

# Effects of prenatal exposure to extremely low frequency magnetic field on testicular tissues of male rats

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## Abstract

**Aim:** Extremely low-frequency magnetic field (ELF-MF) is produced by a large variety of electronic devices in everyday use. This study was therefore undertaken to examine the effects of ELF-MF on oxidative stress parameters in rat testicular tissue.

**Material and Methods:** Twenty-five Sprague Dawley rats were exposed to 50 Hz 500 $\mu$ T ELF-MF 24 hours a day, starting from the prenatal period. The exposure continued for further 60 days following the birth. Testicular tissue samples were collected and analyzed. The levels of an oxidative stress marker MDA, and an antioxidant scavenger GSH were investigated.

**Results:** Long-term exposure to ELF-MF increased MDA levels and decreased GSH levels significantly. No correlation was shown between MDA and GSH levels.

**Conclusion:** According to the results of this study, ELF-MF exposure could impair oxidant-antioxidant function and might increase oxidative stress and lipid peroxidation in the testicular tissue.

**Keywords:** ELF-MF; Magnetic Field; Testicular Tissue; Fertility; MDA; GSH.

## INTRODUCTION

Extremely low-frequency magnetic fields (ELF-EMF) have frequencies up to 300 Hz, which are thought to be harmless for biological tissues (1). As the use of technological devices mainly mobile phones, wi-fi systems, and implantation of base stations on a wide range of locations increase, people are exposed to ELF-MF in an increasing dose and frequency, everyday. Although ELF-MF frequencies are assumed to be a source of non-ionizing radiation, adverse biological effects have been also reported including disturbances in fertility, circulation, behavior, and cell proliferation (2). Furthermore ELF-MFs are classified as potential cancer-causing agent to humans by the International Agency for Research in Cancer (IARC) (3).

Oxidative stress is caused by the production of reactive oxygen species (ROS) as a result of respiratory and metabolic processes within the cell (4). Being involved in cancer, cardiovascular and neurological diseases, infertility, and various systemic disease pathogenesis, oxidative stress in a widely studied subject for scientist, worldwide.

Malondialdehyde (MDA) is a late marker of lipid peroxidation which is an indirect indicator of oxidative stress on the tissue. On the other hand, reduced glutathione (GSH) is a major cellular antioxidant protecting the tissues from the adverse effects of oxidative stress.

It is a well-known issue that the sperm count, motility and quality have decreased in males living in urban area when compared to the age-matched subjects located in rural areas and subjects who lived a few decades earlier (5). The data on the possible duration and distance of the ELF-MF for effecting the sperm cell proliferation in the testes are inadequate and controversial. Moreover, people carry along the mobile phones close to reproductive organs for hours within the day.

In our study, we aimed to effect of ELF-MF in the oxidant and antioxidant systems on the testicular tissue of rats. We hypothesized that, exposure to ELF-MF increase oxidative stress in the male reproductive

tissue, and resulting in decreased fertility. In the context of our study, we measured the effect of 50 Hz ELF-MF on the oxidative stress response of the testis tissue of male rats, starting from the prenatal period.

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## MATERIAL and METHODS

### Subjects and animal care

The experiments were performed on 25 Sprague Dawley rats obtained from the Research Center of Bezmialem University. Experimental protocols were approved by the local ethics committee. To archive prenatal exposure female rats whose pregnancy were confirmed with smear were placed into 50 Hz 500 $\mu$ T ELF-MF radiating magnetic field cages for 24 hours for the duration of pregnancy (21 days). Following the birth, newborn rats (n=15) were exposed to 50 Hz 500 $\mu$ T ELF-MF, 24 hours a day, for additional 60 days. The control group consisted of 10 newborn male rats that were not exposed to the ELF-MF, and subjected to same growth conditions with the study group. Appropriate measurements were performed in order to ensure the lack of exposure to ELF-MF. Animals were kept in a 12 h light/12 h dark environment at a constant temperature of 22 °C and 45% humidity, and they received standard laboratory food and tap water ad libitum.

### Experimental Procedure

#### ELF-MF Exposure

ELF-MF were produced using Merritt coil system which produce 50 Hz frequency and 500 $\mu$ T field surrounded in a frame. Internal coils consisted of 11 turns, and external coils have 26 turns. Each pregnant rat was placed into the separate cages, and the cages were located on the shelves of the ELF-MF producing system within the study period. During the exposure duration, the magnitude of the ELF-MF was checked by using a magnetic field probe, taking measurements for every six-minutes (Narda EHD-50D, Germany).

#### Measurement of tissue MDA and Glutathione levels

Testicular tissues were homogenized in cold sodium phosphate buffer (pH 7.4) containing 1 mmol/L EDTA. The homogenates were then centrifuged at 4,000 rpm for 15 min at 4 °C. The supernatants were separated and used for analysis. Tissues homogenates were used for the measurement of the MDA and, GSH level in control and treated groups.

The lipid peroxide levels in testes tissues were measured using a thiobarbituric acid reactive substance (TBARS) assay, which monitors MDA production, based on the method of Beuge and Aust (6). The amount of MDA was calculated using an extinction coefficient (1.56 $\times 10^{-5}$  M/cm).

The concentrations of GSH in testes tissue were determined in a modified optical test system. In this system, GSH is oxidized by 5.5'-dithiobis-2 nitrobenzoic acid and then reduced by GSH reductase with NADPH as the hydrogen donor. The change of absorption was recorded at 412 nm on visible ultraviolet spectrophotometry. The GSH concentration was determined by using the molar absorption coefficient 13.6 $\times 10^{-4}$  M<sup>-1</sup> cm<sup>-1</sup> (7)

### Statistical analyses

Statistical evaluations of study and control groups were performed with the Student's t test using MedCalc program. The results were presented as mean $\pm$ standard deviation (Lowest-highest value). Significance level was accepted as p<0.05. The correlation analysis of the MDA and GSH levels in the study group was performed using the Pearson's correlation coefficient test. Comparison graphs were drawn using the MedCalc statistical program.

## RESULTS

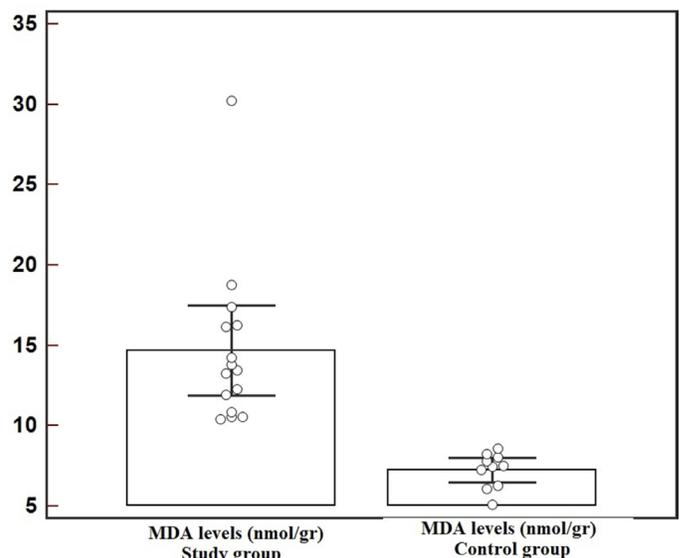
Concentrations of testicular tissue MDA and GSH are summarized in Table 1.

**Table 1. The concentrations of MDA and GSH in tissue homogenates of rat testis**

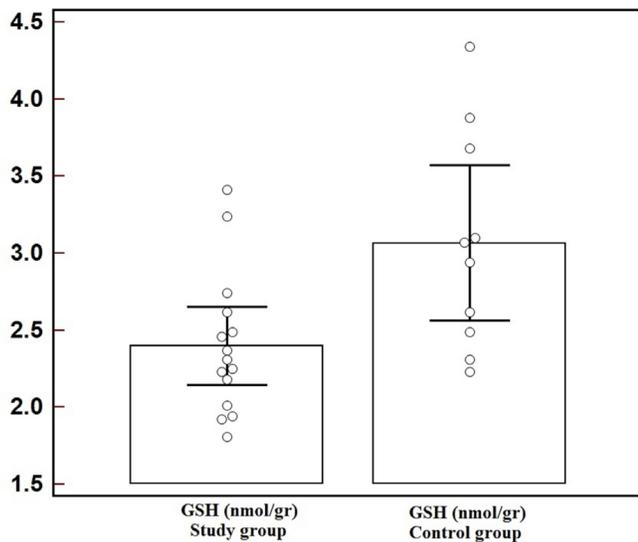
	Control group (M $\pm$ SD)	Study group (M $\pm$ SD)	p value
MDA (nmol/gr)	7.24 $\pm$ 1.09 (5.11-8.60)	14.68 $\pm$ 5.03 (10.41-30.24)	<0.001
GSH (nmol/gr)	3.06 $\pm$ 0.70 (2.23-4.34)	2.39 $\pm$ 0.46 (1.81-3.41)	<0.05

Statistical analyses revealed significant differences for MDA and GSH levels between the study and control groups (p<0.001, p<0.05, respectively). MDA levels were higher, whereas GSH levels were lower in the study group. The mean levels of MDA and GSH in the study group were 14.68 $\pm$ 5.03, and 2.39 $\pm$ 0.46 nmol/gram, respectively. Comparison of distribution data is presented in Figure 1 and 2.

Pearson's correlation test did not show a significant correlation between the MDA and GSH levels (r=-0.082, p=0.77).



**Figure 1.** Comparison graph of MDA levels



**Figure 2.** Comparison graph of GSH levels

## DISCUSSION

As the modern technologies using wireless communication technologies increase, 24 hours exposure to ELF-MF is inevitable mainly people living in the metropolitan regions. A variety of electronic devices in everyday use distribute ELF-MF of different frequencies. Cell phones, base stations, electric bulbs, heaters, wi-fi devices, cordless phones are among the main resources of ELF-MF in the daily basis.

Several diseases of circulatory, nervous, endocrine and reproductive systems due to ELF-MF exposure have been reported in animal and human subjects. It is essential to determine the possible physiological and pathological effects of ELF-MF on different organ system in the organisms.

The effect of long-term ELF-MF exposure on reproductive organs is still unclear and the data are contradictory. Morphological and pathological alterations of the testicular tissue and sperm cells as a result of ELF-MF exposure have been reported (8). Hong et al. showed broken DNA strands of testicular cells in mice exposed to ELF-MF (9). Furthermore, it is possible that ELF-MF increases oxidative stress in the cells resulting in impaired organ and tissue functions (10-13).

Therefore, we aimed to investigate the effects of long-term ELF-MF exposure in terms of oxidative stress on testicular tissue of male rats. According to our findings, long-term 50 Hz 500 $\mu$ T ELF-MF exposure increased MDA levels as the manifestation of lipid peroxidation, and affected antioxidant system by decreasing GSH levels in the testicular tissue of male rats.

MDA is an indirect marker of lipid peroxidation, thus oxidative stress in the cell. And is mainly increased in the tissues with rich lipid content and high oxygen consumption and mitochondria count. One of the main sources of oxidant agents are disturbed metabolic and

respiratory pathways in the cells, resulting in DNA damage and impaired gene expression (14,15). Being the mostly produced lipid peroxidation product, MDA has been shown to be most mutagenic one when compared to other products of lipid peroxidation (16). Taken together, in our assumption, having the mitochondria as the sole energy producer, sperm cells are more prone to MDA production. Besides, it has been reported that when the oxidative damage exceeds the repairing capacity of the cell, apoptotic mechanisms are induced leading to programmed cell death. These mechanisms together might be causing decreased sperm cell number by the triggering effect of ROS.

GSH can be accepted as a scavenger tripeptide for ROS, and with the presence of ROS in the environment, GSH is oxidized into its oxidized form (GSSG). The ratio of GSH to GSSG is used as a marker of oxidative stress (17). Similar to our findings, Canseven et al. found increased levels of MDA and decreased levels of GSH in the heart and liver tissues of guinea pigs with a short-time exposure to 50 Hz ELF-MF of 1, 2 and 3 mT (18). In another animal study of rats exposed to long-term ELF-MF, Manikonda et al. showed increased lipid peroxidation and decreased GSH to GSSG ration in brain regions in a dose-dependent manner (19). Additionally, cell culture studies revealed increased rates of ROS as a result of exposure to ELF-MF in different cell-lines (20,21). Decreased levels of antioxidant system enzymes such as SOD, CAT and Gpx as a result of ELF-MF exposure have been demonstrated on animal and cell culture studies (5).

Among all devices causing ELF-MF, the mobile phones, most widely used devices during the life time recently, are shown to spread three types of emissions including a GSM system a 900 MHz radio frequency, more than 217 Hz pulsing signal and an ELF-MF (22). However, the mentioned study was conducted in 2005, and mobile phone technologies have been largely evolved up to date. Thus, novel findings on the ELF-MF exposure from new smart phones are required in order to get a better understanding on the radiation levels from these devices. Precautionary guidance values have been set limiting the daily exposure to ELF-MF from various devices for more than 4 hours a day have been set by European Academy for Environmental Medicine (EUROPAEM) – EMF working group in 2016. According to those regulations, offered daytime and nighttime exposure values were defined with a mean value of 100 nT, and maximum value of 1000 nT (23).

Significant impairment of the quality, motility and number of sperm cells as a result of environmental conditions is a well-documented fact within the last decades. Akdag MZ et al did not find a positive relationship between the levels of oxidative stress parameters and rate of ELF-MF exposure, however they showed a higher apoptosis score of testicular cells in the study group (24). It has been shown that, a prolonged exposure to 50 Hz 1G ELF-MF is toxic to testicular functions prompting decreased motility,

cell number and testicular weight in rat testes (25). The usual carrying position for mobile devices and portable computers are close to reproductive organs. Therefore, it is possible to assume that, daily used electronic devices might have a negative effect on reproductive function due to their potential on producing ELF-MF.

## CONCLUSION

In conclusion, ELF-MF exposure might be affecting reproductive potential of the sperm cells as a result of increased oxidative stress and lipid peroxidation and decreased antioxidant enzymes and metabolites. However, the division of samples in subgroups in terms of time-of-exposure and dose in our study groups might have yielded different results in terms of MDA and GSH levels. Also, additional studies measuring the levels of different members of the oxidant and antioxidant system members in the tissue samples exposed to different durations and doses of ELF-MF are required.

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