Effects of extremely low-frequency electromagnetic field on fertility and heights of epithelial cells in pre-implantation stage endometrium and fallopian tube in mice

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Objective: To investigate the effects of extremely low-frequency electromagnetic field (ELF-EMF) on fertility and heights of epithelial cells in pre-implantation stage endometrium and fallopian tube in mice.

Methods: Eighty female NMRI mice were randomly divided into 2 groups: control group was not exposed to EMF and experimental group was exposed to 4-hour EMF per day, 6 days a week for 2 weeks to 50 Hz, 0.5 mT EMF. Female mice in two groups were superovulated and mated with male mice over night. At the time of implantation, the blastocysts were obtained from the presumed pregnant mice with vaginal plug by flushing the uterus horns. The samples of uterus horns and fallopian tubes in two groups were taken and were processed for light microscopic studies.

Results: The analysis of mean number of the flushed blastocysts in the EMF group showed significant decrease as compared with the control group ($P<0.03$). Light microscopic study showed that the height of fallopian tube epithelial cells was significantly increased in the EMF group as compared with the control group ($P<0.001$). However the height of endometrial epithelial cells in the EMF group showed insignificant increase as compared with the control group.

Conclusion: The results indicate that ELF-EMF has detrimental effect on female reproductive system in mice by decreasing the number of flushed blastocysts and increasing the height of fallopian tube epithelial cells.

Keywords: epithelium; endometrium; fallopian tube; fertility; mice

极低频率电磁场对小鼠生育能力及着床前期子宫内膜和输卵管上皮细胞高度的影响

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目的：探讨极低频率电磁场（extremely low-frequency electromagnetic field，ELF-EMF）对小鼠生育力和着床前期子宫与输卵管上皮细胞高度的影响。

方法：80 只雌性 NMRI 小鼠随机分为对照组和 EMF 组。对照组小鼠不接受 EMF，EMF 组小鼠接受 50 Hz, 0.5 mT EMF，每次 4 h，每周 6 次，连续 2 周。雄小鼠注射孕马血清促性腺激素和人绒毛膜促性腺激素促其排卵，与雌鼠交配。通过观察阴道栓检查确定小鼠妊娠后，冲洗子宫腔获得胚泡，取子宫角和输卵管

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Life on earth has evolved in a sea of natural electromagnetic fields (EMFs). Over the past century, this natural environment has sharply changed with introduction of a vast and growing spectrum of man-made EMFs, and exposures to extremely low-frequency (ELF) electric and magnetic fields emanating from the generation, transmission, and use of electricity are a ubiquitous part of modern life. Popular media and scientists have raised concerns about possible health hazards of environmental exposure to EMF, especially to 50 and 60 Hz. To date, numerous contradictory results regarding the carcinogenic potential of EMFs have been reported in the literature. More than 50 studies on exposures of animals to EMFs have been performed within the last few years. In vitro studies have explored the potential effects of ELF-EMF on cell proliferation, apoptosis, differentiation, genotoxicity and proto-oncogene modulation. Human data reviewed concern the potential reproductive effects (mainly spontaneous abortions, low birth weight, and congenital malformations) of exposure to various sources of EMFs. It is unclear how ELF-EMF would influence cellular behavior, but a plausible hypothesis is that ELF-EMF affects membrane structure and permeability to small molecules. Some studies have shown that ELF-EMF exposure increased Ca²⁺ currents in neuroendocrine cells due to over expression of voltage-gated Ca²⁺ channels and this occurrence may be correlated with proliferative events. Another interesting hypothesis is that ELF-EMF interferes with chemical reactions involving free radical production. Regarding to the role of the uterus and fallopian tube on fertility and the widespread application of EMF-producing devices, the effects of EMF (50 Hz, 0.5 mT) on fertility and heights of epithelial cells in mice endometrium and fallopian tube in pre-implantation stage were investigated.

1 Materials and methods

1.1 Materials

1.1.1 Animals Eighty female NMRI mice (20 to 25 g) were obtained from the Razi Institute (Karaj, Iran) and housed in groups of five per cage under standard laboratory conditions. They were kept at constant room temperature (21 ± 2) °C under a normal 12-h light/12-h dark cycle with free access to food and water. All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) to minimize their suffering.

1.2 Experimental methods

1.2.1 Mouse model and group To identify possible effects of EMF exposure on pregnancy rate and morphometric changes in uterus fallopian tube epithelium, eighty female NMRI mice were randomly divided into 2 groups: Group I (control group) was not exposed to EMF and Group II (EMF group) was exposed to 50 Hz and 0.5 mT EMF 4 hours per day, 6 days a week for 2 weeks. Female mice in the control group and the EMF group on the 8th day of exposing were superovulated with PMSG (10 IU) and with hCG (10 IU) about 48 h later and mated with male mice over night.

1.2.2 The number of blastocysts Next morning females with a vaginal plug were identified as pregnant mice and the number of pregnant mice (positive plug) in two groups was counted: 102 h after hCG injection, pregnant mice were sacrificed by cervical dislocation and their uteri were dissected apart. Blastocysts were subsequently obtained from these mice by flushing the uterus horns using PBI solution. The number of blastocysts per pregnancy in two groups was counted.

1.2.3 Light microscopic study For light microscopic study, the samples of endometrium and fallopian tubes in two groups were taken, and following tissue processing, serial sections by microscope were prepared. Finally, five slices from every sample were selected (slices 5, 8, 11, 14 and 17). After staining with hematoxylin and eosin (HE), the heights of endometrium and fallopian tube epithelia were compared by using statistical methods in both groups. The heights of epi-
and fallopian tube epithelial cells The height of fallopian tube epithelial cells in terms of micrometer was significantly increased in the EMF group as compared with the control group \( (P < 0.001) \). However, the height of endometrial epithelial cells in terms of micrometer in the EMF group was insignificantly increased as compared with the control group (Table 1). Moreover, qualitative observation of mouse fallopian tube in the control group showed the normal columnar epithelial cells with normal cilia and centrally located nuclei (Figure 1-A), but in the EMF-exposed group, increase in the numbers and heights of cilia and nuclei were observed (Figure 1-B).

<table>
<thead>
<tr>
<th>Group</th>
<th>Height of fallopian tube epithelium (mm)</th>
<th>Height of endometrial epithelium (mm)</th>
<th>Number of blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.37±0.24</td>
<td>20.11±0.44</td>
<td>9.0±4.8</td>
</tr>
<tr>
<td>Experimental</td>
<td>23.83±0.48</td>
<td>20.68±0.57</td>
<td>5.5±5.7</td>
</tr>
<tr>
<td>( P ) value</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
<td>&lt;0.03</td>
</tr>
</tbody>
</table>

Figure 1: Microscopic view of mouse fallopian tube (HE staining, \( \times 40 \))
A: Control group, enfoldling of mucosa with normal epithelial cells. B: EMF group. The arrow shows increase in size of epithelial cells as compared with the control group. Bar in Figure B represents 10 \( \mu m \).

3 Discussion

The results of present study showed that there was insignificant difference in height of endometrial epithelium in the EMF group but the height of fallopian tube epithelial cells in the EMF group was considerably increased as compared with the control group \( (P < 0.001) \). In our best knowledge, there are no morphometric studies about the EMF effects on heights of epithelia in mice endometrium and fallopian tube in pre-implantation stage in literature. and all studies have been focused on blood parameters and sexual hormones in females. The only morphometric study by Rajaei et al. in 2009 showed that when the mice were exposed to EMF (50 Hz, 0.5 mT) 4 hours a day, 6 days per week and for 2 months, the heights of epithelial cells in epididymis and deferens duct in the EMF group were considerably reduced as compared with the control group. This is not in consistent with the results found in the present study. The reason for the difference could be associated with differences in duration of exposures (two weeks versus eight weeks). Similarly, Kim et al. found that following the exposure of mice to the 60 Hz ELF magnetic field (MF) for 16 weeks, the testis weight increased in the 14 \( \mu T \) exposure group as compared with that in the sham control group, while it remained unaffected in the 200 \( \mu T \) exposure group and decreased in the 1st and 2nd generation mice exposed to 60 Hz MF of 0.5 or 1.5 mT for 46 weeks. They concluded that increase and decrease of testis weight may be dependent on exposure dose and time of MF and the increase in testis weight seems to be mediated by a physiologic testicular compensatory mechanism.
for weak harmful stimulus over a long period.

There is a possibility that human tissues try to compensate the destructive role of EMF and when the exposing rate reaches to a threshold, the destructive role of EMF is appeared.

The increase in height of epithelium in this study may be due to detoxification role of fallopian tube epithelium in pre-implantation stage following the short time exposing of mice to EMF. If the exposure time continued till eight weeks, the decrease in height of epithelium might be observed. Although changes in endometrial epithelium between two groups are not significant, the increase in the number of cilia and an epithelial layer might be related with possible effects of EMF exposure on alteration in the pattern of estrous cycle. However, further study is suggested for analysis of changes in hormonal profile.

Extensive literature highlights the role of free radicals in the physiological functions in female reproduction. In consistent of this data, Jung et al. suggested that exposure to 20 kHz EMF may affect normal cycling of the estrous cycle by disrupting the female reproductive endocrine physiology. In another study, Minkina et al. presented that exposure of male rat to electromagnetic field was associated with activation of neurosecretion in hypothalamus, inhibition of adenocorticotropic and gonadotropic functions of hypophysis and adrenal cortex, degenerative and dystrophic changes in gonads. Moreover, the data showed that the mean number of pregnant mice insignificantly decreased in the EMF group as compared with the control group while the mean number of blastocysts after flushing significantly decreased in the EMF group as compared with the control group. In consistent of this data, Zimmermann et al. demonstrated that the numbers of pregnant mice were considerably reduced following exposure to EMF. Al-Akhras et al. showed that exposure to a 50 Hz field reduced male rat fertility and the number of pregnant females when mated with exposed males. In contrast Ohnishi et al. showed that exposure to 50 Hz magnetic fields of 0 mT (sham), 0.5 mT and 5.0 mT for 9 and 2 weeks prior to mating for males and females, respectively, through fertilization and until cesarean sectioning on 18th day of gestation has no major effects on pregnancy and fetus development in mice. In addition Chung et al. showed that exposing pregnant rats to 60 Hz at field strengths up to 0.5 mT from the 6th day of gestation to the 21st day of lactation did not produce any biologically significant effects in dams, F1 offspring, or F2 fetuses. The difference between the findings of the current studies and those of the previous could be due to difference in animals, exposure duration and EMF intensity. It was shown that cell reactions to EMF exposure is depended on duration of EMF exposure and EMF intensity. Finally, it could be deduced that perhaps the damaging effects of EMF radiation is associated with an increase in body temperature and free radical formation, both of which could be considered as detrimental agents to body tissues especially the reproductive system. Hence, in reducing the side-effects of these raditions on reproductive system, avoidance to unnecessary application of such EMF-generating appliances is recommended. In conclusion, the present study showed that the EMF radiation could negatively affect the female reproductive system in mice by increasing the heights of endometrium and fallopian tube in pre-implantation stage. However, regarding the effects of such radiation on human reproductive system, further studies are needed.

The results indicated that ELF-EMF has detrimental effect on female reproductive system in mice by decreasing the number of flushed blastocysts and increasing the height of fallopian tube epithelium.

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