lipids, or detergent.

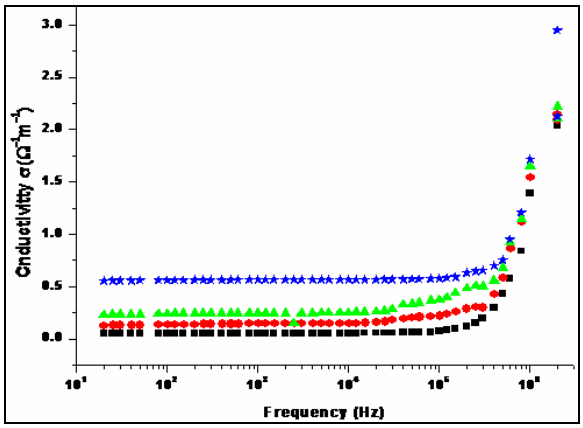

Fig. 4: Electrical conductivity σ as functions of the applied frequency in the range of 20Hz to 100 kHz for eggs that were not exposed (control) and those that were exposed to a magnetic field of 7.5G for different durations in minutes; (control, ■), (20 ●), (40 ▲) and (60*)

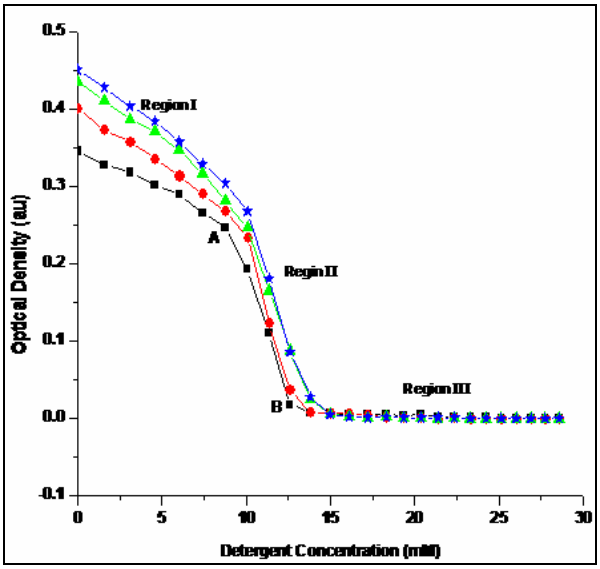

Fig. 5: Variation of the turbidity of RBCs as a function of the detergent concentration (mM) for blood collected from hatched chicks that were not exposed (control) and those that were exposed to a magnetic field of 7.5G during incubation for different durations in minutes; (control ■), (20●), (40▲) and (60*)

Table 1: The values of relaxation time τ, dielectric increment $\Delta\varepsilon = (\varepsilon_\infty - \varepsilon_o)$ and Cole- Cole parameter for all treated groups.

Exposure time (min)	Dielectric increment Δε	Relaxation time τ (ms)	Cole-Cole parameter
0 (unexposed)	(1.3±0.09) x10^6	3.5±0.2	0.15±0.05
20	(2.8±0.10) x10^6	2.6±0.2	0.33±0.05
40	(4.15±0.15) x10^6	1.8±0.08	0.56±0.055
60	(6.0±0.18) x10^6	1.4±.08	0.72±0.061

Values are Means ± SD**,** P-value <0.05 is significant

STATISTICAL ANALYSIS

Each value is expressed as mean and standard deviation (SD). One-way analysis of variance was used to compare each variable in the different studied groups. The Mann-Whitney U-test was used to determine the significant differences among values of different groups. For all statistical comparisons, p value<0.05 was considered significant.

RESULTS

Figs. 3 and 4 show the variation in the relative permittivity ε' and electrical conductivity σ, as a function of the applied frequency in the range of 20 Hz to 100 kHz for eggs that were not exposed (control) and those that were exposed to a MF of 0.75mT for different durations; control, 20, 40 and 60 minutes. The results of this study indicate a dielectric dispersion for Hb in the α-region. Moreover, the electrical conductivity (σ) of the samples is frequency-dependent, and increases as the exposure time increases. From the dispersion curve of Hb, the critical frequency (f_c) can be measured, and the relaxation time τ can be calculated from the equation $\tau=1/2\pi f_c$. The values of τ, the dielectric increment $\Delta\varepsilon = \varepsilon_\infty - \varepsilon_o$ and the Cole-Cole parameter for all groups are given in table 1.

Table 2: Detergent concentrations in the membrane bilayer at break points A and B for different exposure times.

Exposure time (min)	[Detergent] at break point A (mM)	[Detergent] at break point B (mM)
0 (unexposed)	9.25±0.15	12.75±0.23
20	9.5±0.2	13.3±0.23
40	10±0.2	14.0±0.23
60	10±0.2	14.35±0.23

Values are Means ± SD**,** P-value < 0.05 is significant

To identify any damage that might occur in the cellular membrane due to MF exposure, the solubilization of RBC membranes was performed, which may provide a clear picture on the interaction mechanisms of such a field with the biological membranes. Fig. 5 shows the variation in the turbidity of RBCs collected from the hatched chicks as a function of detergent concentration. The results indicate that the solubilization process of RBCs membranes passes through three regions, which termed region I, II and III. In region I, the detergent molecules begin to solubilize the surface proteins but are not incorporated within the bilayer (begin from zero detergent until break point A). In region II, the increasing detergent concentration causes a corresponding increase in the number of detergent molecules incorporated within the bilayer, which leads to solubilization of the membrane, cell rupture, and hemolysis (until break point B). In region III, the increased detergent concentration causes the formation of mixed micelles. Furthermore, the high concentration causes an increase in the detergent-to-lipid ratio and a consequent decrease in the average mixed micelle size. The calculated values of detergent concentration in the membrane bilayer at the break points A and B are given in table 2.

DISCUSSION

The dielectric results indicate that Hb exhibits dielectric dispersion in the frequency range employed in this study. This behavior has been identified as anomalous frequency dispersion and has been previously reported for various biological materials (Shafey *et al*., 2006; Ibrahim *et al*., 2008; Ghannam and Mady, 2012; Ghannam *et al*., 2002). The pronounced increase in σ for the exposed eggs is attributed to the increased Hb macromolecules surface charge density, which results from the formation of highly active molecular species. This speculation is supported by an increase in the relaxation time τ of the exposed groups which indicates an increase in the molecular size or the formation of a new cluster of cells. Moreover, the increase in τ will cause an increase in the dipole moment in the studied Hb groups because the dipole moment is directly proportional to the relaxation time.Consequently, a higher electric conductivity is expected as a direct result of the high surface charge density of the formed clusters during the cell growth.

Results from the RBC membrane solubilization tests confirmed the need for higher detergent concentrations to solubilize the exposed RBC membranes in comparison to the control, indicating that the RBC membranes were more rigid and less soluble in the treated eggs than in the

control group. This decrease in membrane mobility of the RBCs following MF exposure may be due to the disturbance of the ionic motion through the cellular membrane and/or changes in the molecular packing properties of the macromolecules that form the membrane as a result of MF exposure.

CONCLUSIONS

It is concluded that exposure of layer breeder eggs to a MF of 0.75mT at 50 Hz for up to 60min may produce an increase and/or redistribution of the surface charge density of Hb macromolecules, resulting from the formation of highly active molecular species. Moreover, a direct mobility loss of the RBCs in the blood of hatching chicks is occurred due to the eggs' MF exposure, which are induced during incubation prior to hatching.

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