Effect of 50 Hz magnetic field on chicken embryo development and course of hatching

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Abstract
The effect of additional extremely low frequency (ELF) magnetic field (50 Hz) on the development of chick embryo was investigated. The experiment was carried out in three variants for induction values 15, 10 or 5 μT. Magnetic field (MF) in the experimental incubator was generated by a set of three Helmholtz coils. The following analyses were performed: embryopathological analysis; evaluation of the hatched chicks for quality; analysis of the course and synchronization of hatching Early embryo mortality in groups exposed to MF 10 and 15 μT compared to the control was higher by about 3 percentage points. Simultaneously, in these groups the hatching processes accelerated, but the quality of hatched chicks was much lower.

Keywords: chick embryo, extra law frequency magnetic fields, hatch synchronization, embryo development, malformation

Introduction

In the process of evolution, plants and animals have adapted themselves to the presence of some types of natural electromagnetic field (EMF) such as geomagnetic fields, atmospheric radiation, etc. [1], [2], [3]. The intensity of the natural geomagnetic field is 130 V/m for the electric component and 30-70 μT for the magnetic component [4]. Currently the primary role in forming the electromagnetic environment of the Earth is played by fields resulting from the progress of civilization, i.e. "artificial" electromagnetic fields [5], [6], [7]. Among them, special attention, due to their widespread character, must be given to fields generated by the power network current and the equipment relying on it [7]. It seems that of the two components, the magnetic component is more important biologically than the weakly penetrating electric component. This is why experimental methodology allows treating EMF as magnetic field (MF) if the electric component does not considerably exceed the electric component of the geomagnetic field [8], [9].

The role of electromagnetic and magnetic fields as an environmental factor affecting hatching results in house incubators was pointed out by [10] and [11]. This is confirmed by the results of laboratory studies, which point to disturbances in chick embryogenesis (abnormal formation of notochord and spinal cord) under the influence of magnetic field of 50–60 Hz and 1–14 μT [9], [12], [13]. Many authors suggest that the embryo is most sensitive to MF only during the first 24–52 hours of incubation [9], [14], with no disturbances observed in the later stages of embryogenesis [15]. However, these results are in disagreement with [16] and [17] who claims that the normal development of embryo in terms of teratogenic changes should be evaluated at the end of hatching or in late embryogenesis rather than at the first week of embryogenesis.

In light of the above observations, it seemed advisable to undertake further studies concerning the effect of the EMF magnetic component on the development of chick embryos during the whole incubation process.

Materials and Methods

Biological materials

A total of 2160 hatching eggs from the same parental flock of the broiler chicken line between 30 and 43 weeks of age were used in three experiments in triplicate (Tab. 1). Before incubation the eggs were storage 4 day and fumigated with formalin vapours. In one repetition 240 eggs, randomly divided to experimental and control incubator were used.

Incubation parameters

Microclimatic parameters of incubation were 50% relative humidity and 37.8ºC from 1 to 18 days of incubation and 60–75% relative humidity and 37.2ºC from 19 to 21 days of incubation. Embryos of experimental group were exposed to generated by Helmholtz coils the magnetic field (f=50 Hz) with magnetic flux density (B): 5, 10 or 15 μT during the total incubation period (Tab. 1)

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Table 1. Experimental scheme. The chicken embryos of experimental were exposed to magnetic fields generated by Helmholtz coils during the total incubation period. These fields were called "additional magnetic field" (AMF) in contradistinction to magnetic field emitted by standard electric equipment of the incubator *f* – frequency; B – magnetic flux density.

	Experimental group	Control group
Experiment 1	AMF: *f* =50 Hz, B=15 μT 360 eggs (3 repetitions ´120 eggs)	no AMF 360 eggs (3 repetitions ×120 eggs)
Experiment 2	AMF: *f* =50 Hz, B=10 μT DPM; 360 eggs (3 repetitions ´120 eggs)	no AMF 360 eggs (3 repetitions ×120 eggs)
Experiment 3	AMF: *f* =50 Hz, B=5 μT; 360 eggs (3 repetitions ×120 eggs)	no AME 360 eggs (3 repetitions ×120 eggs)

Incubators and electromagnetic fields generator

Eggs were incubated in two Masalles type 65 DIGIT incubators. The experimental incubator was fitted with a set of three EMF generators composed of three parallel φ 51 cm Helmholtz coils, which generated relatively uniform EMF inside the incubator. Further on in the paper, the field emitted by the generators is called additional magnetic field (AMF) in contradistinction to magnetic field emitted by standard electric equipment of the incubator. To suppress the electric component, the coils were screened with earthed copper sheet. The control group was hatched in the standard incubator. Values of EMF induced by the electric equipment of the incubators did not differ between the experimental and control incubators. The induction of magnetic field was adjusted in different variants of the experiment by changing the intensity of current flowing through the coils. Values of the magnetic and electric components of EMF in incubators were measured with Feldmeter and Geo-Graph BPM 3009 (Bio-Physik Mersmann).

Hatch analyses

The course of hatching was monitored from 460 hours of incubation at 4-hour intervals for the number of hatched chicks. This served as a basis for plotting a hatching curve for cumulated percent values and for setting the limits of the second stage of hatching (time between hatching of 10 and 90% of chicks).

The all unhatched eggs were anatomopathological analized and the stage of development, embryo malpositions; the rate of retraction of yolk sac into body cavity; blood vessel lesions and abnormal foetal circulation; the percentage of albumen use and developmental anomalies were determinated. The health of hatched eggs were evaluated by common condition and navel quality. The hatching results were considered to embryonated eggs.

Statistical analizes

The degree of synchronization was evaluated and the course of hatches compared based on regression lines plotted for this stage following the method of [18]. The coefficient *b* of simple regression plotted for the second stage of hatching was taken as a measure of the synchronization degree. To limit the influence of non-experimental factors (e.g. flock age) during the interpretation of results, the effects of different fields on the course of hatching were compared based on differences between the experimental and control groups. The results of hatching were analyzed statistically with the significance test u for the difference of two fractions with large samples and with the $\chi 2$ test.

Results and Discussion

There is still a difference of opinion as to the stage of development in which the embryo is most sensitive to the magnetic field. [19] claim that the developmental response of the embryo depends on the shape and intensity of the magnetic field. An analysis of embryo mortality in the present experiment reveals that the age of embryo mortality changes together with decreased induction values of AMF [Tab. 2]. Chick embryos incubated at 15 μT died mostly during the first 6 days of hatching (58%), while the reduction in incubation value was accompanied by increasingly late deaths of embryos. Embryo deaths were 51% in AMF of 10 μT and 31% in AMF of 5 μT in peak I, and 52% between 18 and 21 days of incubation. This means that in a 5 μT magnetic field, the proportions in the height of particular peaks are reversed in relation to the distribution of mortality considered as physiological by [20].

Table 2. Hatching results [% of embryonated eggs]

	Experiment 1				Experiment 2				Experiment 3			
	AMF -15 µT		Control group		AMF -10 µT		Control group		AMF -5 µT		Control group	
	number	%	number	%	number	%	number	%	number	%	number	%
Setted eggs	360	-	360	-	360	-	360	-	360	-	360	-
Embryonated eggs	335	100.00	343	100.00	327	100.00	329	100.00	320	100.00	304	100.00
I peak of mortality	29	8.73^{abCDE}	20	5.83^{af}	18	5.48^{bg}	13	3.95^{C}	9	2.81^{Dfg}	11	3.62^{E}
Interpeak period	3	0.90	7	2.04^{a}	4	1.23	2	0.62^{a}	5	1.56	2	0.66^{a}
II peak of mortality	18	5.42	19	5.54	13	3.99	14	4.24	15	4.69	14	4.61
Dead embryos, days 1-21	50	15.06^{aBCD}	46	13.41^{efg}	35	10.70^{a}	29	8.81^{Be}	29	9.06^{Cf}	27	8.88^{Dg}
Hatched chicks	285	84.94 ^{aBCD}	297	86.57^{efg}	292	89.30^{efg}	300	91.19^{Be}	291	90.94^{Cf}	277	91.12^{Dg}
Culled chicks	51	15.36^{aB}	48	13.95^{C}	53	16.19^{dE}	36	10.88^{ad}	46	14.34^{F}	26	8.55^{BCEF}
Healthy chicks	230	69.58^{AbC}	249	72.63^{DE}	239	73.11^{G}	264	80.30^{ADf}	245	76.56^{bh}	251	82.57^{CEGh}

small letters – values in the row marked with the same small letters differ significantly ($p \leq 0.05$)
CAPITAL LETTERS – values in the row marked with the same capital letters differ highly significantly ($p \leq 0.01$)

It is suggested that the differences in the distribution of mortality depending on the value of the active field are related to apoptosis. It was noted that especially during the ontogenesis, the embryo naturally loses many cells, thus eliminating mutated and useless cells. This provides more favorable conditions for development of cells with a proper receptor programmed. Among the apoptosis stimulating factors are reactive free oxygen radicals, the reactivity of which increases the magnetic field [21], [22]. Therefore it cannot be ruled out that the intensity of this process will depend on the field induction value. It is possible that a field of higher induction stimulates apoptosis and mortality, during the first 48 hours of incubation, of weak embryos with anomalies and delayed embryogenesis. While a field of lower induction would not be expressed so strongly and the embryos with small anomalies would stand a chance of further development [23]. The latter field would either stimulate the repair processes (apoptosis of damaged cells only) or the natural embryo selection would occur as late as between 18 and 21 days of incubation.

In the present experiment, an analysis of developmental anomalies of embryos dead during the last two days of incubation showed significant differences between the experimental and control groups only for percentage of embryo malpositions in eggs. It should be pointed out, however, that the degree of these defects did not significantly differ among the individual variants of the experimental groups and ranged from 3.06% to 4.06% in relation to PD eggs [Tab. 3].

The greatest effect on the final results of hatching was exerted by chick culling, which in relation to PD eggs ranges from 14.38 to 16.21% in the experimental groups and from 8.81 to 13.41% in the control groups [Tab. 2]. The most frequent reason for culling newly hatched chicks was identification of grey umbilicus defect. There was no incidence of omphalitis, which is a frequent cause of culling in hatches with abnormal synchronization of hatching [24], [25].

Table 3. Incidence of developmental anomalies [% of fertilized eggs]

	Experiment 1				Experiment 2				Experiment 3			
	AMF -15 µT		Control group		AMF -10 µT		Control group		AMF -5 µT		Control group	
	number	%	number	%	number	%	number	%	number	%	number	%
Embryonated eggs	335	100,00	343	100,00	327	100,00	329	100,00	320	100,00	304	100,00
Malformation	2	0.60	2	0.58	2	0.61	3	0.91	5	1.56	4	1.32
Underdevelopment	1	0.30	3	0.87	2	0.61	1	0.31	0	0.00	1	0.32
Unretracted yolk sac	7	2.11	6	1.75	6	1.83	4	1.22	6	1.88	3	0.95
Shell membrane hemorrhage	0	0.0	1	0.29	0	0.00	0	0.00	0	0.00	0	0.00
Malpositions	11	3.31^{a}	17	4.96^{bc}	10	3.06^{d}	2	0.61^{acde}	13	4.06^{ef}	3	0.99^{bf}

small letters – values in the row marked with the same small letters differ significantly ($p \leq 0.05$)

Table 4. The course and synchronization of chick hatching relative to variant of the experiment

Variant of experiment	Course of hatching				Hatching synchronization							
	first hatching	X_{mean}	s	last hatching $H_{10\%}$	II stage of hatching process						Degree of synchronization	
					s	$H_{90\%}$	s	H_{II}	s			
	[hours of incubation]				[hours of incubation]				[h]		b	s_b
Experimental groups												
Exp. 1 AMF – 15 µT	482	499.55**	6.30	510	490.99	2.00	507.73	2.64	16.74	1.29	5.01	0.51
Exp. 2 AMF – 10 µT	482	500.18*	6.33	518	491.71	0.62	509.34**	0.12	17.63	0.51	4.71*	0.16
Exp. 3 AMF – 5 µT	478	499.80**	6.26	518	491.99*	0.23	507.91*	0.54	15.92*	0.42	5.19*	0.16
Control groups												
Exp. 1	486	502.86	6.45	518	494.37	1.15	511.18	2.32	16.81	0.86	4.99	0.44
Exp. 2	478	501.67	7.65	518	492.63	0.43	512.32	1.19	19.69	1.18	4.07	0.15
Exp. 3	482	502.49	7.29	522	492.99	0.25	511.98	1.57	19.00	1.59	4.42	0.21
Differences between experimental and control groups												
Exp. 1 D-K	-4	-3.32^{a}		-8	-3.38^{CD}		-3.45		-0.07^{ef}		0.02^{hi}	
Exp. 2 D-K	-4	-1.51^{ab}		-0	-0.92^{C}		-2.98		-2.06^{eg}		0.66^{h}	
Exp. 3 D-K	-4	-2.69^{b}		-4	-1.00^{D}		-4.07		-3.08^{fg}		0.77^{i}	

Differences between experimental and control groups:
*- significant at p≤0.05;
** - highly significant at p≤0.01;
Differences between experiments:
small letters – groups marked with the same small letters differ significantly (p≤0.05)
CAPITAL LETTERS – groups marked with the same capital letters differ highly significantly (p≤0.01)

Hatching synchronization is a natural tendency for hatching at the same time. It takes place through regulation (acceleration or delay) of the growth rate probably in response to vibrations, ultrasounds and sounds of varying frequency generated by other hatching chicks . In all the groups subjected to magnetic field, acceleration in the growth of embryos was found, manifesting itself in the earlier onset of the hatching process [Tab. 4]. However, this phenomenon was not always equivalent to the increase in the hatching synchronization degree. A significant increase in the synchronization process was noted in hatches exposed to 5 and 10 μT fields, but this tendency was observed for a 15 μT field. [13] suggested that earlier hatching of a group of chicks can result from a genetically conditioned, higher sensitivity to magnetic field. In order to obtain the heterosis effect in a commercial flock of broilers the reproductive lines have to be genetically distant, their offspring are characterized by large genotypic variation. Therefore it cannot be ruled out that a certain combination of genes may cause a special excitation of the embryo growth under the influence of additional magnetic field. This hypothesis is supported by the findings of [25] who found significant differences in the degree of embryo growth stimulation in magnetic field depending on the origin of one of two different flocks. While other authors reported [26], [27], [28] [29] that electromagnetic field accelerates growth avian embryos in relation to the control group, but this was accompanied by markedly lower viability. In looking for reasons behind this phenomenon, the authors indicate that the response of organism to the field depends not only on its intensity and length of exposure, but also on the time of exposure and sensitivity of individual organs.

Conclusions

Summarizing, chick embryo seems sensitive to weak extremely low frequency electromagnetic fields. This factor can induce early embryo mortality but other side accelerate the embryo development.

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