Folia biologica (Kraków), vol. **53** (2005), No 1-2

Effect of Electric Power Network Frequency Magnetic Field on Embryonic Development of *Ascaris suum* (Nematoda)

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Accepted January 25, 2005

KUŹNA-GRYGIEL W., GONET B., JABOROWSKA M., KOŁODZIEJCZYK L. 2005. Effect of electric power network frequency magnetic field on embryonic development of *Ascaris suum* (Nematoda). Folia biol. (Kraków) **53**: 101-105.

Fertilised *Ascaris suum* eggs were subjected to an alternating electromagnetic field of frequency 50 Hz and density 2 mT for 60 days. The developing embryos in both control and experimental cultures were examined daily under a microscope. The experiment resulted in an accelerated rate of embryogenesis in the eggs incubated in the electromagnetic field, higher rates of malformed embryos as well as much higher mortality rate of L2 larvae.

Key words: Electromagnetic fields, eggs, embryogenesis, *Ascaris suum.*

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An increasing intensity of magnetic fields produced by electric power networks is one of the consequences of human interference in the environment. As research has shown, artificially created magnetic (electromagnetic) fields disturb the geomagnetic field, which affects sense of direction in many living organisms, be it unicellular organisms (BLAKERMORE *et al.* 1980), fish (FORMICKI *et al*. 1999), birds (GOULD 1982), or mammals (MATHER & BAKER 1981).

In recent years, a number of research centres have carried out investigations on the effects of static or alternating magnetic fields on various stages of animal ontogenesis. It has also been demonstrated that embryonated organisms are more susceptible to magnetic fields than mature ones (WINNICKI *et al*. 1996). So far, the effects of magnetic fields have been studied in terms of embryonic development chiefly in insects (RAMIREZ *et al*. 1983; CAMERON *et al*. 1993) and various vertebrate species (JUUTILAINEN *et al*. 1986; BERMAN *et al*. 1990; FORMICKI *et al*. 1999; FORMICKI & WINNICKI 1998).

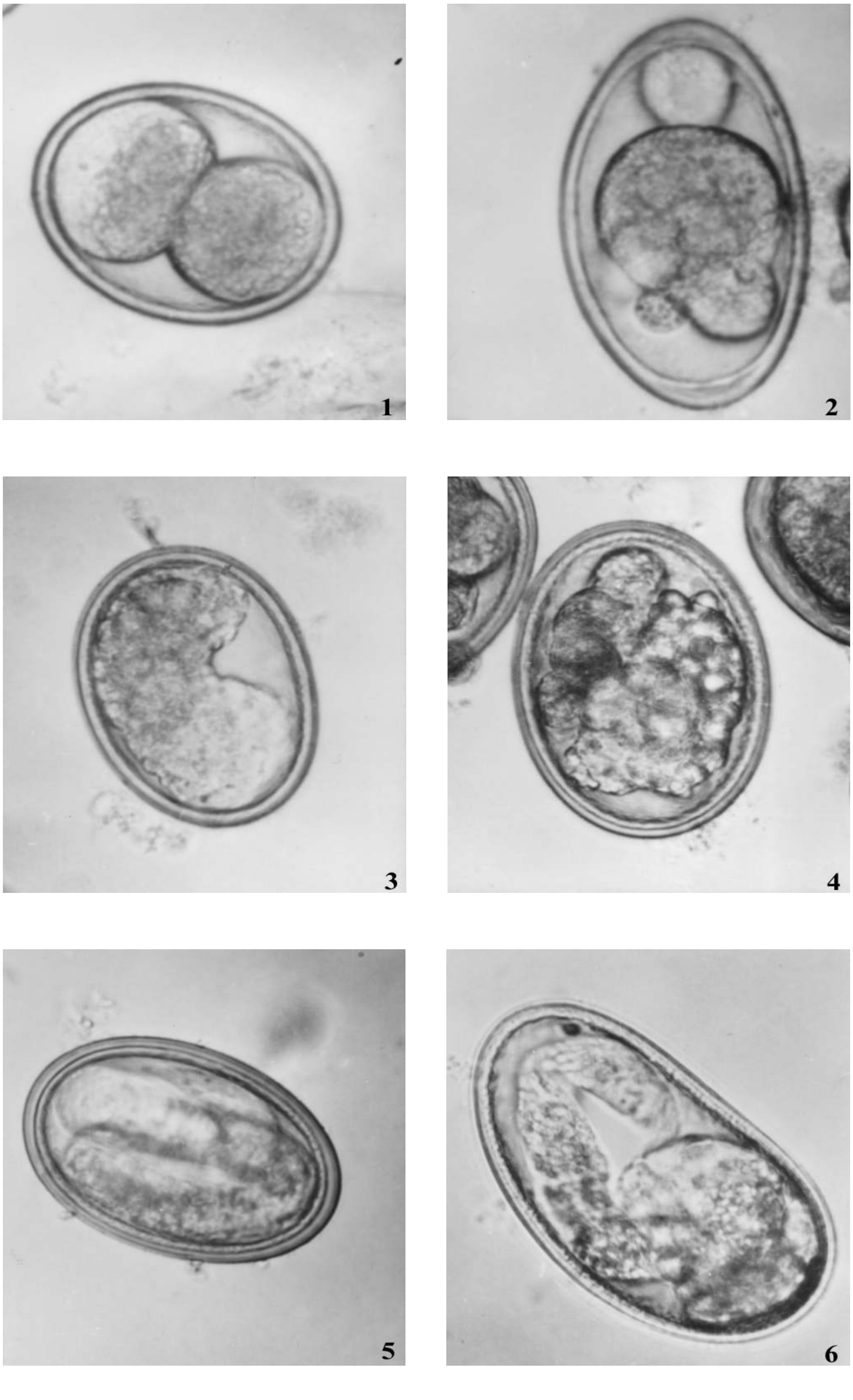
Little is known about the effects of magnetic fields on embryogenesis of invertebrates. Parasitic geohelminths, such as *Ascaris suum* whose egg have transparent egg shells making it possible to observe individual stages of embryonic development under a microscope, are particularly useful for such studies.

The aim of this study was to evaluate the effect of a slowly alternating electric power frequency magnetic field on the embryonic development of *Ascaris suum*.

Material and Methods

The material comprised fertilised *Ascaris suum* eggs, which had been collected from the rear fragment (about 2 cm) of the uteri of 50 mature females. The roundworms had been collected from intestines of pigs slaughtered at the Meat Processing Plant in Szczecin.

Egg cultures, three control and three experimental treatments, were performed in petri dishes at 21-22°C. Collected eggs were placed in dishes with 15 ml of Ringer solution of pH 7.2. There were approx. 3000 eggs in 1 ml of the suspension. The experimental cultures were placed under an alternating power network frequency magnetic field of 50 Hz, produced by Helmholtz coils. Field induction was 2 mT in the volume of the petri dishes. The control cultures were in the range of this magnetic field. Microscopic observations of embryonic development were carried out every



Figs 1-6. Fig. 1. Regular zygote division in a control *Ascaris suum* egg, x 1000. Fig. 2. Unequal divisions of blastomeres in an *Ascaris suum* egg incubated in an electromagnetic field, x 1000. Fig. 3. Egg of *Ascaris suum* at the gastrula stage from the control culture, x 1000. Fig. 4. Malformed gastrula in an *Ascaris suum* egg cultured in an electromagnetic field, x 1000. Fig. 5. Egg of *Ascaris suum* containing L2 larva from the control culture, x 1000. Fig. 6. Malformed L2 larva in an *Ascaris suum* egg cultured in na electromagnetic field, x 1000.

Table 1

Comparison of developmental rate of *Ascaris suum* eggs in control cultures (C) and in the electromagnetic field (EMF)

Day of culture:		0 2 4 6 8 10 12 14 15 16 18 21 23 25 28 30.....................60
Zygote	C	………………………….........…..
	EMF	……......…………
Cleavage	C	...………………… ↑ B (75%) ……...
	EMF	………….…. ↑ B (82.5%) ….....…..
Gastrulation	C	………………......... ↑ G (78.5%) ….
	EMF	………..…… ↑ G (85.2%) ………….
L2 larvae	C	…………………………… ↑ L (100%) ……...............…..
	EMF	……………… ↑ L (100%) ………………………......................

↑ – the highest percentage of *Ascaris suum* eggs
B – blastula
G – gastrula
L – larvae

day at the same time for 60 days. *A. suum* eggs are known to develop asynchronously. Therefore, each day, 0.1 ml of each culture was sampled and 200 randomly collected eggs in each sample were classified according to their stage: (I) zygote, (II) cleavage, i.e. from two blastomeres (Fig. 1) to blastula, (III) gastrula (Fig. 3), and (IV) L2 larva (Fig. 5). Moreover, in each stage (I-IV) the percentage of eggs with dead and malformed embryos was determined. In order to test the viability of embryos, the eggs were stained with iodine solution (ZAMA & VISUVALINGAM 1967), for which the shells of eggs containing dead embryos are permeable.

Results

A comparison of the rates of *Ascaris suum* development in experimental cultures placed in the electromagnetic field, and in control cultures, is presented in Table 1. In the control cultures, the first zygote divisions were observed on day 4 of incubation (Fig. 1), while in those subjected to the electromagnetic field, already on day 2. In the control cultures, the last eggs of *A. suum* at the zygote stage were found on day 15, whereas in the experimental cultures, such stages were observable until day 10 only. In the control cultures, a majority of eggs (75%), attained the blastula stage on day 14 of development, while in the cultures in the EM field, 83% of eggs were recorded as blastula as soon as on day 10, i.e. 4 days earlier than in the control cultures. Gastrulation was accelerated by one day in the experimental cultures as compared with the control. Namely, in the control cultures most eggs in the gastrula stage were found on day 17 (Fig. 3), while in the experimental – on day 12 of incubation. In the control cultures, developing eggs reached the stage of L2 larva after 26 days, while in the experimental cultures – after 19 days. Therefore, the formation of larvae in the experimental cultures was extended by another 2 days. On the whole, the entire embryonic development in the experimental cultures, i.e. from zygote to L2 larva, was shorter by 7 days compared to the control cultures.

Morphological abnormalities of *Ascaris suum* embryos are shown in Figs 2, 4 and 6, and the prevalence of such malformations is presented in Table 2.

During cleavage, experimental culture eggs showed about a 5-fold higher percentage of eggs with malformed embryos. Deformities in this period of development were manifested in the form of uneven divisions of zygotes and blastomeres

Table 2

Prevalence of eggs with deformed embryos and dead eggs in control cultures (C) and cultures in the electromagnetic field (EMF)

Developmental stage	Culture	Eggs with deformed embryos	Eggs with dead embryos
Cleavage (zygote→ blastula)	C	0.7%	0.2%
	EMF	3.4%	1.2%
Gastrulae	C	0.9 %	0.4%
	EMF	1.5%	1.5%
2 larvae (32 day culture)	C	0.2%	0
	EMF	2.7%	1.7%
L2 larvae (60 day culture)	C	0.2%	0.4 %
	EMF	3.4%	9.4%

(Percentages calculated from mean numbers of eggs with deformed or dead embryos from three cultures).

(Fig. 2). In the gastrula stage, the prevalence of defects was much lower than during the cleavage stage. Abnormal gastrulae were deformed and were composed of cells of different sizes (Fig. 4). Among 32 and 60 day-old larvae the percentage of malformations was similar, which most frequently resulted from partial differentiation of the gastrula (Fig. 6).

The percentage of dead embryos in the control cultures during cleavage, gastrulation, and in 32-day old larvae was similar. In the 60-day cultures, the percentage of dead larvae was higher by more than 9%. In many eggs, dead and decomposing larvae were found.

Discussion

The results of this study have demonstrated that the electromagnetic field of a power supply network (50 Hz, 2 mT) accelerates the rate of embryonic development and elevates the incidence of developmental defects in *Ascaris suum*. Changes in embryonic developmental rate may be a result of disturbances in each stage of the cell cycle in dividing cells.

In the light of these results, it can be presumed that an electromagnetic field causes perturbations to the DNA repair mechanisms, which can lead to aggregated damage and developmental defects in embryos. Recent studies by TAKASHIMA *et al.* (2003) have shown that a 30 mT magnetic field suppresses DNA repair processes in yeast. MIYAKOSHI *et al.* (2000) have recorded increased rates of DNA damage in human glioma MO54 cells in fields of 5-400 mT. The results of contemporary studies on an effect of electromagnetic field on cell cycle and cell proliferation are ambiguous.

CAMERON *et al.* (1993) have shown the suppressive effect of a 60 Hz magnetic field on the synthesis of histones and embryonic cell proliferation in sea-urchin morulae. CRIDLAND *et al.* (1999) have revealed an extended G1 phase of the cell cycle in a culture of human fibroblasts under the impact of a magnetic field of 50 Hz, 20-200 μT. TOFANI *et al.* (2002) have demonstrated that 5.5 mT magnetic field had a suppressive effect on cancer growth in mice, intensification of apoptosis in cancer cells, as well as on reduced immunoreactivity of the p53 protein. It may be presumed that accelerated embryonic development in *Ascaris suum*, and thus faster divisions of embryonic cells, may be associated with magnetic field-induced stimulation of protein biosynthesis, which has been demonstrated by BLANK and GOODMAN (1997).

Also, the high proportion of *Ascaris* embryos with developmental defects that we found may have resulted from a mutagenic effect of the applied electromagnetic field. Other studies, carried out on chicken embryos, have shown the mutagenic effect of 100-Hz magnetic fields of 1-13.9 μT (DELGADO *et al.* 1982; UBEDA *et al.* 1983). Similarly, a genotoxic effect of a 50 Hz magnetic field of 1 mT was observed by SIMKÓ *et al.* (1998) in human amniotic cells. ZHANG *et al.* (2003) found an influence of a strong static magnetic field on induction of mutations in *Escherichia coli* through intensified production of superoxide radicals. On the other hand, investigations by STRONATI *et al.* (2004) on an effect of a 50 Hz, 1 mT magnetic field on *in vitro* cultured human blood cells have not confirmed genotoxic effects. The authors found only slightly reduced cell proliferation.

These hypotheses require confirmation in further investigations, especially in markers of disturbances of the cell cycle e.g. mutations of the p53 protein.

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