Effect of extremely low-frequency electromagnetic field exposure on Leydig cell counts in male Swiss webster mice

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Abstract. Widespread use of electronic technology increases the risk of future health problems, including those involving the reproductive system. Animal-model studies have not yet comprehensively examined the effect of electromagnetic fields by studying exposure over consecutive generations. The present study investigated the effect of continuous, extremely low-frequency electromagnetic field (ELF-EMF) exposure on the Leydig cells in 3 generations of male Swiss Webster mice. The mice were distributed into 3 groups with different exposure levels (3, 4, and 5 kV) and a control population (0 kV). The numbers of Leydig cells in all the exposed groups were significantly reduced compared with the control group (p < 0.05). There were also significant decreases in Leydig cell counts in each subsequent generation of the 3 and 4 kV exposure groups (p < 0.05). It was concluded that ELF-EMF exposure negatively affects the Leydig cell population, thereby reducing spermatogenic capacity.

1. Introduction

Infertility is one of the main health issues encouraging couples of childbearing age to check the condition of their reproductive system. International survey results in 2007 estimated that 56% of couples worldwide seek treatment for infertility problems [1]. Surveys by the World Health Organization of 1990–2004 indicated that 1 in 4 couples were affected by infertility that persisted until 2010 [2]. On the other hand, there has been an increase in the number and types of electronic devices in everyday life that can potentially affect biological activity in the human body [3]. Research on the in vitro stage of experiments at the animal level have proven that electromagnetic wave exposure can affect the functioning of the nervous system and memory formation, hormonal activity, cardiovascular function, and cell growth rates in embryos and malignancies [4–8].

Electromagnetic wave exposure can occur at home, workplace, and public facilities [9]. Men who are highly active [mobile] are particularly at risk of increased exposure to electromagnetic waves because the location of the testis is only covered by the skin, compared with women for whom the ovum is in the pelvic cavity. The testes contain Leydig cells that play an essential role in spermatogenesis. We therefore conducted
an experimental study to determine the effect of extremely low-frequency electromagnetic waves (ELF-EMF) exposure on the Leydig cell counts in male mice. We used Leydig cells as a representative model to investigate the possible implications on the male reproductive system. This study aimed to demonstrate if there were any effects of ELF-EMF exposure on Leydig cells and how this was, in turn, affected by the different stress levels applied across different generations. We found significant reductions in Leydig cell counts over 3 successive generations of mice continuously exposed to ELF-EMF. This has important implications for male infertility, and future studies are necessary to further explore this issue.

2. Methods
Swiss Webster mice were selected from the animal house of the Department of Biology, Faculty of Medicine, Universitas Indonesia, after a thorough application of several health criteria including the absence of congenital abnormalities. This parental group was then randomly divided into a control group with zero exposure to extremely low frequencies of <300 Hz (0 kV/10 cm) and 3 experimental groups with different exposure levels (voltage of 3 kV/10 cm with a magnetic field strength of 5.5 μT, 4 kV/10 cm with a magnetic field strength of 5.4 μT, and 5 kV/10 cm with a magnetic field strength of 5.3 μT). The appropriate sample size of 7 mice in each group was determined using Federer’s formula. Parental mice produced the first derivative mice (F1) that comprised the first generation of each of the 4 groups. Each group of F1 mice was exposed to the relevant level of ELF-EMF for 2.5 months. The male and female F1 mice were then randomly selected for mating to produce the F2 generation, which was then exposed to 2.5 months of the relevant level of ELF-EMF. The same procedure was performed with the F3 generation. Seven mice from each generation of each exposure group were randomly selected to undergo euthanasia and testicular surgery. Paraffin blocks of testicular tissue sections were prepared and stained. Observations of Leydig cell counts were performed using a light microscope at 400× magnification by counting the cell numbers in 20 fields of view for each sample preparation. The results were then processed using SPSS 16.0 software and tested for normality using the Komogorov–Swirnov test. The statistical significance of differences across groups was analyzed using the Kruskal–Wallis test, and post-hoc Mann–Whitney U-test was used to analyze the significance of differences between groups.

3. Results
Microscopic examination of testicular tissue sections indicated significant reductions in Leydig cell counts for all generations and all exposure levels compared with the control (Table 1).

| Generation | Exposure levela | Controlb |
|------------|----------------|----------|
|            | 3 kVb          | 4 kVc    | 5 kVd    |
| F1         | 34             | 34       | 24       |
| F2         | 34             | 34       | 25       |
| F3         | 30             | 29       | 24       |

aControl groups had significantly higher Leydig cell counts than each of the experimental groups (p < 0.05).
b,c Consecutive generations had significant reductions in Leydig cell counts (p < 0.05).
dAll generations of the 5 kV group had significantly lower Leydig cell counts than those of the 3 and 4 kV groups (p < 0.05).
The Leydig cell count was significantly higher in the control group that did not receive any added ELF-EMF exposure than in any of the experimental groups (p < 0.05; Figure 1). Among the experimental groups, the groups exposed to a voltage of 3 kV/10 cm with a magnetic field strength of 5.5 μT in each generation had the highest Leydig cell count, whereas the groups exposed to 5 kV/10 cm with a magnetic field strength of 5.4 μT had the lowest Leydig cell count. No differences were observed between generations of the control group; however, increasing reductions in Leydig cell counts were observed over consecutive generations of the exposed groups, particularly for the 3 kV (p < 0.05) and 4 kV (p < 0.05) exposure groups.

![3 Generations of male swiss webster male](image)

**Figure 1.** Comparison of the Leydig cell counts between generations in the control and intervention groups.

Figure 2 shows a comparison of the Leydig cell count between the generations in each group. The control group showed a higher Leydig cell count than the intervention group. In the intervention group, the highest Leydig cell count appeared in the F1 generation and the lowest in the F3 generation in each group. In contrast with other intervention groups, the highest exposure group (5 kV/10 cm with a magnetic field strength of 5.3 μT) had the highest Leydig cell count in the F2 generation. The result of the analysis using Kruskal–Wallis test showed that there were at least 2 significant different groups (p = 0.000).

![Exposure group](image)

**Figure 2.** Comparison of the Leydig cell counts between the control group and the intervention in each generation.

Statistical analysis using Mann–Whitney U-test in a group of mice exposed to 3kV voltage EMV and 5.5 μT magnetic field strength showed a significant decrease in the Leydig cell count from the F3 group to the F1 and F2 groups. This was also found in mice in a higher exposure group of 4kV with a magnetic field strength of 5.4 μT. In the 5 kV group with a magnetic field strength of 5.3 μT, there was a decrease in the Leydig cell counts in the F2 and F3 generations compared with the previous generation, but not statistically significant. The decrease in each generation was significant when compared with the control group without exposure.
Based on the Leydig cell counts in each exposure group, there were no significant decreases among any of the generations in the 4kV group compared with those in the 3kV group. There were, however, significant differences among generations of the 5 kV group and those of the 3 and 4 kV groups (p < 0.05).

4. Discussion
In this study, we exposed Swiss Webster mice to ELF-EMF from an embryonic age to 2.5 months. In the experimental groups, mice were exposed to electromagnetic waves with a voltage of either 3, 4, or 5 kV/10 cm with magnetic field strengths between 5.3 and 5.5 µT. Continuous exposure and observation of up to 3 generations of mice (F3) was performed to determine the cumulative effects of exposure over several generations, which we hypothesized would increase the risk of effects on the reproductive system, particularly on the Leydig cells in the testes.

Despite numerous similar animal-model studies published to date, differences of opinion still remain regarding the influence of EMF on mice. Some of these studies used EMF, whereas others used magnetic fields alone. The wave frequency used also varied from radiofrequency waves to ELF waves. In addition, different magnitudes of stress and magnetic force were applied, with different durations and onset of exposure. This variety of methods used may, at least in part, explain the inconsistencies between these studies.

This study found a decreasing numbers of Leydig cells at higher EMF stress levels, with significant reductions in the 5kV exposure group compared with the 3 and 4 kV groups, and significant reductions were observed between all experimental groups and the control group for each generation.

Previous studies have suggested several possible mechanisms that may lead to reductions in the Leydig cell counts; one of these relates to decrease in luteinizing hormone (LH) levels. Several studies have reported an association between EMF exposure and gonadotropin hormone levels. This was evidenced, for example, by the low LH levels observed in mice exposed to electromagnetic fields that conferred high doses of radiation [10]. However, other studies using waves on cell phone frequencies did not show significant changes in LH levels [11].

LH acts as a receptor on the surface of the Leydig cell membrane. LH is a hormone involved in the development and steroidogenesis activity of Leydig cells. LH is also known to cause Leydig cell growth at the end of fetal development to produce testosterone as a marker of birth masculinity. Decreased LH levels due to EMF exposure may therefore be one of the mechanisms involved in the decreased Leydig cell counts observed in our study.

However, other studies using different sources of stress and magnetic field strengths did not show any effect of EMF on gonadotropin hormone levels. Effects of EMF on spermatogenesis were hypothesized to occur directly and not as a result of decreased gonadotropin hormone activity. Other studies have also refuted any changes in melatonin levels caused by EMF exposure.

The decrease in the Leydig cell count may also have been caused by the direct destruction of Leydig cells without hormonal mediation. This is evidenced in previous studies on mice using 1000 or 2000 Hz magnetic exposure, which are higher frequencies than those within the ELF range [12]. Cell damage activates apoptosis, which may also explain the decreased numbers of Leydig cells after exposure. This theory is supported by a study by Kumar et al. in Wistar rats exposed to microwaves of 2.45 GHz [13]. Other studies using radio frequencies of 2,450 MHz similarly showed damage to DNA in the testes, whereas at frequencies of 900 MHz and 1.7 GHz, DNA damage occurred in spermatozoa in the cauda epididymis [14]. Despite the frequency differences used in our study, i.e., extremely low frequencies <300 Hz, these studies suggest another possible mechanism involved in the reduction of Leydig cell counts. Interestingly, another study with an ELF of 100 Hz showed a reverse effect that could help treat infertility caused by previous higher-frequency exposures [13].
In addition to the direct effects of cell damage, EMF exposure also affects steroidogenesis. EMF generated by cellular phone signals can inflict damage on the protein kinase C (PKC) enzyme [15]. Leydig cells are an important center for the production of specific steroid hormones. PKC in Leydig cells promotes increased cholesterol intake, and this cholesterol is then transported by the StAR protein into the mitochondria to be converted into pregnenolone. Subsequent processes produce progesterone and then testosterone. Therefore, reductions in the number and activity of Leydig cells would certainly affect testosterone levels.

Some researchers argue that EMF exposure often presents an unrepresentative histopathological picture of Leydig cell activity. Leydig cell counts in exposed mice are often found to be normal or not significantly different from those in non-exposed mice. Therefore, several researchers have also measured steroidogenesis activity in Leydig cells by growing Leydig cell cultures and assessing their results. A study by Forgacs et al. showed response of testosterone in Leydig cells cultured from mice that had had their entire bodies exposed to magnetic waves with a magnitude of 100 µT and a frequency of 50 Hz (classified as extremely low frequency), although no changes were observed in basal cell production and serum testosterone levels [16]. Similar studies using higher wave frequencies such as 1,800 MHz (GSM-like microwave exposure) did not, however, show any significant changes in testosterone levels in Leydig cell cultures compared with the non-exposed group [17].

Contrary to the results of our study, other researchers have observed increases in Leydig cell counts regardless of their associated pathophysiology due to EMF exposure. This is supported by the majority of current research results. Studies by Saadeldin et al. Kim et al. and Kalan et al. demonstrated higher Leydig cell counts in the EMF-exposed experimental groups than those in the control group [18,19,20]. The study by Saadeldin et al. [18] used histopathology to similarly prove the increased activity of Leydig cells based on the increased reactivity of their cytoplasm to PAS staining compared with cells in the control group. Leydig cell hyperplasia is associated with increased testosterone levels as compensation for the damaged spermatogenesis process. This compensation has also been demonstrated in a study linking EMF exposure with increased ROS and p53 levels, both of which play a role in oncogenesis [13].

Certain theories discussed above appear to contradict the results obtained in this study. These discrepancies may be due to the different ELF of electromagnetic waves used in our study, which might not have induced any compensatory response toward spermatogenesis damage following ELF-EMF exposure.

Nevertheless, there are a number of studies that demonstrate a reduction in Leydig cell counts following EMF exposure. For example, a study by Saygin et al. [21] using mice exposed to 2.45 GHz electromagnetic radiation for 60 min per day for 28 days showed a significant decrease in Leydig cell counts in the experimental group compared with the non-exposed controls.

In addition to comparing different voltage exposures, this study compared the cumulative effects of exposure over consecutive generations. We observed successive reductions in Leydig cell counts over each generation, with the lowest Leydig cell counts obtained in the F3 groups for the 3 and 4 kV exposure groups. This effect was, however, not observed in the 5 kV exposure group with a magnetic field strength of 5.3 µT. Therefore, there is a potential for inherited exposure effects that accumulates in each generation.

Leydig cells first differentiate during the fetal period. EMF exposure during embryogenesis affects the number and activity of Leydig cells at birth, thus affecting the steroidogenesis capacity of the reproductive organs when they undergo maturation in the adult phase. This is consistent with a previous study that used 3 µT magnetic force exposure with 50 Hz frequency during gestation from day 0 to birth [22]. Decreased numbers of Sertoli cells and damage to the gonocytes and seminiferous tubules were observed. Although no changes in the Leydig fetal cell population could be concluded, there was potential for a decreased number of these cells in these individuals as adults. This is because Sertoli cells play a role in signaling that determines the growth, development, and steroidogenic activity of adult Leydig cells [23]. Another study by Sari et al. [24] used Swiss Webster mice exposed to ELF-EMF and investigated the differences in
frequency of chromosomal damage between the F1, F2, and F3 generations. The incidence rate was not significantly different among generations with the same exposure level; however, significant increases in chromosomal damage were observed between the experimental and control groups. Further research is needed on the cumulative effects of EMF exposure over time and across generations, with a particular focus on the number and activity of Leydig cells.

5. Conclusions
ELF-EMF exposure at voltages of 3, 4, and 5 kV was observed to reduce the Leydig cell count in Swiss Webster mice. Cumulative reductions in the Leydig cell counts were also observed over 3 generations of mice. Such reductions may have been caused by various factors such as increased levels of Leydig cellular damage, leading to increased levels of apoptosis, and DNA damage in the gametes, leading to inherited effects.

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