Effect of extremely low frequency electromagnetic field exposure on pachytene spermatocyte cell quantity in Webster strain male mice

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Abstract. Many electronic devices that are used in daily life can produce an extremely low frequency (ELF) electromagnetic field that may disturb human reproductive organs. Several studies have shown disturbances in spermatogenesis, which decreases sperm production. We aimed to determine the accumulative effect of exposure to a low frequency magnetic field on pachytene spermatocyte cells in mice. The cells were exposed to electromagnetic fields of 0 kV (control), 3 kV/10 cm 5.5 µT (Group 1), 4 kV/10 cm 5.4 µT (Group 2), and 5 kV/10 cm 5.3 µT (Group 3) in first (F1), second (F2), and third (F3) generation mice. The cell number decreased in all exposure Groups compared to the control Group (\(P < 0.05\)). Group 1 exposure had no accumulative effects on any generation (\(P > 0.05\)). Group 2 exposure showed an accumulative effect on the F1 and F2 generations, while Group 3 exposure had an accumulative effect on F2 and F3 generations. Moreover, for all three groups, exposure was correlated with proportional decrease in cell number. ELF electromagnetic field exposure caused decreased pachytene spermatocyte cell numbers and had an accumulative effect on each generation.

1. Introduction

Human love of technology, especially for highly functional electronic devices, is apparent. However, the high rate of use of these electronic devices can impact human health owing to exposure to electromagnetic field radiation [1]. Electromagnetic fields comprise naturally (earth’s magnetic field with 0.1–0.5 kV/m power) or artificially (electronic devices such as radio, washing machine, refrigerator, television) sourced electromagnetic waves. Electronic devices produce extremely low frequency (ELF; 3–3,000 Hz) electromagnetic waves [2,3].

Kim and Cho reported that workers tend to be exposed to electromagnetic fields more often than nonworkers [4]. The biological effects of exposure to electromagnetic field were firsts studied by Wetheimer and Leeper, who demonstrated a risk of leukemia in children exposed to electromagnetic filelds [5]. Jing-Wen Sun et al. concluded that exposure to electromagnetic field increased the risk of breast cancer in men [6].

The Indonesian Doctors Association (IDI) and World Health Organization state that electromagnetic waves have various health effects, including disruption of the blood circulation system, heart and blood...
vessels, nerves, and endocrine system, and hypersensitivity [7,8]. One of the most affected systems is the reproductive system. One study found that electromagnetic waves resulted in the disruption of testosterone and follicle-stimulating hormone levels in male rabbits, and also caused physiological changes in male germinal behavior and structure, including a significantly decreased epididymis and vas deferens in mice. In humans, significant use of cell phone reduces sperm motility [8,9].

The use of various electronic devices sometimes is continuous, from waking up until even after bedtime. Effects of exposure to electromagnetic fields are no longer temporary, but continuous and cumulative. Therefore, the present study was designed to examine the effect of very low frequency electromagnetic field exposure on the number of pachytene spermatocytes male stem cells of first (F1), second (F2), and third (F3) generation Webster strain mice using field strengths of 0 kV (control), 3 kV/10 cm 5.5 µT (Group 1), 4 kV/10 cm 5.4 µT (Group 2), and 5 kV/10 cm 5.3 µT (Group 3).

2. Methods

2.1 Study design

The study protocol had been approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital. This study used 24 male Webster Strain Mus musculus L mice (age, 3 months; weight, 30–40 g). The Federer formula was used to determine sample size: \((t - 1)(n - 1) \geq 15\) \((t = \text{treatment}, n = \text{samples size per Group})\). This study had four experimental groups (control + Groups 1–3); thus, in this study, \(t = 4\). Further, the sample size required for each treatment was 6 mice of each generation, so the four generations (parental, F1, F2, F3) resulted in a total of 24 mice. The animals were maintained in a special electromagnetic field exposure cage at 22°C with good air circulation, 12-hour dark and light cycles, and standard nutrition consumption. At euthanization, mice were anesthetized using ether.

This study used a random stratification design with three generation variables (F1–F3) and four voltage variables (controls, and Groups 1–3).

2.2. Obtaining F1, F2, and F3 Generation Mice

Male and female mice (a parental pair) were obtained from the animal house of the Biology Department, Faculty of Medicine, Universitas Indonesia, and were exposed to an electromagnetic field of 3 kV/10 cm with magnetic power of 5.5 uT. These mice then produced a new generation (F1), which had already been exposed to electromagnetic fields which were previously exposed to electromagnetic fields at conception. Those mice were raised to adulthood (2.5 months old), and then a male and female pair were randomly selected. During growth and development, the mice were continuously exposed to a 3 kV/10 cm electromagnetic field. These F1 mice then produced the F2 generation, and this method was continued until the F3 generation was produced. F1 and F2 generation mice that were not selected to produce the next generation were euthanized to obtain samples. This process was also followed using the other voltage variables.

2.3. Exposure of Mice to Electromagnetic Fields

When the mice were 2.5 months old, the parental mice were placed into four different cages covered with a wire netting. Three of the cages were electrified with a current that produced electromagnetic field strengths of 0 kV (control), 3 kV/10 cm 5.5 µT (Group 1), 4 kV/10 cm 5.4 µT (Group 2), and 5 kV/10 cm 5.3 µT (Group 3). All four cages were placed on an aluminum plate, which worked as negative electrode, while the wire netting covering the cage worked as a positive electrode. These electrodes were installed with a 10 cm gap between them and were connected to a high voltage electric generator, which was also connected to a regulator that worked to stabilize the voltage.
2.4. Surgery and specimen preparation
The exposed mice were euthanized with ether before undergoing surgery. Then, the mice were laid out on a surgical table (a Styrofoam plate coated with aluminum foil), and the abdomen was swabbed with alcohol. Surgery was performed with sterilized scissors and tweezers.

The testicle was surgically removed and fixed for 24 h using a biological nitrogen fixation (BNF) solution. Next, the BNF solution was removed and the specimen was washed with graded alcohol: 70%, 80%, 90%, 100%, xylol 1, and xylol 2 (one hour each), and 1:1 xylol:paraffin for 30 min. Then paraffin 1, 2, and 3 infiltrations were done in the oven for 1 h. The specimen was pressed into a paraffin block until frozen and affixed firmly on the holder. Lastly, the specimen was soaked in xylol 1 and 2, each for 20 min.

2.5. Cutting and Staining of Microscopic Specimens
Paraffin specimens were cut using a microtome and soaked in xylol 1 and 2, each for 20 min; then the specimens were stained with Ehrlich’s hematoxylin for approximately 5 s, and were washed with tap water for 10 min and distilled water for 1 min, and stained in different concentrations of alcohol (30%, 50%, 70%, 80%, and 90%), each for 1 min each, and in xylol 1 and 2, each for 10 min.

2.6. Collecting and Analyzing Data
The testicular specimens were microscopally observed at 100× to evaluate pachytene spermatocyte cells. Data were collected and statistically analyzed using SPSS v.16 for Windows. Inferential statistical analysis was performed using a bivariate (crosstab) test between the independent and dependent variables to determine the $P$ value.

3. Results
The exposed (Groups 1–3) and nonexposed (control) groups were compared to determine differences. Comparisons also were performed up to the next generations to determine whether there was any cumulative effect.

![Figure 1](image)

**Figure 1.** Average number of pachytene spermatocyte cells after every treatment

There was a significant decrease ($P < 0.05$) in the average number of pachytene spermatocyte cells in the three exposure Groups compared to the control Group (Figure 1). The Kolmogorov–Smirnoff
normality test showed an abnormal data distribution. Therefore, a nonparametric Mann–Whitney U-test was used to test the hypothesis of two independent samples (Table 1).

### Table 1. P values of mann–whitney U-test

| Control | 3 kV F1  | 4 kV F1  | 5 kV F1  |
|---------|---------|---------|---------|
|         | 0.000   | 0.000   | 0.000   |
| Control | 3 kV F2  | 4 kV F2  | 5 kV F2  |
|         | 0.000   | 0.000   | 0.000   |
| Control | 3 kV F3  | 4 kVF 3 | 5kVF3   |
|         | 0.000   | 0.000   | 0.000   |

| 3 kV F2  | 3 kV F3  |
|----------|----------|
| 0.0966   | 0.187    |

| 3 kV F2  | 4 kV F3  |
|----------|----------|
| 0.002    | 0.000    |

| 4 kV F2  | 5 kV F3  |
|----------|----------|
| 0.636    | 0.000    |

| 3 kV F1  | 5 kV F1  |
|----------|----------|
| 0.051    | 0.000    |

| 4 kV F1  | 5 kV F1  |
|----------|----------|
| 0.051    | 0.000    |

| 3 kV F2  | 5 kV F2  |
|----------|----------|
| 0.000    | 0.005    |

| 4 kV F2  | 5 kV F2  |
|----------|----------|
| 0.000    | 0.034    |

| 3 kV F3  | 5 kV F3  |
|----------|----------|
| 0.000    | 0.000    |

| 4 kV F3  | 5 kV F3  |
|----------|----------|
| 0.000    | 0.000    |

Based on the cumulative effect results, no significant relationship was found in the median strength of Group 1 exposure from F1 to F2, F2 to F3, and F1 to F3. However, based on the average seen in Figure 1, there was a decrease in average number of cells, even though it was not statistically significant. A similar result also was noted in the magnetic field power of Group 2 from F2 to F3 and Group 3 from F1 to F2. Meanwhile, a change was noted in the shape of the testicular tissue. Normal seminiferous tubules were moon-shaped (Figure 2), but the shape became irregular (Figure 3, Figure 4).

**Figure 2.** Testicular tissue of unexposed mice (control)
4. Discussion

4.1. Effects of Electromagnetic Fields of Various Voltages on the Number of Pachytene Spermatocyte cells

In our study, decrease in pachytene spermatocyte cell numbers was caused by electromagnetic field exposure, which affects various cellular mechanisms, including changes in endocrine hormonal regulation, gonad function, embryonic development, pregnancy, and fetal development [8, 10–13]. A histopathological study by Khayyat showed significant changes in the microscopic structure of mice tissues, which include seminiferous tubular atrophy, interstitial tissue extension between seminiferous tubules, germinal cell necrosis of seminiferous tubules, decreased Sertoli cells, decreased spermatogenesis rate, and germinal cell degeneration [14]. Continuous exposure to an electromagnetic field may induce germ cell apoptosis in mice as demonstrated by Lee et al. [15]. Apoptosis may spontaneously occur and may be more frequent when spermatogenesis has reached the spermatocyte stage. This may lead to weight loss of the testis. Tenorio et al. used a 60 Hz electromagnetic field with a magnetic field power of 1 mT and demonstrated decreasing diameter, area, height, and total volume of seminiferous tubules, and number of Leydig cells; thus, inhibiting progression and development in the testes [16]. Microscopically, Khaki et al. also observed damage to the prostate gland in mice exposed to electromagnetic fields [17].

4.2. Increased Radical Free Induction, Ca2+ Influx, and Changes in Protein Structure

At the cellular level, electromagnetic fields induce increased free radicals, and Ca²⁺ calcium ions can inhibit cell growth, alter protein structure, and damage DNA [8]. One mechanism illustrating the effects of exposure to electromagnetic field is the formation of a pair of free radicals because of changes in equilibrium reaction in cells. Electromagnetic fields with a frequency of 50/60 Hz can prolong the presence of free radicals in cells and increase their concentration. Increased concentration of free radicals, reactive oxygen (ROS) or nitrogen (RNS) species, can induce oxidative and nitrosative stress thus leading to tissue damage [18]. Structural molecules such as lipids, proteins, carbohydrates, and
amino acids may be damaged due to reaction with ROS/RNS. Changes in enzyme activity, gene expression, and membrane structure and DNA damage are the main effects of ROS/RNS. Electromagnetic fields can induce oxidative stress by forming lipid peroxidation and reducing gonadotropin-stimulating hormone (GSH) levels as a free radical feeder [19,20].

4.3. DNA damage
Exposure to nonionized electromagnetic fields can cause DNA, inducing cancer. Also, DNA damage can cause cellular malfunction, leading to apoptosis. Exposing vero cells to ELF electromagnetic fields resulted the ROS production, which then led to DNA damage [21].

4.4. Decreased Hormone Activity
Several studies have reported that exposure to electromagnetic fields could disrupt the secretory functions of the pineal gland in humans and several other species [22]. In addition, exposure to electromagnetic fields also may affect the cycle of melatonin secretion in some species. For example, Long-Evans mice were exposed to a 50 Hz electromagnetic field for 6 weeks, resulting in decreased pineal gland activity and melatonin levels [23]. In cows, 60 Hz electromagnetic field exposure for 4 weeks was found to interfere with circulation of melatonin levels, estrus levels and estrus cycles [24]. A similar result also was obtained in adult Djungarian hamsters [25]. Melatonin is secreted by the pineal gland and acts as an antioxidant [21-26]. Melatonin has a potential function as an anti-apoptotic sperm cell with its free radical destruction mechanism [27,28]. In addition, Meo et al. reported that long-term (3 months) exposure to cellular phones resulted in decreased testosterone levels in Wistar albino mice [29]. In male mice, testosterone is the main sex hormone that has a role in the reproductive system. The same result also was obtained by Kumar S [30].

4.5. Leydig Cells and Sertoli Cell Degeneration
Leydig cells located in the interstitial testes have an important role in hormonal regulation, which is the secretion of testosterone. This secretion is stimulated by the luteinizing hormone (LH) produced by the anterior pituitary. Meanwhile, Sertoli cells that trigger spermatogenesis are stimulated by follicle-stimulating hormone (FSH) and produce estrogen. Sertoli cells also produce special nutrients to keep sperm needs sufficient [31,32]. Exposure to electromagnetic fields can induce apoptosis in both Leydig and Sertoli cells.

4.6. Increase in Temperature
Hyperthermia or heat increase in the testes can lead to abnormalities in spermatogenesis and result in abnormal sperm production [33]. Certain heat and chemical inductions may lead to changes in seminiferous tubular structure [15]. In some studies, a very high frequency induction of 2.45 GHz triggered cellular stress in the thyroid gland of mice but did not trigger apoptosis [34]. Other studies using a frequency of 2.45 GHz affected spermatogenesis and caused apoptosis in testicular tissue due to heat and stress [35].

4.7. Increase in Testicular Blood Barrier Permeability
Testicular blood has an important role in the reproductive system of male mice. A variety of factors increase the permeability of the blood barrier thus generating antisperm antibodies that are key to infertility in male mice [36]. Exposure to an electromagnetic field may damage the intercellular linkage of the blood barrier, lowering the testicular defense system [37]. This may lead to apoptosis or spermatogenic cellular necrosis [37].

4.8. Effects of Electromagnetic Fields on the Number of Pachytene Spermatocyte cells in F1, F2, and F3 Generation Mice
The negative effects of exposure to electromagnetic exposure can disrupt spermatogenesis. As described earlier, it lowers sexual hormonal activity, damages germinal cells, and induces apoptosis. As a result,
the development of pachytene spermatocyte cells may also be inhibited, leading to a decrease in the number of cells [8,10,12].

In the mice exposed to 3 kV/10cm 5.5 µT, no statistically significant change was observed in the F1, F2, or F3 generations. This suggested that 3 kV/10 cm 5.5 µT exposure did not have an accumulated effect on any generation of the mice in this study. The underlying mechanism of the absence of such effect was that the applied voltage was not sufficient enough to cause the accumulative effect on the next generation. In addition, the mechanism of cell adaptation to electromagnetic exposure and cellular DNA repair can manage the negative effects of electromagnetic exposure [13,38].

In mice exposed to 4 kV/10 cm 5.4 µT, there was a significant decrease in the amount of spermatocyte cells from F1 to F2, but not from F2 to F3. This observation may be explained by the influence of physiological and pathological adaptation of body. The degradation of Sertoli and Leydig cells thus lowering the function can trigger positive feedback on the hypothalamus. The hypothalamus will secrete gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary to secrete FSH and LH. FSH will increase Sertoli cell activity, while LH will increase Leydig cell activity. Thus, a decrease in the amount of pachytene spermatocyte cells from F2 to F3 will appear to be less significant [31,32].

Mice exposed to 5 kV/10 cm 5.3 µT showed no significant changes from F1 to F2. Significant change was seen only from F2 to F3. This incidence differed from exposure to 4 kV (Group 2). One factor that could cause this difference was the genetic variation that caused high levels of reproductive hormone activity in mice. In addition, DNA repair activities also could occur so quickly that mice had strong defense mechanisms against electromagnetic exposure. However, in the F2 to F3 exposure, the defense mechanism decreased and the cumulative effect appeared as a significant decrease in the amount of pachytene spermatocyte cells [21,37]. Upon comparing the stress applied to the first generation, there was no significant difference between electromagnetic field with strength of 3 kV/10 cm 5.5 µT and 4 kV/10 cm 5.4 µT. However, the difference became significant when compared to 5 kV/10 cm 5.3 µT field strength. Unlike the F2, the effect of exposure to electromagnetic fields of any strength resulted in significant differences. Similar results also were obtained for F3. The 3 kV/10 cm 5.5 µT and 4 kV/10 cm 5.4 µT electromagnetic fields were not significantly different. Thus, exposure to 3 kV / 10cm 5.5uT and 4 kV / 10cm 5.4uT in F1 had no significant effect. However, for F2 and F3, all the three electromagnetic field groups had a significant impact. This may be attributable to the accumulative effects of exposure to the electromagnetic field.

5. Conclusion
ELF electromagnetic field exposure decreased the number of pachytene spermatocyte cells and had an accumulative effect on its generation. Higher voltage levels and decreasing the generation of mice resulted in narrowed diameter of seminiferous tubules.

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