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Effect of exposure to extremely low electro-magnetic field during prenatal period on mice spleen

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Total body weight of newborns, the volume of spleen, and the number of megakaryocytes decreased following the exposure to ELF-MF ($6x10^{-3}$ T and 50 Hz) at 1-5, 6-10, 11-15, and 16-20 days of pregnancy of mice. The complete period of gestation was sensitive to ELF-MF exposure; the initial days were more prone to exposure. The results suggest that the use of ELF-MF producing instruments should be limited during gestation.

Keywords: Electromagnetic field, Megakaryocyte, Mice, Spleen, Stereology

The overuse of cell phone and other instruments that generate extremely low electromagnetic fields (ELF-MF) made researchers interested in exploring the biological effects of these fields. Epidemiologically, the effects of these fields on embryogenesis have suggested a possible relationship between ELF-MF and adverse pregnancy outcomes in humans[1-3].

In vivo studies have suggested that ELF-MF disturb the development of non-mammalian embryos leading to induction of structural anomalies[4,5] and markedly depress levels of cellular products[6]. In mammals results have been inconsistent, some studies reported no or only slight effects on fetuses that included slight limb anomalies and body weight reduction[7-9]. But others have shown that prenatal exposure to these fields induces adverse effects such as growth retardation, delayed puberty and reduced IGF-1 levels, damage of hypothalamus, pituitary gland and ovaries in female newborn Wistar rats[10]. Moreover, only 48 h exposure to these fields in pregnant rats increases incidence of non-vital fetuses, neonates, or infants[11]. At cellular level many studies have shown that both prenatal and post-natal exposure to these fields induces noxious effects in mammals[10,12-15].

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It seems that the effects of electromagnetic fields on embryonic development differ based on the type of species, parameters of the field and the type of tissue. Thus, to achieve more correct data, the more numerical modeling provides the most suitable analysis tool for more accurate estimation[16]. Two recent studies carried out stereological technique to evaluate quantitative changes induced by these fields[13,14].

The present study has been undertaken to see the effects of ELF-MF on the embryogenesis of mammals particularly on the development of mouse spleen. Stereological method has been used to evaluate the quantitative effects of ELF-MF on the volume of spleen and the number of megakaryocytes .

Materials and Methods

Forty-eight female NMRI (Naval Medical Research Institute) mice (10 weeks of age, 30±4 g) purchased from Razi Institute (Karaj, Iran) were maintained in the animal house at Arak University of Medical Sciences. All animals received care as recommended by the research committee of the University. The mice were maintained on a regular diet and water at a 12:12 h L:D cycle without any stressful stimuli. at 23°±2°C. Experiments were started after one week adaptation. Following mating, the beginning of pregnancy was determined by daily evaluation of sperm presence in vaginal smear by

light microscopy analysis (ZEISS, magnification ×100). Fertilized mice were divided randomly into following 6 groups of 8 mice in each. Group 1(control): mice were placed in switch off electromagnetic housing while group 2 (sham): mice had no treatment at all. In the four experimental (E) groups pregnant mice were exposed to an established pulsed ELF-MF exposure system (6 mT and 50 Hz for 10 h a day for 5 days) at 1-5 (E1), 6-10 (E2), 11-15 (E3), and 16-20 (E4) days of gestation.

Pulsed ELF-MF exposure system

The ELF-MF used in the present study was produced by a pair of Helmholtz coils able to generate a highly homogeneous field (with homogeneity 5/1000). The characteristics of the system were as follows:

(i) Power supply: 220 V in, 25 V out, permanent current intensity 3 Ampere.
(ii) Multi-meter to control the intensity of the current entering the instrument.
(iii) A 50 Hz sinusoidal oscillating ELF-MF was produced by a 380 round turn coil twisted around a cylinder (19 cm diameter and 15.5 cm length) and containing a chamber to house mice in the center of the cylinder, where the maximum even ELF-MF (6 mT) was recorded.
(iv) A Teslameter (Compensation-51662) for precise measurement of magnetic field intensity in the chamber.

Stereological analysis of megakaryocytes population

Newborn pups (n=180) were weighed. Their spleens were removed and fixed in 10% formalin. Tissue sections of spleens were prepared according to stereological methods and stained with H&E[17-19].

Cavalieri method was used for the numerical evaluation of newborn spleen megakaryocytes[17]. Briefly, 30-40 microscopic fields were selected in each section via a systematic subsample. The number of the megakaryocytes was estimated using ''dissector'' method at the final magnification of 3500 by means of counting frame with inclusion (right and upper) and exclusion (left and lower) borders that was superimposed on the images[17-19].

To estimate the volume of spleens, 15 double serial tissue sections of each sample were selected and stained[11-13]. The stereological probe (consisting of 25 points) was superimposed upon the images of the tissue sections viewed on the computer monitor. To take advantage of the probe, for each couple of sections, two randomly chosen images belonging to the two sections were projected over a computer monitor, one as reference and the other to look up[17-19]. The volume of spleen was estimated using the formula:

$$Vv = Pstructure/Preference$$

where 'Pstructure' and 'Preference' are the number of test points falling on the structure's profile and on the reference space, respectively.

To gain the proper estimation of the volume of spleens and the number of cells, five microscopic fields were chosen from each section, projected and counted.

The data are reported as means±SD. Statistical analysis was done by One-Way Analysis of Variance (ANOVA) using SPSS 16.0 for Windows XP (SPSS Inc., Chicago, IL). P value < 0.05 was considered significant.

Results

The mean weight of the newborns decreased in ELF-MF exposed groups (E1-E4) and the change was higher in E1 group (treatment at 1-5 days) (P<0.024) (Fig. 1A). The mean volume of the spleens and megakaryocytes number of the newborns decreased and this reduction was more prominent for E1 group (Fig. 1B and C). The mean volume of the spleens and the number of megakaryocytes of the newborns were calculated by stereological method based on tissue section samples (Fig. 2).

Discussion

The results showed that ELF-MF disturbed the development of spleen by decreasing the spleen volume, and the number of megakaryocytes.

There are some reports about the effects of ELF-MF on the embryonic development of spleen and lymphoid organs. Shafey *et al.*[20] have shown that exposure to ELF-MF (60 Hz) during chicken pregnancy increased the weight of spleen, lymphoid organs and total body of newborns. However, post-natal birds at 42nd day of age had significantly (P < 0.01) lower total body weight, lymphoid organ, and bursa of Fabricius weight when compared with those of 21st day of age, suggesting that although the weight of spleen increased during first days of postnatal life following prenatal exposure to ELF-MF, it reduced later to normal size[20]. Moreover, exposure to ELF-MF (60 Hz) during 18-21 days of gestation

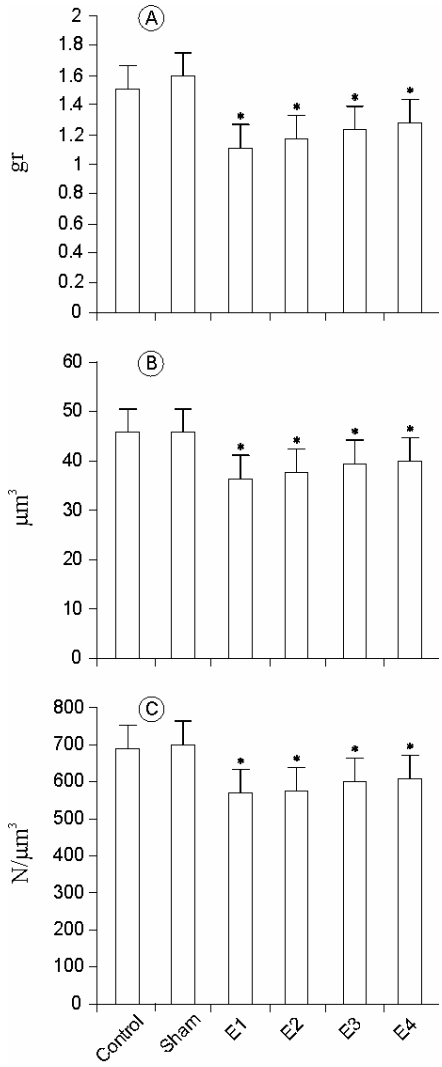

Fig. 1—Effect of maternal exposure to low electro-magnetic fields on (A) body weight (g), (B) spleen volume (μm^3) and (C) number of megakaryocytes ($N/\mu m^3$) in newborn mice (*$P< 0.05$).

did not promote inflammatory reactions such as gross or microscopic changes in the spleen[21].

Another study showed that the volume of spleen in exposed newborn animals was reduced. This result was in contrast with that of Cicekcibasi *et al.*[22] who showed that 50 Hz magnetic fields did not reduce the weight of spleen in adult mice[22].

In present study the number of splenic megakaryocytes of newborn mice was reduced by prenatal exposure to ELF-MF. The *in vivo* studies have showed some controversy with this data. Gorczyńska[23] showed that static magnetic field (0.05 and 0.3 T) decreases the megakaryocytes in bone-marrow of guinea pigs. But in contrast, Prisco *et al.*[24] showed that 900 MHz ELF-MF did not affect the bonemarrow precursor cells of mice. However, these two studies were undertaken on adult samples and could not find any previous data for embryo exactly for the spleen.

Although the mechanisms by which ELF-MF induce cytototoxicity is not completely understood, apoptosis is considered as main cellular event by many authors. *In vivo* studies showed that 50 Hz 0.2, 3.2 or 6.4 mT for 2 weeks or 4 weeks blocked the cell cycle in S phase in testes of mice[25] and 60 Hz 14 mT magnetic field for 8 weeks induced apoptosis in testicular germ cell in mice[12].

In vitro studies confirmed *in vivo* experiments that showed induction of apoptosis in K562 human leukemia cell line by ELF-MF (1 mT, 50 Hz)[26], in

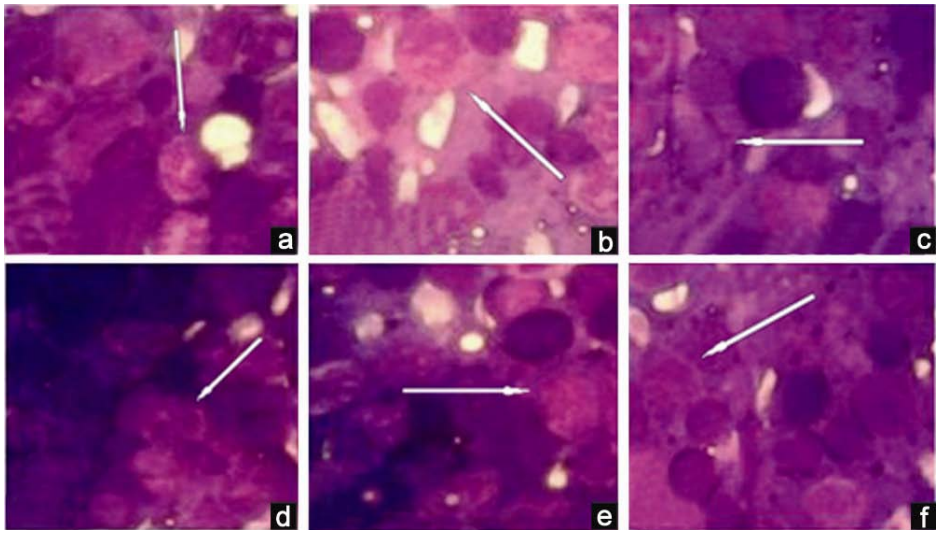

Fig. 2—Effect of maternal exposure to low electro-magnetic fields on spleen of newborn mice [a=sham, b=control, c=E1, d=E2. e=E3, f=E4. (H&E × 40). The arrows show the megakaryocytes.

isolated human osteoclasts by pulsed electromagnetic fields (7.5 Hz)[27], in isolated liver cells and peripheral blood sampled from newborn mice by ELF (50 HZ)[28], in human normal and cancer cells by time-varying magnetic fields (MFs) (60-Hz 6 mT)[29], and also induction of micronuclei in rat bonemarrow by (910-MHz)[30]. In sub cellular level, regulations of many molecules were considered for induction of apoptosis. The studies suggest formation of free radicals such as reactive oxygen species (ROS)[26,31], heat-shock protein 70 (hsp70)[26,32], cyclic adenosine mono phosphate (cAMP)[31] and also P53 gene[33-35] and its related gene; P38[24] and other regulating genes of apoptosis such as bcl- 2 and bax[33]. But an *in vivo* experiment found that these fields had no effect on the expression of P53 gene in testes of rats[30] and also an *in vitro* experiment showed that these fields did not inhibit cell proliferation of isolated mouse bonemarrow mesenchymal stem cells[31]. Thus, decreased number of megakaryoctes of newborn mice in ELF-MF exposed groups can be explained by apoptosis process and complete data could be achieved by evaluating ROS and expression of P53 and other related genes.

The present study was conducted to show the effect of 5 days exposure of ELF-MF (50 Hz) during gestational period. Exposure to 900 MHz electromagnetic fields during total time of gestation induced pyramidal cell loss in the hippocampus of newborn rats[36]. On the other hand, Okudan *et al*.[37] have shown that both the static and 50 Hz EFs influence the early development of rat bones at exposure time in 14 days before and after their birth. In fact, duration of exposure during gestational period is more prominent than the frequency and other physical parameters of ELF-MF. The present results also confirm that exposure to ELF-MF during 5 days may induce side-effects like exposure during total period of gestation. These findings are in agreement with those showing that exposure for 1 day to ELF-MF lead to increase cAMP but 2 or 3 days decreases the concentration of this intracellular secondary messenger[31].

The present results showed that the weights of newborns in all experimental groups were reduced; demonstrating that the whole gestational period is sensitive to these fields for weight loss of delivered newborns. These results are in parallel with the study done on rats subjected to a 35 mW/cm^2 ELF-MF[38] and with the one on mice treated with 20 kHz fields[39], but not in agreement with the results on rats treated with 20 kHz fields[40]. The reduction of the weight of newborn mice may be justified with induction of apoptosis by ELF-MF like the one explained for decreasing the number of megakaryocytes.

In conclusion, this study showed that ELF-MF has a general cytotoxic effect on the total body weight of embryo. These fields may reduce the function of hematopoetic organs, as indicated by reduction in spleen volume and number of megakaryocytes that can be explained by occurrence of cell death. Taking together, using ELF-MF producing instruments like mobile phones and microwave ovens should be limited during gestation. The first trimester of gestation is important because the side-effects of these fields on the embryos are prominent and the mothers usually are unaware of pregnancy.

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