Development of preincubated chicken eggs following exposure to 50 Hz electromagnetic fields with 1.33-7.32 mT flux densities*

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The effects of applying extremely low-frequency (50 Hz) electromagnetic fields (ELF-EMF) for 24 hr and different densities (1.33-7.32 mT) were examined on healthy, freshly fertilized white leghorn chicken eggs (55-65 g). Results showed no increase in the rate of abnormalities in exposed groups, but were only significant in 4.19, 5.32 and 5.86, 6.65 mT densities. Alizarin red S and alcian blue 8G/X staining showed some embryos with extra ribs, defects in ribs and vertebrae, anuria and abnormal beaks. Study of egg weight, after 9 days of incubation, showed no significant differences between control, sham-exposed and experimental groups. Analysis of crown-rump, beak-occipital length and weight of embryo, showed significant decrease in weight at 4.39 and 5.52 mT intensities, comparing with control and sham-exposed groups. These results revealed that 50 Hz electromagnetic fields can even induce developmental alterations in preincubated chick embryos and confirm that its strength could be a determinant factor for the embryonic response to extremely low frequency EMFs (window effects) prior to incubation.

Keywords: Preincubated Chick embryo, ELF-EMF, Abnormalities.

Environmental electromagnetic fields (EMF), such as those from electric power transmission and distribution lines, have been associated with increased risk of leukemia, cancer of the nervous system, and lymphomas.1-5

Very weak low frequency pulse magnetic fields can induce significant effect on development of chick embryo, exposed at first 24 and 48 hr of incubation.6-12 These effects are dependent on the frequency, flux densities and wave form.7,10,13

Studies of the effects of 50-100 Hz electromagnetic fields on the embryos of various species (fly, sea urchin, fish, chicken, mouse and rat) indicate that early stages of embryonic development are responsive to fluctuating magnetic fields.14-16 The first 24 hr of incubation was reported to be crucial and could have some effects on the orientation of the embryo in relation to the direction of the field.14-17 But some reports did not find any differences between exposed and control unexposed chicken eggs.9,18-22 An alteration on cell proliferation, myoblast membranes, cytotoxic effects, cell surface, chromosomal aberrations, sister chromatid exchange, cellular death of human lymphocytes, tissue spaces, bone matrix formation, dose-dependent increase of micronuclei in bone marrow cells and proteoglycan composition have been reported.14,21,23-29

In most of these studies, chick embryos were killed at the end of two days exposure period, and their morphology described. In this way, the information obtained was based on the short-term effects of magnetic fields and did not allow the study of the development of morphological anomalies and other alterations induced by the fields; thus, it is possible that slight abnormalities could have been repaired or reversed in later embryonic phases, following treatments.14 In the present study, however, the eggs were exposed to electromagnetic fields, 24 hr prior to incubation (15°C); the eggs of experimental groups were then incubated (38±0.7°C) in the absence of field exposure, for 9 days (stage 35).30 At the end of this period, embryos have been studied to detect possible morphological and skeletal abnormalities, compared with previous post-incubated exposed-eggs.

Materials and Methods

A total of 307 freshly fertilized white leghorn hen eggs (55-65 g), obtained from Bonyademostazafan farm (Karaj, Tehran, Iran) were transported to the laboratory (15±0.5°C) and placed at their long axis horizontally, for less than 60 hr.
In 15 different experiments, 15 different flux densities (1.33, 1.38, 1.46, 2.66, 2.76, 2.93, 3.39, 4.14, 4.39, 5.32, 5.52, 5.86, 6.65, 6.90, and 7.32 mT) were used \((n = 10-16)\). There were also sham-exposed \((n = 38)\) and control \((n = 65)\) groups available\(^{32,33}\).

In each experiment, five eggs were placed inside a cylindrical coil \([42 \text{ cm in length (L)}, 9.6 \text{ cm inside and } 11.5 \text{ cm outside diameter, made of 980 (N) turn of } 2.5 \text{ mm enameled copper wire}]\), with their long axis parallel to the radius of annulus towards north-south geomagnetic direction. The EMF in the coil was created by the currents provided with 15 different densities and 50 Hz frequency; their values in the center of the coil were calculated according to:

\[ B = Mo.N.I/L, \]

where \(B\) is flux density, \(I\) is the intensity of electrical current applied to the coil \((Mo = 1.26 \times 10^{14})\). However, to calculate the value of magnetic field at a distance of 5 and 10 cm from the center of the coil, \(K\) was calculated as: \(K_s = 0.9427\) and \(K_{10} = 0.9079\) in the mentioned formula, respectively \((B=KI)\).

To create one-way wave and regulating the densities, an equipment with one key, for both activation and deactivation, a round screw for influx \((B=KI)\), and a sensor were installed inside the incubator \((38° \pm 0.7°C, \text{ and } 65\% \text{ RH})\), so that the magnetic flow was parallel to the embryo's long axis and perpendicular to the egg's long axis \((\text{longitude } 35°40' \text{ and latitude } 51°25')\). The air conditions \((one, 5 \text{ cm from the entrance of the coil and others beneath the coil})\) and a sensor were installed inside the incubator to maintain and monitor the temperature.

The unexposed groups included control \((outside the coil)\) and sham-exposed eggs \((inside the coil, with no exposure)\); exposed and unexposed eggs were then incubated \((38° \pm 0.5°C, 65\% \text{ RH})\) for 9 days. At the end of this period, all eggs were weighed and embryos were removed from their shells, immersed in Tyrode solution\(^{34}\) and studied blind\(^{33}\).

Embryos were scored for several gross anatomical features \((eyes, \text{ beak, developmental stage, tail, central nervous system, limbs}), \text{ stage of death (early and/ or late death), abnormalities and lengths of crown-rump (CR) and top of the beak to occipital bone (BO).}\)

Criteria for normality required normal embryological development of each of those features\(^{30,35}\).

To study possible skeletal abnormalities, the embryos were stained with alizarin red S \((E. \text{ Merk Darmstadt})\) and alicion blue 8 GX \((Riedle-DeHaen AG, D. 3016 \text{ Seelzel})\) and preserved in glycerine; due to binding to calcium and hyaluronic acid, bones and cartilages were stained red and blue, respectively. Thus, the number of vertebrae and ribs, abnormalities in sternum, pelvis, vertebræ, head, beak and tail were examined under stereo microscope.

The frequencies of abnormal embryos in the experimental \((exposed)\), sham-exposed and control groups were compared by using stag software and chi-square \((\chi^2)\) test. The differences between the values for the different variables of exposed, sham-exposed and control eggs were compared by one-way variance analysis, using SPSS software. Differences, having \(P \leq 0.05\) were regarded as significant. Values were given as a mean \(\pm SE\).

**Results**

**Dead embryos**—Number of embryos with early death \((before stage 13, \text{ or on day two of incubation})\) and late death \((after day two of incubation)\) were variable; some with late death and abnormalities were considered as abnormal embryos; early death occurred in experimental \((except in 1.33 \text{ and } 1.46 \text{ mT})\) and sham-exposed groups. Late death also happened in all three groups, except in 1.38, 1.46, 2.66, 5.52, and 6.90 mT exposed-eggs, which had abnormalities in 1.46, 4.39 and 6.65 mT, too.

**Growth retardation**—There were delays in growth amongst the experimental groups, in 1.38 and 1.46 mT and had not reached stage 35 of development\(^{31}\).

**Morphological abnormalities**—The abnormalities observed included monophthalmia \((5 \text{ embryos})\), with three embryos lacking right eye \((Fig. 1a)\), one with no left eye in 1.46 mT, and one with anuria and skeletal anomalies occurred in 6.9 mT intensity. There was only one case of monophthalmia in control and sham-exposed groups, which had abnormal beaks and brain \((Fig. 1b)\). These embryos had crossed-beaks, with their upper beak tilted towards the side of brain with abnormalities, which also happened in sham-exposed embryos with anophthalmia.

There was one embryo with underdeveloped left cerebral hemisphere, in 6.65 mT \((Fig. 2)\). The most frequent abnormalities seen in all groups were gastrochisis \((in 1.33, 1.38, 1.46, 2.66, 2.76, 2.93\) and...
5.52 mT) and thoracogastroschesis (in 1.33, 1.38, 1.46, 2.93, 5.52 and 5.86 mT (Table 1); hearts were outside the thorax, skeletal anomalies and anuria (in 2.93 mT) happened in embryos with thoracogastroschesis. In embryos with gastroschesis, liver, stomach, intestine, crop and spleen had projected outside the body cavity (Fig. 3)

Anuria occurred in 1.33 and 2.93 mT, but atrophied tail was observed only in 1.33 mT; these embryos had no sacral, tail and lumbar vertebrae.

In one case, in addition to lack of hindlimb bud (4.39 mT), caudal part of trunk and tail had been deleted and forelimb was abnormal.

Qualitative characteristics—There were no significant difference between eggs' weights of experimental and control groups (Fig. 4); but, maximum decrease in body weight occurred in embryos with early death, although it had no significant difference with sham-exposed and control eggs (Fig. 5). There was significant decrease in the weight of embryos in 4.39 and 5.52 mT (Fig. 6a).

There were no effects on lengths of CR and BO, but there was an insignificant increase in the length of BO in 6.9 mT density (Figs 6b and c).

Skeletal structures—There were seven embryos with extra ribs in 5.32, 5.52, 5.86, 6.65, 6.9 and 7.32 mT flux densities. In 6.9 mT intensity, the embryos had no sternal ribs in each side (Fig. 7). In 6.65 mT, one embryo had some sort of projection on the right side of ribs. There was bifurcated rib 6 (Fig. 8) and one extra rib on the first lumbar vertebra; left side lacked sternal rib.

In 5.52 mT, embryo had no sternal rib 7, but one perforated rib; in one of the control embryo, rib 5 had slit.

Embryos with monophthalmia and anuria, had one pair of extra rib; both ends of right rib 7 were bifurcated and left rib had three branches; there were no tail and sacral vertebrae in these embryos. Lack of lumbar vertebrae and abnormal lumbar vertebrae (in 4.14, 5.32, 5.86 and 6.9 mT intensities) were also observed.
The neural tube was open in sacral region; sternum was either bifurcated or had cleft in embryos with thoracogastroschesis.

There was significant difference on the percentage of abnormal embryos in 4.14, 5.32, 5.86 and 6.65 mT densities, comparing with control embryos (Fig. 9).

Discussion

Electromagnetic fields have some negative effects on the electrostatic characteristics of macromolecules (glycoproteins, proteoglycans and glycosaminoglycans), organogenesis, tissue spaces, morphogenesis, cell surface and differentiation which have enough energy to directly destroy DNA and chromosomes; it can produce free radicals and other cofactors, which would damage DNA, lipids, proteins...
and macromolecules. It can also change cell signals, binding to surface hormones, neurotransmitters, antibodies at the cell surface, bone matrix formation and bone marrow cells.

By effecting cell migration in chick embryos, EMF causes distortion in germ layers and their derivatives. Abnormalities created by EMFs are dependent on the frequency, intensity, wave forms and local geomagnetic fields.

By exposing fertile chicken eggs to EMFs, 24 hr prior incubation (for the first time), it was found out that there was significant increase in the number of abnormal embryos, in 4.14, 5.32, 5.86 and 6.65 mT flux densities. There were some abnormalities (anuria,
atrophied tail, late death and gastroschesis) in 1.33 mT density, while in the similar study by Lahijani and Moham\textsuperscript{32}, on first 24 hr of incubation, there were no increase in the rate of anomalies; late death occurred only in 31% of the embryos. In 1.38 and 1.46 mT intensities, abnormal embryos with early death, late death, growth retardation, gastroschesis and monophthalmia were formed, while early death, late death, abnormal tail and growth retardation only happened in mentioned intensities, on first 24 hr of incubation; 37 and 36.3% of embryos (respectively), were abnormal. In 2.66 mT intensity, percentage of normal embryos created was similar to previous researches (80% and 75%, respectively).

In 2.76 mT, 33.3% embryos were abnormal, comparing with research of Lahijani and Moham\textsuperscript{32} with 50% abnormal embryos; rest of the embryos (50%) grew with monophthalmia, gastroschesis, crossed-beaks, early death and late death.

In 2.96 mT density, there were 40% of abnormal embryos in pre-incubated exposed-eggs, comparing with 30% in post-incubated eggs; 80% of embryos were normal; while it was about 68.7% in 3.39 mT intensity, in post-incubated exposed-eggs, but number of embryos with early and late death amongst abnormal embryos was different between these two groups. Number of abnormal embryos in 4.14 mT density, in both pre and post-incubated exposed-eggs were almost the same (55.5 and 56.3%, respectively); presence of skeletal abnormalities (bifurcated rib 6, extra rib, and abnormal sacral vertebra) occurred only in preincubated embryos; 36.3% of embryos were abnormal with malformed forelimbs, in 4.39 mT flux density. In 5.32 mT, 55.5% of embryos were abnormal, with 50% of them having skeletal anomalies (extra vertebra between vertebrae 6 and 7, and abnormal sacral vertebra); among postincubated groups, 56.3% of the embryos were abnormal, and one embryo had extra rib\textsuperscript{32}.

In 5.52 mT flux density 40% of the embryos were abnormal, comparing with post-incubated embryos with 37.5% of abnormalities. In 5.86 mT, 50% of embryos were abnormal, one embryo with extra rib on one side and one with thoracogastroschesis; in similar case of post-incubated exposed-eggs, only 25% of embryos were abnormal, and one embryo had abnormal tail. In 6.65 mT flux density, 50% of embryos were abnormal; skeletal anomalies included extra rib in lumbar region, atrophied tail and underdeveloped cerebral hemisphere; in post-incubated eggs, 35.7% of embryos were abnormal; malformed lumbar vertebra was observed too. Abnormalities observed in 6.9 mT intensity included extra rib in lumbar region, bifurcated vertebra, branched rib, lack of sacral and tail vertebrae. There was 35.7% of abnormal embryos in this group, while in post-incubated eggs, it was 57.1%; 30% of embryos were abnormal in 7.32 mT, one embryo had extra rib. In post-incubated group, it was 36.4%; one embryo with monophthalmia and the rest with skeletal anomalies.

Degraded glycosaminoglycans, abnormal gaps, necrotic zones and reduction in the head mesenchymal cells have also been proposed for the occurrence of abnormalities in the head, beak and face regions\textsuperscript{11,14}.  

Fig. 9—Nine-days old chick embryo with extra rib (arrow point) (x 7).
Fig. 10— Nine-days old chick embryo with bifurcated rib (arrow) (x 7).
Defects in the tail region could be due to the influence of EMFs on the number of somites, which confirms previous studies. On the other hand, it was demonstrated that EMFs have some effects on the ectomesodermal wall of gut regions, which could cause gastroschesis and thoracogastroschesis.

Quantitative investigations showed that there was no difference between the body weight of eggs of experimental, sham-exposed and control groups; comparison of the weight of embryos of most experimental groups showed significant decrease ($P \leq 0.05$) in 4.39 and 5.52 mT intensities, comparing with controls, while in comparable postincubated exposed-eggs, there was decline in weight of some embryos and increase in others.

Flux densities 1.33-7.32 mT, had no effects on the lengths of CR and BO of experimental groups, whereas, except in one case, reduction in CR and BO lengths have been reported in post-incubated exposed-eggs.

The reports have demonstrated that EMFs have no influence over embryo's weight, but in previous studies, results were controversial.

EMFs affected length of CR and BO has been diminished, because of inductive effects of EMFs on forebrain and optic vesicles.

But why EMF has affected preincubated eggs? Answer to this question may lie in the reports that EMFs can influence intracellular DNA, RNA, proteins, metabolism, cell division and cell growth.

With respect to morphogenetic fields, which play an important role in developmental processes, disturbances could influence the development of organs later on.

As the results and figures show, the effects of EMFs on preincubated chicken eggs are not dependent on doses and confirm Ubeda's theory, even in preincubated eggs, which usually are not placed in an incubator ($38^\circ\pm0.7^\circ$C).

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