



Effects of prenatal exposure to electromagnetic fields on biomarkers of oxidative stress and fertility of F1 generation *In-vitro*

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Abstract

The aim of the present study was to evaluate the effects of Electromagnetic Field (EMF) in the prenatal period on biomarkers of oxidative stress and fertility of F1 generation. **Material and Methods:** The pregnant rats divided to 3 group's treatment (EMF exposure), Sham (off -EMF) and control. After delivery the blood sample of mothers provided and pups were kept until maturity age, and then their fertility was investigated. **Results:** CAT in treatment group was significantly decreased but no differences in GPX were observed. In treatment group decrease in multicellular embryos and abnormality in cleavage pattern was found. **Conclusion:** Exposure in EMF cause oxidative stress and reduce fertility.

Keywords: EMF, Fertility, F1 generation, Oxidative Stress, Prenatal

Introduction

Electromagnetic fields (EMFs) may cause an increased activity in free radicals in living organisms and can affect the biological systems (Gulnihal *et al*, 2008). EMFs interact with human tissues and may have adverse effects on fertility and reproduction. During the last decades it has been a growing concern on the effects of electromagnetic radiations on biological systems in general. This is because of the global introduction of electronic devices on a massive level for communications and data transmission, industry applications, medical/diagnostic and therapeutic purposes that are now new sources of Extremely low Frequency- Electromagnetic Fields (ELF-EMF) and radiofrequency microwave radiation (RFR) (Behari and Rajamani, 2012). During recent decades much interest has centered on power lines and their possible association with the health effects including heart functions cell stress (Tokalov and Gutzeit, 2004), memory disorders (Moustafa *et al*, 2002), melatonin and its relation to sleep and circadian rhythm (DeBruyn *et al*, 2001),

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cancers (Kheifets *et al*, 1995), male and female reproduction and fertility (Al-Akhras *et al*, 2006). Interesting researches are performed in relation with effects of magnetic fields on cellular stress, reactive oxygen species (ROS) and free radicals, reproductive and fertility (Mohammad Saad, 2003). EMF results in excessive formation of ROS which would result in irreversible tissue damage (Ferniekim and Reynold, 2005). The effects of ELF-EMF are the modification of the normal balance of the free radicals such as superoxide anion (O_2^-), hydroxyl radicals (OH^\bullet) in the organism and modifying the normal pathways of the elimination of these substances (Drogue, 2002). It is well established that changes in intracellular ROS concentration plays an essential role in the modulation of antioxidant defenses (Gulnihal *et al*, 2008). A living organism has defense systems against different oxidants for e.g. antioxidant vitamins (A, C and E), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxides (GPX) that protect cells against lipid peroxidation. These antioxidant defense systems can be deteriorated by a magnetic field which leads to oxidative stress (Lee *et al*, 2004). CAT is one of the major enzymes which normally exist in the cells and can be stimulated by variety of factors (Valko *et al*, 2007). Epidemiological studies on birth defects and abortions in pregnant women working

in offices revealed that the EMF generated from a computer monitor can negatively affect human reproduction (Bergqvist, 1984). Many people still believe that, because the energy of the fields is too low to give significant heating, they cannot have any biological effect. However, the evidence that alternating electromagnetic fields can have non-thermal biological effects is now overwhelming (Goldsworthy, 2012). Increased risk of miscarriage caused by ELF-EMF exposure during pregnancy in women, changes in uterus and ovary in rats, congenital deformities and developmental delay in the offspring of mice, reduced fertility in female rats are some outcomes of these investigations in females (Li *et al.*, 2002). Also, EMF affects reproductive system and could lead to sub fertility, implantation disorder and congenital malformations (Chernoff *et al.*, 1999). Use of electronic household items and cell phones are reported to decrease fertility potential in men by decreasing sperm count, motility, viability, inducing pathological changes in sperm and testes morphology, and so on (Erogulet *et al.*, 2006). Recently it was demonstrated that the exposure for at least 1 h to a field of 1mT intensity, 50 Hz frequency reduced the fertilizing ability of boar mature spermatozoa, tested under *in vitro* fertilization conditions (IVF), by interfering with different cell activity such as energy metabolism, ionic homeostasis and membrane integrity (Bernabo *et al.*, 2007). According to these considerations, the present research objective was to study the effect exerted by ELF-EMF in prenatal period on biomarkers oxidative stress in pregnant rats and fertility F1 generation in *In-vitro* condition.

Material and Methods

Animals

In the present research, 18 female Wistar rats and 9 male rat weighting 200-250 g with 2-3 month age were studied. The rats were supplied from animal house of the histology Department Faculty of Medicine, Tabriz University of Medical Sciences. The rats were housed in plastic cages and kept under 12 h light/dark conditions under 20-22°C, 50-60% humidity and free access to food and water. The rats were mated and pregnancy was determined by detection of vaginal plagues. The pregnant rats were divided 3 groups of 6 rats in each group, including: experimental, sham and Control.

Study design

The rats in treatment group were randomly exposed to 3mT EMF produced by 50Hz for 4 hours/day during the pregnancy period. The sham groups were kept in a similar condition without exposure to EMF. The control group was kept in standard condition. After delivery, all rats were bleed from the eye angle were kept in -80°C freezer for biochemical analysis and detection of CAT and GPX. For study of fertility F1 generation in *In-vitro* condition, the neonates were kept up to adolescence and at that time IVF assay with sperm and oocyte preparation were performed.

Biochemical analysis

CAT level

Activity of catalase was estimated by measuring its ability to decompose hydrogen peroxide formed due to metabolic reactions into water and oxygen. Hydrogen peroxide has absorbance at 240 nm and the decrease in optical density (OD) is an indicator of the decomposition of hydrogen peroxide by catalase. The decomposition of the substrate (H₂O₂) was recorded spectrophotometrically (Schimadzu UV340) at 240 nm for 60 s and 30 s time interval. Catalase activity was expressed as K/g hemoglobin (Luck, 1965, Aebi, 1984).

GPX level

1unit of glutathione peroxides forms 1.0 μmol NADP⁺ from NADPH per min at pH 8.0 at 25°C. Enzyme units were defined as the number of micromoles of NADPH oxidized per minute. Results were interpreted as International units per unit per gram of Hb (U/gHb) (Beutler, 1984).

Statistical analysis

The data were analyzed and compared with control and sham group through statistical analysis using with SPSS v.19 software by using T-test and P<0.05 is considered as significant.

Survey *In-vitro* fertilization (IVF)

Sperm preparation

At first in adult F1 male's epididymis was removed, then, it was fragmented in Hams F/10 media. Finally it was placed in an oven at 37 °C for 40 min, and then oocytes were fertilized with sperm.

Oocyte preparation

In the adult F1 female for induce of super-ovulation, HMG hormone was injected intra-peritoneal and after 48 hours HCG hormone was injected. Female rats were anesthetized and their ovaries and fallopian tubes were removed and



oocytes were isolated in Flushing medium under a light microscope.

IVF assay

In-Vitro fertilization (IVF) in Universal IVF Medium was performance, then fertilized eggs were preserved in oven CO₂, 37°C. After 24 and 48 hours the number of multicellular embryos and their cleavage pattern was assessed by light microscope.

Results and Discussion

Biochemical analysis showed that in the treatment group CAT level when compared with the control group was found significantly decreasing, while CAT level in Sham group was not found significantly different from the control group. GPX level were found not significantly different between the groups (Table 1).

Table 1: levels of CAT and GPX in control, sham and treatment groups

Groups	CAT (k/g Hb)	P-value	GPX (U/g Hb)	P-value
Treatment	132.10 ± 2.49	0.023 *	28.95 ± 7.43	0.85
Control	140.42 ± 2.66		28.1 ± 7.87	
Sham	139.89 ± 2.54	0.35	30.78 ± 4.07	0.47

Values represent the means ± SD,* P <0.05

The results of present study state that maternal exposure to ELF-EMF from 0 day of gestation lead to decreasing of CAT level in treated groups in

comparison with control group (Table 1). These results indicated that, acute exposure to EMF may modulate the oxidative stress of free radicals by reducing the activation of CAT which is free radical scavenger. Changes in activities of antioxidant enzymes were found as follow 10-day or 2-month exposures to MFs at 0.5-50 μT. (Markkanen, 2009). In the present study, the GPX level was not found significantly different between groups (Table 1). It has been showed that chronic (90 days) exposure to 50 Hz EMF does not cause oxidative stress. They showed that the concentration of antioxidant enzymes in the blood of mice did not increase (Martinez *et al*, 2010). It showed that SOD and GPX activities altered in expose of ELF-EMFs, However not found significant changes in catalase levels (Tiwari *et al*, 2013). Some studies have shown increased oxidative-stress in human fibroblasts under ELF-EMFs exposure (Focke *et al*, 2010). Levels of catalase, GPX and SOD were significantly increased in the liver and lungs of mice exposed to EMF for 8 weeks (Martinez *et al*, 2010).The different results of different studies can be explained by the differences in the duration exposure time, intensity and frequency of EMF.

The photomicrograph observations and investigation of percentage multicellular embryos (blastocysts) showed that rats exposed to magnetic fields during the development period in comparison with control group have impaired fertility and their fertility has decreased (Table 2) (Fig 1, 2). Results in sham group were found not significantly different from the control group.

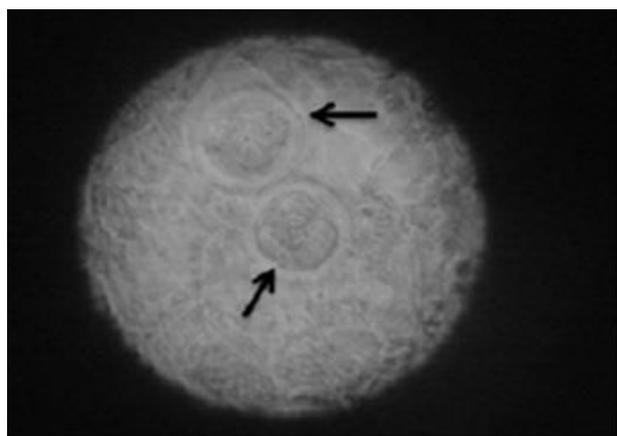


Fig 1: Multicellular embryo in treatment group after 48 hours in *In-vitro* culture medium
 Note blastocyst with irregular cleavage (arrow)



Fig 2: Multicellular embryos in control group after 48 hours In *In-vitro* culture medium
 Note blastocyst with normal cleavage (arrow)



Table 2: Comparison percentage of multicellular embryos in IVF culture medium in treatment (Exposure EMF) with control group

Groups	Number of oocyte preparation	Number of proliferated oocyte	Percentage of multicellular embryos
Treatment	16	9	56%
Control	26	20	77%

The results of present study showed that rats exposed to magnetic fields during the development period at the time of puberty, have impaired fertility. The teratogenicity effects of chemical and physical environmental agents are often related to their ability to damage DNA (Aydin, 2007). In the present investigation, significant decreases in fertility and catalase activities were observed in puberty of F1 generation. Reactive oxygen species (ROS) and oxidative damage to bimolecular as a mechanism of chemical and physical environmental agents may contribute to male infertility by reducing sperm function (Atessahin *et al.*, 2006).

When female mice were super ovulated and mated following exposure to a 50Hz, EMF at 0.5mT 4 hours per day, 6 days a week for 2 weeks, and the number of blastocysts was significantly decreased (Borhani *et al.*, 2011), that this suggests exposure to EMF in the pre-implantation stage could have detrimental effects on embryo development. Male and female rats exposed to magnetic fields 50Hz for 90 days showed a decrease in fertility (Al-Akhras *et al.*, 2001). It showed that magnetic field exposure interrupts the process of the gamete fusion but did not influence gametes, embryos, or embryonic development (Borhani *et al.*, 2011). It showed that *in-vitro* exposure to 50 Hz magnetic field did not induce considerable difference in the number of mouse early blastocyst and late blastocysts (Hannele *et al.*, 2001). In parallel, Pafkova *et al.*, (1996) found no significant alterations in embryogenesis of rat and chick embryos, exposed to 50 Hz EMF. Importantly, the reproductive hazard of EMF exposure in non-pregnant young women has not been studied well. Together, the effect of EMF exposure on embryonic development is still controversial, but some negative effects of EMFs have been reported in some animal models (Gye and Park, 2012).

Conclusion

The results of this study indicated that ELF-EMF in prenatal period cues of oxidative stress in maternal and decrease of fertility in F1 generation that such as study of Behari and Rajamani, (2012) maybe cause of Sperm DNA damage that it regarded as a potential risk factor to the development of normal embryos leading to impaired embryonic development.

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