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Effect of extremely low frequency electromagnetic fields on the diameter of seminiferous tubules in mice

P D Esa¹, D A Survandari^{2*} and P Sari²

¹Medical Education Master Program, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430, Indonesia ²Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430. Indonesia

*E-mail: anitabio@yahoo.co.uk

Abstract. Electromagnetic field radiation has the potential to disrupt the reproductive system, especially spermatogenesis, because the more superficial position of the male testes compared with the female ovaries increases the chance of exposure. Therefore, this study aimed to determine the effect of extremely low frequency electromagnetic field exposure on the diameter of seminiferous tubules over 3 generations of Swiss Webster mice. Using an experimental study design, we exposed the mice to 3 different voltages and magnetic fields: 3 kV/10 cm with a magnetic field of 5.5 iT; 4 kV/10 cm with a magnetic field of 5.4 iT; and 5 kV/10 cm with a magnetic field of 5.3 iT. The data were analyzed using the Kruskal-Wallis or Mann–Whitney test, as appropriate, and significance was set at an α value of 0.05 with 95% confidence intervals. This study found a significant decrease in the diameter of the seminiferous tubules in the intervention group compared with the control group (p < 0.05), and this decrease tended to be in direct proportion with increasing voltage and number of generations.

1. Introduction

Electromagnetic fields (EMFs) can occur naturally or be produced by man-made electrical equipment [1]. The electric component of the field is formed by a voltage difference, with higher voltages forming stronger fields and the intensity decreasing as the distance to the source increases. In contrast, the magnetic component of the field occurs when the electrical current flows with its power in line with the high current flow [2]. Although humans are exposed to the Earth's natural EMF of 0.1-0.5kV/m, modern lifestyles are leading to increased exposure to man-made EMFs [1]. These are typically extremely low frequency (ELF) or very low level EMFs with frequencies in the range of 3-60 Hz. Typically, ELF-EMFs produced by military communications devices result in the smallest exposure [1], while those produced by everyday human appliances are associated with much higher exposures (e.g., microwave ovens, washing machines, refrigerators, televisions, mobile phones, and computers) [2,3].

The association between EMF exposure and human health has been widely studied in recent years, gaining worldwide attention [2]. According to the Indonesian Doctors Association and the World Health Organization, potential consequences of EMF exposure include leukemia, malignant lymphoma, heart rhythm disturbance, nerve degeneration, disordered melatonin metabolism,

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hypersensitivity, and male reproductive system disorders [3]. In children, continuous exposure to EMFs <0.4 micro-Tesla (μ T) have been reported to be needed to prevent leukemia, whereas for adult health, continuous exposure to EMFs <0.2 μ T has been reported to be needed to prevent leukemia, breast cancer, and brain cancer [4]. Other epidemiological studies indicate that exposure to EMF radiation by mobile phone use can have a significant effect on sperm concentration and motility [5]. Indeed, numerous studies have looked at the effects of EMF exposure on the male reproductive system, with experimental studies in animals showing significant decreases in seminiferous tubule diameters, testosterone levels, and average germinal cell heights [1]. However, EMF exposure has only been assessed in single generations in mouse testes.

In this study, the aim is to expose 3 generations of mice to very low EMFs to explore the effects across generations and to check the effect of EMF exposure on the seminiferous tube diameters of the mice in each generation.

2. Methods

2.1. Study design

This was an experimental study to determine the effect of ELF-EMF exposure on the diameter of the seminiferous tubules in Webster mice and to determine the effects of first (F1), second (F2), and third (F3) generation exposure to EMF. Exposure was performed under 3 ELF-EMF conditions, as follows: 3 kV/10 cm, $5.5 \mu\text{T}$; 4 kV/10 cm, $5.4 \mu\text{T}$; and 5 kV/10 cm, $5.3 \mu\text{T}$. Under each ELF-EMF, the parents were exposed until the first offspring were generated (F1), and the F1 offspring were then mated and exposed until the F2 generation was formed. This was repeated to create the F3 generation. Just after exposure in each generation, mice were randomly selected for further mating or euthanasia by stratification (random sampling). The mice testes were then removed and paraffin sections were prepared and stained with the code on each mouse. Group allocation was by stratified random sampling.

2.2. Setting

This study was conducted at the Faculty of Medicine of the Universitas Indonesia from May 2, 2011, to April 23, 2012. Animal exposure and euthanasia were performed in dedicated facilities for animal care, histology slides were prepared in the histology laboratory of the Department of Medical Biology, and data retrieval was conducted at the Laboratory of Medical Biology.

2.3. Outcome variables

The dependent and independent variables were the seminiferous tubule diameters and the EMF voltage, respectively. The control variables were the environment and how the mice were kept.

2.4. Animals

We included 28 male Webster mice (*Mus Musculus L*) aged 2–4 months old and weighing 30–40 g, which were obtained from our Department of Medical Biology. The sample size was calculated using the Federer formula. In this research, the experimental animals were exposed to 3 kV, 4 kV, 5 kV, and control conditions (t = 4), and we required 6 animals for each exposure plus 1 to allow for deaths (n = 7). All experimental animals in this study were kept in plastic enclosures and rooms maintained at 22° C with good air circulation. A 12-hour light–dark cycle was used and animals are given regular nourishment and water ad libitum. Cages were acclimatized a week before use and cleaned each week.

2.5. Procedure

2.5.1. Creating the experimental groups. The ELF-EMF Groups in Each Generation. The F1 generation was created by mating male and parental mice inside the cage while exposed to a 3 kV/10 cm EMF with a strength of 5.5 μ T. Mice were mated in advance to obtain the F1 generation from a pair of male and female parental mice reared in the animal house of our biology department. Parental

mice were drawn at random from mice that appeared healthy and had no congenital abnormalities. The F1 generation was exposed from the embryonic stage until age 2.5 months (adulthood). A random pair of F1 generation male and female mice from each enclosure were then taken and used to obtain the F2 generation, if they showed no congenital anomalies. The remaining 2.5-month-old mice in the F1 generation were exposed to a 3 kV/10 cm EMF with a strength of 5.5 μ T until the F2 generation was obtained. This method was repeated for the F3 generation, and the entire method was repeated with different mice to obtain F1, F2, and F3 generations under the other EMF exposures (i.e., 4 kV/10 cm, 5.4 μ T; 5kV/10 cm, 5.3 μ T).

2.5.2. ELF-EMF exposure of mice. Four adult male and female mice (age 2–3 months) were put in cages covered with wire netting. Three were exposed to the ELF-EMFs and progressed from generation F1 to F3. The fourth enclosure was placed on an aluminum plate (the negative electrode) and wire netting was placed over the aluminum plate (the positive electrode) at a distance of 10 cm. Both electrodes were connected to a high-voltage power generator that was connected to a regulator that could be adjusted in the range 1–10 kV.

2.5.3. ELF-EMF measurement. Magnetic field measurements were performed for exposures under 3 voltages and strengths (3 kV/10 cm, 5.5 μ T; 4 kV/10 cm, 5.4 μ T; and 5 kV/10 cm, 5.3 μ T) in the space between the positive and negative electrodes. The Gaussmeter equipment was prepared and connected to a power source, before the stick/sonde was exposed to the area or circle of the magnetic field. This was directed to the space where the EMF was present (between the 2 electrode plates or where the mice were exposed) to confirm the strength and voltage. The tool was equipped with a powerful magnetic field measuring a regulator arranged for the strength of the magnetic field:

- 1. For high-strength magnetic fields, the multiplication meter was set to $1 (\times 1)$.
- 2. For medium-strength magnetic fields, the multiplication meter was set to $0.1 (\times 10^{-1})$.
- 3. For low-strength magnetic fields, the multiplication meter was set to 0.01 ($\times 10^{-2}$).

2.5.4. Euthanasia. Experimental (male) mice were randomly selected for euthanasia at each generation and in each exposure condition. Ether anesthesia was given to avoid undue suffering: the mice were placed in a bottle with cotton-containing ether and a wide opening, which was sealed about 30 s to induce anesthesia. When the mice were unconscious, grab the mice from the bottle and place it on the surgical table in the form of an aluminum-coated Styrofoam plate with the supine position. performing antisepsis, the mice were ready for surgery. Surgery was performed using scissors and sterile tweezers and was only done to remove the testes. Fixation of the hands and feet of mice using a pin needle. It is then applied to the ventral part of the mice for antisepsis action. After that the animal dissected and taken his testicles.

2.5.5. Tissue preparation. Mice testes were fixed in BNF solution for 24 hr, before they were washed in graded alcohol (70%, 80%, 100%, xylol 1, and xylol 2) for 1 hour each. They were then place in and paraffin: xylol (1: 1) for half 1 hr, paraffin was infiltrated in the oven for 1 hr, and finally blocked into paraffin and frozen. The frozen paraffin box was embedded in a holder then soaked with xylol 1 and 2 for 20 minutes per treatment. Paraffin cutting was done microscopically, with tissue samples stuck on glass slides and colored in xylol 1 and 2 for 20 minutes. The slides were then immersed in 100%, 95%, 80%, 70%, 50%, and 30% alcohol for 1 min each, before performing Hematoxylin Ehrlich staining for 5 s. The next step was to wash the preparation under running water for 10 min and distilled water for 1 min. The preparations were dipped in alcohol concentrations of 30%, 50%, and 70% and dyed with eosin for 1 min. Then, they were immersed in 70%, 80%, and 96% alcohol for 1 min after dipping in xylol 1 and 2 for 10 minutes each. Three glass slides treated in each generation were then glued and labeled with a code, based on stratified random sampling. The observations (20 fields of view per sample) were performed with a 40 \times 10 light microscope in our Medical Biology IOP Conf. Series: Journal of Physics: Conf. Series **1073** (2018) 062043 doi:10.1088/1742-6596/1073/6/062043

laboratory. Seminiferous tubule diameters were measured with a micrometer (scale, 1: 0.01 mm) and recorded with the relevant slide code.

2.6. Statistical analysis

The data were analyzed using the Kruskal–Wallis or Mann–Whitney test, as appropriate, and significance was set at an α of 0.05 with 95% confidence intervals.

3. Results

3.1. Results of exposure in each generation

At varying ELF-EMF levels there were variations in of the decrease in seminiferous tubule diameter from the F1 to the F3 generations. However, statistical analysis indicated that the results were significant in each exposure group and generation compared with control (p < 0.05). Indeed, all treatments produced a significant decrease in seminiferous tubule diameter compared with controls (Table 1).

Table 1. The Mann–Whitney test on every exposure group and generation on control.

Р	F1			F2			F3		
	3 kV	4kV	5kV	3 kV	4kV	5kV	3 kV	4kV	5kV
Control	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000



Figure 1. Mouse exposure to various voltages in generation F1 compared with control.

Figures 1–3 summarize the results for the average seminiferous tubule diameters in each generation. In Figure 1, the significant decrease in average seminiferous tubule diameter can be seen at 3 kV/10 cm and 4 kV/10 cm compared with control. There was a slight but significant increase in the 5 kV/10 cm exposure group compared with the other groups (p < 0.05). In the F2 generation (Figure 2), there was a higher average decrease compared with the F1 generation, but the same overall pattern remained, and

the slight increase in the 5 kV/10 cm exposure group remained significant compared with the control group (p < 0.05). Figure 3 shows the clearest pattern for decrease in seminiferous tubule diameter as the voltage of the ELF-EMF increased.





Figure 2. Mouse exposure to various voltages in generation F2 compared with control.



Figure 3. Mouse exposure to various voltages in generation F3 compared with controls.

3.2. Differences in intergenerational accumulation of negative effects by voltage group

The results of the Mann–Whitney test for differences in intergenerational exposure by voltage group are shown in Table 2. Exposure to 3 kV did not produce a statistically significant decrease in the number of cells between the F1 and F2 generations (p > 0.05). Although this indicated that exposure to 3 kV did not cause a significant accumulation of negative effects between the F1 and F2 generations, there was a non-statistical decrease in the average tubule diameter that suggests this has started. However, there was a significant decrease in tubule diameter between the F2 and F3 generations (p < 0.05), indicating that there was an accumulation of negative effects. It was notable that these differences became significant between both the F1 and F2 generations and the F2 and F3 generations at 4 kV exposure (p < 0.05), indicating statistically significant accumulation of negative effects between each generation. Unfortunately, the average decrease in tubular diameter between the F1 and F2 generations was lost in the group exposed to 5 kV (p > 0.05), despite a non-statistically significant decrease in average tubular diameter between the generations. Between F2 and F3, exposure to 5 kV again led to a significant decrease in tubule diameter (p < 0.05).

Generation			Voltag	e (KV)		
group	3 KV		4 KV		5 KV	
	F ₂	F ₃	F_2	F ₃	F_2	F ₃
F_1	0.182	0.013	0.293	0.045	0.744	0.000
F_2		0.072		0.455		0.000

Table 2. The Mann–Whitney test for intergenerational exposure by voltage group.

3.3. Comparison of voltage exposure in each generation

The results of the Mann–Whitney test between exposure groups for each generation are shown in Table 3. As shown, the average decrease in tubular diameter between the 3 kV and 4 kV exposure was statistically significant in the F1 generation (p < 0.05), but although the average tubule diameter increased between the 4 kV and 5 kV groups in the F1 generation, this was not statistically significant (p > 0.05). In the F2 generation, the average tubule diameter decreases were statistically significant between both the 3 kV and 4 kV exposures and between the 4 kV and 5 kV exposures (p < 0.05), clearly showing smaller average tubule diameters with higher voltages. In the F3 generation there was only a slight and non-significant increase in the average tubule diameter between the 3 kV and 4 kV exposure, in this generation, the average tubule diameter between the 3 kV and 4 kV exposure groups (p > 0.05). However, in this generation, the average tubule diameter between the 3 kV and 4 kV exposure groups (p > 0.05). However, in this generation, the average tubule diameter between the 3 kV and 4 kV exposure groups (p < 0.05), again confirming that higher voltages were associated with smaller average tubule diameters.

Voltage (KV)			Generati	on group		
	F ₁		\mathbf{F}_2		\mathbf{F}_3	
	4 KV	5 KV	4 KV	5 KV	4 KV	5 KV
3 KV	0.000	0.000	0.004	0.002	0.805	0.000
4 KV		0.143		0.796		0.000

 Table 3. Mann–Whitney test between exposure groups for each generation.

3.4. Histology

Figures 4–6 show representative testicular tissue samples for the study mice. In the testicular tissue of both the control and exposed mice, the seminiferous tubules retain their spherical appearance with a regular size and shape. However, the testicular tissue in F1 mice exposed to a 3 kV EMF shows that, although the seminiferous tubules had almost the same size, they had developed a less regular shape.

In the testicular tissue of mice in the F3 generation, exposure to a 5 kV EMF produced marked changes in the seminiferous tubules, leading to irregular shapes and non-uniform sizes.



Figure 4. Unexposed (control) tissue.



Figure 5. F1 generation testicular tissue for mice exposed to 3kV.



Figure 6. F3 generation testicular tissue for mice exposed to 5kV.

4. Discussion

4.1. Mechanism of decreasing seminiferous tubule diameter by ELF-EMF voltage

The results showed consistent decreases in the seminiferous tubule diameters as the voltage increased, irrespective of the generation. This has been reported to be caused by free radical cellular and calcium ions levels induced by EMF exposure, leading to inhibited cell growth, failure of protein folding, and DNA chain termination. EMF exposure can also interfere with cell-dependent calcium ion signaling, causing apoptosis, and can cause rearrangements of DNA segments in testicular cells. An epidemiological study also showed that EMF exposure from mobile phones in trouser pockets could decrease the sperm concentration [6]. However, other mechanisms must be in play because it is known that EMF exposure is associated with a reduction in the diameter of seminiferous tubules. An explanation for this is that EMFs can increase the permeability of the blood-testis barrier in mice [7], with increased testicular blood flow allowing more toxic substances to enter the seminiferous tubules and kill germ cells.

4.2. Effect of ELF-EMF exposure on decreasing tubule diameter among generations

In general, there were significant decreases in tubule diameter with increasing ELF-EMF voltage in each generation when compared with the control group. Specifically, the increase in voltage in each generation tended to be directly proportional to the seminiferous tubule diameter. Given that the effects of EMFs are also dependent on the magnitude of the voltage used, it was plausible that negative effects on the cell would increase with higher voltages [1]. Exposure to EMFs would ultimately lead to decreased germ cell division, decreased sperm formation, and decreased testicular development. This is consistent with research showing that increasing voltages are associated with fewer sperm cells and decreasing seminiferous tubule diameters in the testes [8,9]. Nevertheless, we did observe a slight increase in diameter at the 5 kV/10 cm exposure level between the F1 and F2 generations that was inconsistent with this evidence. This may have been because of an adaptation mechanism in the mice after initial exposure to the EMF. We posit that this mechanism did not occur in all mice and was dependent on each mouse's genome, which will have occurred through natural selection [10].

4.3. Effect of ELF-EMF exposure on tubule dropout among generations

The figures and statistical analyses clearly showed that the seminiferous tubule diameters were directly proportional to the generation, with the number of germ cells and the tubule diameters decreasing progressively between generation F1 and F3. Thus, the F3 generation tended to have fewer cells than the F2 generation, which tended to have fewer cells than the F1 generation cells. This indicated that the negative effects of EMF exposure were passed to downstream generations in a cumulative manner and were exacerbated as exposure continued [1]. The number of cells formed in the lumen is known to be closely related to the seminiferous tubule diameter, with wider diameters when more cells are formed, and smaller diameters when less cells are formed.

5. Conclusion

This study showed that there was a significant decrease in seminiferous tubule diameter in mice following exposure to EMFs of different strengths. These decreases in diameter tended to increase proportionally, not only as the voltage increased, but also as damage accumulated from the first to the last generation.

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