Effects of Low Frequency Magnetic Fields on Chick Embryos. Dependence on Incubation Temperature and Storage of the Eggs

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Chick embryos were exposed to sinusoidally oscillating 100 Hz magnetic fields during their first two days of development. The magnetic field strength was 1 A/m. Incubation temperatures of 36.3, 37.0, 38.0 and 38.5 °C were used and the duration of the storage of the eggs before incubation was varied from 1 hour to 4 days. After the incubation, the embryos were examined for abnormalities. When the temperature was 36.3 or 37.0 °C and the eggs were stored for one day or less, the effect of the magnetic field was statistically significant. In these conditions, the percentage of abnormal control embryos was low, 8% in 36.3 °C and 5% in 37.0 °C. In the exposed groups the corresponding percentages were 23% (36.3 °C) and 25% (37.0 °C). However, higher temperature and storage of the eggs for 3 to 4 days increased the percentage of abnormal embryos in both the exposed and control groups. The difference between the exposed and control embryos was not significant in these conditions. The results demonstrate the importance of the handling of the eggs in this kind of experiments.

Introduction

Increased number of abnormalities has been reported in chick embryos after exposure to weak pulsed magnetic fields at pulse frequencies 10, 100 and 1000 Hz [1, 2]. Similar effects were found in our experiments with sinusoidal, rectangular and pulsed waveforms [3] and several frequencies between 1 Hz and 100 kHz [4]. However, Maffeo *et al.* [5] reported that chick embryo development was not affected by pulsed low frequency magnetic fields. The difference between these results may partly be due to different magnetic field intensities. In our experiments, the minimum r.m.s. magnetic field strength required to produce embryological effects was about 1 A/m [3]. Maffeo *et al.* expressed the field strengths as peak values, which means that their r.m.s. values were much lower. They used peak values of 1.2 μT or 12 μT during the rectangular pulse. Pulse duration was 0.5 ms and pulse frequency 10, 100 or 1000 Hz. Thus, the r.m.s. field strength was more than 1 A/m (= 1.26 μT) in only one of the six field strength/frequency combinations used by Maffeo *et al.* However, their results suggest no effect on embryological development with this 12 μT/1000 Hz field.

As some of our unpublished experiments suggested that there could be considerable variations in the sensitivity of the embryos to the magnetic fields, we decided to study whether small variations in the incubation temperature and differences in the length of the storage of the eggs before incubation could explain the differences between the results of various experiments.

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Materials and Methods

Fertilized eggs of the breed Mäkelä 16 (a variety of white leghorn) were obtained from a farm specialized to produce chickens and fertilized eggs (Ollilan siitoskanala Ky, Hirvilahti, Kuopio). The eggs were incubated in a room with temperature and humidity control and forced air circulation. The temperature was measured continuously with a Ni-100 probe and recorder. The functioning of the humidity control was checked daily with a Humicap (Vaisala, Finland) probe. There were short-term variations within ±0.5 °C in the recorded temperature. These variations were not seen in the reading of a bulb thermometer with a heat capacity larger than that of the Ni-100 probe. As the heat capacity of the eggs is still larger, it can be concluded that their internal temperature was practically constant.

The eggs were exposed to a sinusoidally oscillating magnetic field during the whole incubation period. The r.m.s. field strength was 1 A/m and the frequency was 100 Hz. The exposure system has been described in detail elsewhere [3]. Briefly, it consisted of a function generator, a power amplifier and coils

with a rectangular cross section. Four identical coils were used with ten eggs in each. The magnetic field strength inside the coil was calculated as described by Saali *et al.* [6]. Calculations and measurements showed that the homogeneity of the field was better than ±2% within the volume occupied by the embryos. The orientation of the eggs was vertical, with the air-space end up. Also the orientation of the magnetic field lines of force was vertical. The control eggs were placed in similar carboard holders as the exposed eggs. The background (50 Hz) magnetic field strength at the site of the control eggs was about 0.015 A/m, measured with a calibrated coil.

Four independently collected groups, 20 eggs in each, were used per experiment. Each group was divided to 10 exposed and 10 control eggs. Two of the egg groups were collected the day before the experiment (stored at 15 °C at the farm), and the other two were collected in the morning immediately before the experiment. The age of the eggs was 1 to 25 hours when the incubation was started. All experiments were started at about 12 o'clock. Four similar experiments were done with four different temperatures. The average temperatures (from the temperature recorder) were 36.3 °C, 37.0 °C, 38.0 °C and 38.5 °C. In order to get embryos at about the same stage of development, longer incubation times were used in lower temperatures. The duration of the incubation was 50 h at 38.5 °C, 52 h at 38.0 °C, 55 h at 37.0 °C and 57 h at 36.3 °C. Two additional experiments were done in order to study the effect of long storage. The eggs were stored at room temperature (about 20 °C) for 3 days. In addition, part of these eggs had been stored for 1 day at 15 °C at the farm. Thus, the total storage time was 3 or 4 days. The incubation temperatures were 37.0 °C and 38.0 °C, and the incubation times 52 h and 55 h, respectively.

For blind analysis of the embryos, the eggs were coded with random numbers in the beginning of the incubation. After the incubation, the eggs were opened in physiological saline and the embryos were removed onto a watch glass. The embryos were observed in transmitted light under a stereomicroscope, and classified as normal or abnormal as described by Juutilainen *et al.* [3]. In addition, the severity of the malformations was classified as follows:

MILD: Any kind of mild malformation.

SEVERE: The head and the anterior part of the neural tube strongly abnormal. Somites are present, posterior part of the embryo can be normal.

STRONGLY RETARDED: Development apparently disturbed at an early stage. The embryo is an almost totally disorganized mass of cells with no somites.

For further analysis of the type of the malformations, a short written description was made of every abnormal embryo. The developmental stage of each embryo was determined according to the Hamburger and Hamilton [7] scale, except for the strongly retarded embryos, whose developmental stage could not be determined. No histological analysis of the embryos was made, since the aim of these experiments was only to evaluate the usefulness of the observation of whole 2-day chick embryos as a rapid and simple test for studying the effects of magnetic fields on development.

All tests of statistical significance were done with the chi-square test for fourfold tables.

Results

The frequency of abnormal embryos increased with increasing temperature both in exposed and control eggs (Table I). The frequency was always

Table I. The frequency of abnormal embryos in the eggs stored for 1 to 25 h before incubation.

T [°C]	Control	Exposed [H = 1 A/m]	Difference (exposed-control)	p
36.3	3/ 40 (8%)	9/ 39 (23%)	6 (15%)	< 0.1
37.0	2/ 37 (5%)	9/ 36 (25%)	7 (20%)	< 0.05
38.0	6/ 36 (17%)	12/ 37 (32%)	6 (15%)	N.S.
38.5	9/ 38 (24%)	15/ 40 (38%)	6 (14%)	N.S.
Σ	20/151 (13%)	45/152 (30%)	25 (17%)	< 0.001

T = temperature during incubation; p = significance of the difference between exposed and control embryos; N.S. = not significant.

higher in the exposed embryos and the absolute difference between exposed and control embryos was independent on temperature. This suggests that the effects of temperature and the magnetic field exposure are additive and independent on each other. The statistical significance of the magnetic field effect decreased with increasing temperature, because the relative difference between exposed and control embryos decreased.

No significant difference between control and exposed embryos could be shown in the eggs stored for 3 to 4 days before incubation (Table II). This is due to the high percentage of abnormalities in the control embryos. The combination of long storage time and high temperature had the most dramatic effect: In the group incubated at 38 °C, more abnormalities were found in the controls than in the exposed eggs. Also very short storage of 1 to 4 h seemed to produce slightly more abnormalities than storage for 20 to 25 h (data not shown, the difference is not significant). This is consistent with the belief of commercial chicken producers that best results are obtained when the eggs are stored for one day before starting the incubation (personal communication, Mrs. Anja Venäläinen, owner of Ollilan siitoskanala Ky).

Both "mild" and "severe" malformations appeared to be increased in the magnetically exposed embryos (Table III). However, the "strongly retarded"-type abnormality was associated with long storage time and high incubation temperature, but not with exposure to magnetic field.

The analysis of the type of the abnormalities shown in Table IV was made on the basis of the written descriptions of the abnormal embryos. The "strongly retarded" embryos were excluded from this analysis because of their totally disorganized appearance. "Abnormalities in the neural tube" does not exclude abnormalities in other organ systems (somites, vessels, heart). They were included in this group because they were far less common and never occurred without simultaneous defects in the neural tube, which was therefore thought to be the primary target organ of the magnetic field effect [3]. Abnormalities in the neural tube were very significantly increased in the embryos exposed to the magnetic field. Another abnormality that seemed to be associ-

Table II. The frequency of abnormal embryos in the eggs stored for 3 to 4 days before incubation.

T [°C]	Control	Exposed [H = 1 A/m]	Difference (exposed-control)	p
37.0	3/34 (9%)	10/39 (26%)	7 (17%)	< 0.1
38.0	13/39 (33%)	11/39 (28%)	−2 (−5%)	N.S.
Σ	16/73 (22%)	21/78 (27%)	5 (5%)	N.S.

Table III. The frequency of embryos classified to different classes according to the severity of the abnormality.

T [°C]	Duration of storage	Mild		Severe		Strongly retarded	
		Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
36.3	< 1 d	2	5	1	4	0	0
37.0	< 1 d	1	2	1	7	0	0
	3–4 d	2	3	1	7	0	0
38.0	< 1 d	1	5	4	7	1	0
	3–4 d	5	4	3	5	5	2
38.5	< 1 d	2	9	6	4	1	2
	Σ	13	28	16	34	7	4
		$p < 0.025$		$p < 0.01$		N.S.	

Cont., control; Exp., exposed.

Table IV. The frequency of different types of abnormalities.

T [°C]	Duration of storage	Abnormalities in the neural tube		Abnormal torsion of the embryo		Other abnormalities	
		Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
36.3	< 1 d	3	9	0	0	0	0
37.0	< 1 d	2	5	0	3	0	1
	3–4 d	3	10	0	0	0	0
38.0	< 1 d	5	9	0	3	0	0
	3–4 d	7	8	0	1	1	0
38.5	< 1 d	8	13	0	0	1	0
	Σ	28	54	0	7	2	1
		$p < 0.001$		$p < 0.01$		N.S.	

The strongly retarded embryos (Table III) were excluded from the data of this table.

ated with the magnetic field exposure, was abnormal torsion of the embryo. As it is possible that the preparation causes small disturbances in the shape of the embryo, only embryos with very strong curvature were classified in this group.

The stage of development of the embryos was not significantly affected by the exposure to magnetic field (Table V). The reduced rate of development at low temperatures was not fully compensated by longer incubation times.

Table V. The developmental stage of the embryos expressed as the number of somites ± 2 S.E.

T [°C]	Duration of storage	Control	Exposed
36.3	< 1 d	14.2 ± 0.83	13.8 ± 0.77
37.0	< 1 d	15.7 ± 0.68	16.5 ± 0.85
	3–4 d	18.5 ± 0.90	17.6 ± 1.01
38.0	< 1 d	17.9 ± 0.67	18.0 ± 0.71
	3–4 d	18.4 ± 0.95	18.9 ± 0.84
38.5	< 1 d	17.2 ± 0.94	17.0 ± 0.65

Duration of the incubation: 57 h at 36.3 °C, 55 h at 37.0 °C, 52 h at 38.0 °C, 50 h at 38.5 °C.

Discussion

The results support our earlier results and suggest that a sinusoidally oscillating magnetic field with a frequency of 100 Hz and field strength of 1 A/m disturbs the development of chick embryos. An alternative explanation to the effect could be altered temperature conditions due to the exposure system. In our earlier experiments, we found that exposure with the same exposure system to 100-Hz magnetic fields at 1, 10 or 100 A/m did not increase the internal temperature of the eggs. Furthermore, sham-exposure or exposure to 0.1 A/m did not increase the frequency of abnormalities, whereas exposure to 1, 10 or 100 A/m did [3, 4]. The present results further support the conclusion that the effect of the magnetic field exposure is not thermal in nature. Both the magnetic field and high temperature increased the frequency of abnormal embryos. However, their effects were additive and independent of each other. Moreover, the magnetic field did not affect the rate of development, whereas even a small difference in temperature has a strong effect on the rate of development. Possible mechanisms of the magnetic field effects have been discussed in our earlier papers.

When fresh eggs and low incubation temperatures were used, the frequency of spontaneous abnormalities appeared to be small, and the effect of the magnetic field exposure was clear. However, high incubation temperature and long storage of eggs before incubation increased the number of abnormalities in the control embryos, which made it difficult to find statistically significant differences between the exposed and control embryos.

Analysis of the type of the abnormalities can be used to distinguish the effects of the magnetic field from other abnormalities. Abnormalities in the neural tube appeared to be typical to the magnetic field effect. However, also high temperature increased this type of abnormalities. Abnormal torsion of the embryo seemed to be the most specific effect caused by the magnetic field: no abnormalities of this type

were found in the control embryos. However, the frequency of this abnormality was too small (7/230 = 3%) for use as an indicator of the magnetic field effect. Strongly retarded development was associated only with long storage and high incubation temperature but not exposure to the magnetic field.

The type of abnormalities associated with the magnetic field exposure are consistent with the hypothesis that low frequency magnetic fields affect the regulation of growth and cell division. Both abnormalities in the neural tube and abnormal torsion of the embryo can be thought to be due to unbalanced growth, *i.e.* differences in the growth rate of different tissues or parts of the embryo. However, this hypothesis is not supported by the fact that the magnetic field did not affect the general rate of development.

In the present experiments, the magnetic field exposure was found to increase the frequency of abnormal embryos by 17% (from 13% in controls to 30% in the exposed embryos). The corresponding increment calculated from our earlier experiments with field strengths from 1 A/m to 100 A/m and frequencies from 16.7 Hz to 100 kHz, is 22%, or 24% if only experiments with 1 A/m are included [4]. Although this difference from the earlier results may be due to differences in the frequencies used, it may also indicate changes in the eggs used. Two changes have occurred between these experiments at the farm used as egg supply. The old hens have been replaced by younger ones of the same variety, and the care of the hens has become more regular due to changes in the personnel of the farm. These changes have resulted in less deaths of hens and increased hatchability at the farm (personal communication, Mrs. Anja Venäläinen).

The results may explain part of the discrepancies between different studies of chick embryo development in magnetic fields. Additional differences may be caused by genetic variations and differences in the environment of the hen and cocks. In order to investigate the importance of these factors, experiments in independent laboratories are strongly encouraged. In further experiment of this type with chick embryos, the incubation temperature should be less than 38 °C, and only fresh eggs should be used. Because of possible variations in the sensitivity to magnetic fields, large enough egg groups from several hen and cocks should be used.

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