

# Development disorders and increased mortality in chick embryos exposed to electromagnetic fields of 10 $\mu$ T and 30 Hz

Miguel López-Soler, Olga Roda-Murillo, M<sup>a</sup> Teresa Pascual Morenilla, Miguel Guirao Piñeyro, Indalecio Sanchez-Montesinos, José A. Roda Moreno

Department of Human Anatomy and Embryology, Neurosciences Institute, University of Granada, Granada, Spain

## SUMMARY

Study of the body weight, third-toe length, transversal and anteroposterior cranial diameters, brain weight and mortality of embryos of *Gallus domesticus* at 15 and 21 days of incubation showed that their exposure to electromagnetic fields of 10  $\mu$ T intensity and 30 Hz frequency accelerated their development and produced an increase in mortality at the end of incubation.

**Key words:** Chick embryo – Development – Magnetic fields

## INTRODUCTION

Major concerns are currently being expressed about the possibility that low-frequency electromagnetic fields (EMFs) may produce health disorders (Hug et al., 2006; Li et al., 2009). The studies published are contradictory, with some authors reporting that they are harmful and others finding no biological alterations. Epidemiological and experimental studies have related exposure to electromagnetic fields to an increased risk of adverse pregnancy outcomes and a higher miscarriage rate (Juutilainen et al., 1993; Delpizzo, 1994; Wilson et al., 1996; Li et al., 2002; Charter et al., 2006; Mezei et al., 2006). How-

ever, whereas some experimental studies have reported major developmental disorders in EMF-exposed chick embryos (Juutilainen et al., 1987; Martin, 1988; Ubeda et al., 1994), others have found no negative biological effects in chick embryos incubated under exposure to EMFs (Maffeo et al., 1984; Cox et al., 1993; Yao et al., 2007). With this background, the present experimental study was designed to contribute data on the possible action exerted by magnetic fields on embryonic development by studying their effects on the body weight, third-toe length, transversal and anteroposterior cranial diameters, brain weight, and mortality of chick embryos.

## MATERIALS AND METHODS

One hundred and sixty-five fertilized eggs of *Gallus domesticus* (Leghorn HR7 variety) were incubated at 37.8°C  $\pm$  0.4°C and a relative humidity of 60-70% in two model 65 Masalles incubators equipped with forced ventilation and automatic voltage (1 volt/h). One incubator contained a control group of 72 eggs and the other contained a treatment group of 93 eggs, which were exposed to a uniform magnetic field produced by two Helmholtz coils at an intensity of 10  $\mu$ T and frequency of 30 Hz.

On days 15 and 21 of incubation, embryos were extracted from each group for measurement of the following parameters: body weight, third-toe length, transversal cranial diameter (distance between the two external auditory openings), anteroposterior cranial diameter (distance between the bony ridge below the posteroinferior edge of the fontanelle and anterior root of crest), brain weight, and mortality. All these parameters are related to embryonic development.

A uniform magnetic field was generated at the incubation site using two identical Helmholtz coils of 1.40 cm diameter, each with 500 turns of 2 mm diameter copper wire, a resistance of 12 Ω, and self-induction of 0.6 H. All of the treated eggs were incubated within a magnetic field that varied by less than 2% from its central point. Exposure was continuous along embryonic development. The magnetic field is directly proportional to the intensity of current passing through the coil, and a high current is needed to supply them directly because of their high inductive impedance. This was resolved by supplying the coils through a circuit that incorporated a bank of condensers. The magnetic fields were measured using two digital Teslameters (Phyme model 13610.93 and Chauvin-Arnoux model C.A 40).

Body weight was measured with a Nettler PL200 digital balance.

## RESULTS

The results listed in Table 1 show that there were no differences in the body weight of the embryos with their yolk sac between the treated and control groups at 15 days of incubation. However, when the same embryos were weighed without their yolk sac the weight of the treated embryos was significantly higher than that of the controls. At 15 days, the brain weight of treated embryos was also significantly higher than that of controls.

At 21 days of incubation, after hatching, no significant differences were observed between the treated and control embryos in either brain weight or total body weight.

The skeletal parameters studied (third-toe length and transversal and anteroposterior cranial diameters) were significantly increased in the treated versus control embryos at 15 days of incubation (Table 2). However, these differences were not significant at day 21.

There was no significant difference in the mortality rate between the treated and control embryos at day 15 of incubation, but the treated embryos showed a significantly higher mortality rate versus controls at day 21 of incubation (Table 3).

**Table 1.** Different weights of chick embryos exposed to electromagnetic fields of 10 μT 30 Hz or used as controls.

	Day 15 of incubation			Day 21 of incubation		
	Controls		Treated	Controls		Treated
		Difference			Difference	
Weight with yolk sac (g)	26.188 ± 2.956 (n=20)	NS	27.981 ± 3.104 (n=20)			
Weight without yolk sac (g)	12.160 ± 1.007 (n=20)	P<0.001	13.884 ± 0.684 (n=20)	42.012 ± 4.408 (n=20)	NS	39.391 ± 3.790 (n=20)
Brain weight (g)	0.491 ± 0.028 (n=20)	P<0.001	0.568 ± 0.036 (n=20)	0.811 ± 0.061 (n=20)	NS	0.840 ± 0.040 (n=20)

Note. Ratios are means ± SD.

**Table 2.** Skeletal measurements in chick embryos exposed to 10 μT 30 Hz electromagnetic field or used as controls.

	Day 15 of incubation			Day 21 of incubation		
	Controls		Treated	Controls		Treated
		Difference			Difference	
Third toe length (mm)	10.233 ± 0.863 (n=20)	P<0.001	11.831 ± 0.689 (n=20)	17.745 ± 0.771 (n=20)	NS	17.696 ± 0.986 (n=20)
Transversal cranial diameter (mm)	10.259 ± 0.757 (n=20)	P<0.001	12.165 ± 0.717 (n=20)	14.765 ± 0.597 (n=20)	NS	14.920 ± 0.928 (n=20)
Anteroposterior cranial diameter (mm)	20.189 ± 0.565 (n=20)	P<0.001	22.606 ± 0.838 (n=20)	24.714 ± 1.075 (n=20)	NS	24.537 ± 1.476 (n=20)

Note. Ratios are means ± SD

**Table 3.** Mortality in chick embryos exposed to 10  $\mu$ T 30 Hz electromagnetic field or used as controls.

	Day 15 of incubation			Day 21 of incubation		
	Controls		Treated	Controls		Treated
		Difference			Difference	
Mortality (%)	4.762 (n=21)	NS	0 (n=20)	21.569 (n=51)	$P < 0.001$	54.795 (n=73)

Note. Ratios are %

## DISCUSSION

In this study, chick embryos exposed to an electromagnetic field of 10  $\mu$ T intensity and 30 Hz frequency showed a greater brain weight as compared with controls after 15 days of development, and their body weight was higher than that of the controls after removal of the yolk sac. This finding indicates a greater absorption of the yolk sac by the treated embryos.

The increased skeletal measurements recorded in the treated embryos at 15 days of incubation compared with the controls may result from the stimulation of bone formation by the EMF via the promotion of osteoblastic differentiation and/or activation (Yamamoto et al., 2003; Chiu et al., 2007).

According to the above findings, an acceleration of development was produced in the treated embryos, although this effect was not macroscopically observable at the end of the incubation period.

The very high mortality among treated embryos at the end of incubation suggests a possible relationship between the acceleration of development and biochemical, cytological and/or histological changes. Other authors have also found an increase in mortality after exposure to magnetic fields (Martin, 1988; Grandolfo et al., 1991; Veterány et al., 2001). These issues were not addressed by the present study but will be the subject of future research by our group.

## REFERENCES

- CHATER S, ABDELMELEK H, PEQUIGNOT JM, SAKLY M, RHOUMA KB (2006). Effects of sub-acute exposure to static magnetic field on hematologic and biochemical parameters in pregnant rats. *Electromagn Biol Med*, 25: 135-144.
- CHIU KH, OU KL, LEE SY, LIN CT, CHANG WJ, CHEN CC, HUANG HM (2007). Static magnetic fields promote osteoblast-like cells differentiation via increasing the membrane rigidity. *Ann Biomed Eng*, 35: 1932-1939.
- COX CF, BREWER LJ, RAEMAN CH, SCHRYVER CA, CHILD SZ, CARSTENSEN EL (1993). A test for teratological effects of power frequency magnetic fields on chick embryos. *IEEE Trans Biomed Eng*, 40: 605-610.
- DELPIZZO V (1994). Epidemiological studies of work with video display terminals and adverse pregnancy outcomes (1984-1992). *Am J Ind Med*, 26: 465-480.
- GRANDOLFO M, SANTINI MT, VECCHIA P, BONINCONTRO A, CAMETTI C, INDOVINA PL (1991). Non-linear dependence of the dielectric properties of chick embryo myoblast membranes exposed to a sinusoidal 50 Hz magnetic field. *Int J Radiat Biol*, 60: 877-890.
- HUG K, RÖÖSLI M, RAPP R (2006). Magnetic field exposure and neurodegenerative diseases: recent epidemiological studies. *Soz Präventivmed*, 51: 210-220.
- JUUTILAINEN J, LAARA E, SAALI K (1987). Relationship between field strength and abnormal development in chick embryos exposed to 50 Hz magnetic fields. *Int J Radiat Biol Relat Stud Phys Chem Med*, 52: 787-793.
- JUUTILAINEN J, MATILAINEN P, SAARIKOSKI S, LAARA E, SUONIO S (1993). Early pregnancy loss and exposure to 50 Hz magnetic fields. *Bioelectromagnetics*, 14: 229-236.
- LI DK, ODOULI R, WI S, JANEVIC T, GOLDITCH I, BRACKEN TD, SENIOR R, RANKIN R, IRIYE R (2002). A population-based prospective cohort study of personal exposure to magnetic fields during pregnancy and the risk of miscarriage. *Epidemiology*, 13: 9-20.
- LI P, MCLAUGHLIN J, INFANTE-RIVARD C (2009). Maternal occupational exposure to extremely low frequency magnetic fields and the risk of brain cancer in the offspring. *Cancer Causes Control* {Epub ahead of print}
- MAFFEO S, MILLER MW, CARSTENSEN EL (1984). Lack of effect of weak low frequency electromagnetic fields on chick embryogenesis. *J Anat*, 139: 613-618.
- MARTIN AH (1988). Magnetic fields and time dependent effects on development. *Bioelectromagnetics*, 9: 393-396.
- MEZEI G, BRACKEN TD, SENIOR R, KAVET R (2006). Analyses of magnetic-field peak-exposure summary measures. *J Expo Sci Environ Epidemiol*, 16: 477-485.
- UBEDA A, TRILLO MA, CHACON L, BLANCO MJ, LEAL J (1994). Chick embryo development can be irreversibly altered by early exposure to weak extremely-low-frequency magnetic fields. *Bioelectromagnetics*, 15: 385-398.
- VETERÁNY L, VETERÁNYOVÁ A, JEDLICKA J (2001). Effect of magnetic fields on embryonic mortality. *Cesk Fysiol*, 50: 141-143.
- WILSON BW, LEE GM, YOST MG, DAVIS KC, HEIMBIGNER T, BUSCHBOM RL (1996). Magnetic field characteristics of electric bed-heating devices. *Bioelectromagnetics*, 17: 174-179.
- YAMAMOTO Y, OHSAKI Y, GOTO T, NAKASIMA A, IJIMA T (2003). Effects of static magnetic fields on bone formation in rat osteoblast cultures. *J Dent Res*, 82: 962-966.
- YAO K, YU Y, WANG K, YE J, LU D, JIANG H (2007). Absence of effect of power-frequency magnetic fields exposure on mouse embryonic lens development. *Bioelectromagnetics*, 28: 628-635.