The effect of continuous exposure to electromagnetic field on four successive generations of mice

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Abstrak

Tujuan penelitian ini adalah untuk mengetahui efek biologik akibat dari pemajanan medan elektrostatik terhadap 4 generasi mencit. Empat puluh delapan ekor mencit Strain Swiss Webster, masing-masing 24 ekor jantan dan betina, umur 3 bulan, dengan berat badan antara 35 - 40 g, ditempatkan pada suatu ruangan yang terkontrol, dan diberi diet makanan standar serta minum secukupnya. Mencit dibagi menjadi 6 kelompok, masing-masing terdiri dari 4 pasang mencit. Kelompok pertama dipajan medan elektromagnit pada tegangan 1 kV/10 cm, kelompok kedua pada tegangan 2 kV/10 cm, dan kelompok ketiga pada medan elektromagnit dari tegangan 3 kV/10 cm. Ketiga kelompok tersebut disebut kelompok eksperimen. Tiga kelompok lainnya digunakan sebagai kelompok kontrol, masing-masing untuk kelompok eksperimen pertama, kedua, dan ketiga. Setiap pasang mencit dimasukkan ke dalam kandang plastik (26x20x11cm) dengan penutup terbuat dari kawat kasa. Selanjutnya, kelompok mencit eksperimen ditaruh di atas lempeng elektroda negatif, yang letaknya sejajar dengan elektrode positif, berjarak 10 cm di atas elektroda negatif. Kedua lempeng elektroda tersebut dibuat dari aluminium. Kedua elektroda tadi dihubungkan dengan stepup transformer, kemudian dengan sumber listrik PLN. Semua pasangan mencit, baik dari kelompok eksperimen maupun kelompok kontrol, dibiarkan kawin, bunting, dan melahirkan generasi pertama sampai generasi keempat. Keempat generasi dari kelompok eksperimen terus mengalami pemajanan medan elektromagnit, sedangkan keempat generasi kelompok kontrol tidak dipajan medan elektromagnit. Selama penelitian berlangsung, semua mencit berada di ruangan bersuhu 26^0 C dengan waktu pencahayaan gelap-terang 12:12 jam. Hasil penelitian menunjukkan, terjadi penurunan fertilitas pada keempat generasi kelompok eksperimen, tetapi tidak diikuti dengan perubahan rasio seks. Sebaliknya, pemajanan tersebut cukup efektif untuk menimbulkan beberapa macam kelainan kongenital, seperti mikroftalmi, mata berwarna putih, kaki belakang pendek, kerdil, dan tumor pada generasi jantan maupun betina yang menyebabkan laju kematian setelah berumur 3 - 4 bulan cukup tinggi, terutama pada generasi ke-3 dan ke-4. Pada kelompok kontrol tidak ditemukan kelainan kongenital maupun tumor. Dari penelitian ini dapat disimpulkan, bahwa beberapa kejadian yang diperoleh pada penelitian ini diduga karena terjadi perubahan-perubahan materi genetik pada sel sperma atau telur selama spermatogenesis atau oogenesis, yaitu berupa efek mutagenik. Interaksi antara medan elektromagnit dengan sel hidup dapat menimbulkan efek biologik pada sel, jaringan, maupun organ, sehingga merupakan konsekuensi bagi seluruh kehidupan organisme. ***(Med J Indones 2002; 11: 3-10)***

Abstract

The objective of this study is to know the biological effects of electromagnetic field treatment on four successive generations of mice. Fourty eight male and female mice of Swiss Webster Strain, 3 months of old, and 35 - 40 g body weight, were kept in a controlled environment and fed a standard diet. Mice were divided into 6 groups of four couples each. The first group was exposed to electromagnetic field of 1 kV/10 cm, the second group to 2 kV/10 cm, and the third group to 3 kV/10 cm. The remaining 3 groups were served as untreated controls of the first, second, and third group, respectively. Each couple of mice was placed in a cage (26x20x11 cm) with wire metal cage tops. The cages of experimental groups with mice inside, were then put on the negative terminal plate of a pair of parallel aluminium plate electrodes. These cages were perpendicular to the positive electrode plate at a distance of 10 cm. Subsequently, the electrodes were connected to stepup transformer as an alternating current power supply. All mice belonging to experimental and untreated control groups were allowed to mate, gastate, and deliver the first up to fourth generations. During investigation, all generations of experimental groups were continuously treated to electromagnetic field, while generations of untreated control groups received no treatment to electromagnetic field, During the study, all mice were housed in a room having a temperature of 26^0 C and a light - dark cycle of 12:12 hours. The results of this study showed that exposure of mice to electromagnetic field results in reduced fertility with no change in sex ratio of the offspring. Exposure to electromagnetic field, however, were effective in inducing congenital anomalies, such as micropthalmy, white eyes, short hind legs, dwarf mice, and tumors in both sexes of the offspring which caused of death after 3 - 4 months of old. A large mortality rate were found, especially in the third and fourth generations. No congenital anomalies and tumors were noted in untreated controls. In conclusion, we suggest that several facts which found in this study were the result of changes in genetic material of the sperm or eggs during spermatogenesis or oogenesis, respectively, i.e. a mutagenic effect. This interaction between electromagnetic field and the living cell, may then cause biological effects on cells, tissues, and organs, so that finally there are consequences for the whole organisms. ***(Med J Indones 2002; 11: 3-10)***

Keywords: *electromagnetic field, fertility, mortality rate, congenital anomalies, tumor, mice*

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The increasing demand for electrical energy resulting from population growth and industrial expansion will require the transmitting of electricity at higher voltages over existing and new right of way. Associated with these higher voltages, will higher electric strengths, to which people and other life form may be exposed. Knowledge of the effects of electric fields on living organisms is still scanty as is our knowledge about the tolerance of human being, animals and plants to various frequencies of these fields. Hitherto, mainly phenomenological observation have been made, which clearly indicate that the processes of live may be influenced by electric fields. Biological effects of low level electromagnetic radiation have become the focus of a number of studies.

There are some reports on the biological effects of electric fields. It has been reported that the reproductive risks of low-frequecy electromagnetic fields (EMF) caused reduced fertility and congenital anomalies in the offspring.[1,2,3]

An epidemiological study from the greater Denver area in Colorado showed that an excess of electrical wiring configuration suggestive of high current flow (60 Hz) near the homes of children who had developed cancer as compared to the homes of control children.[4] Recent epidemiological findings indicate an increase risk of breast cancer in workers occupationally exposed to electromagnetic field.[5] Moreover, chromosomal aberration in lymphocytes of employees in transformer and generator production exposed to EMF and mineral oil also have been reported.[6,7] The following study was designed to examine the possible impact in successive generations of mice from the continuous exposure to EMF.

METHODS

Fourty eight adult male and female mice (initial parents) of Swiss Webster Strain, 3 months of old, and 35 - 40 g body weight, were kept in a controlled environment and fed a standard diet. Mice were divided randomly into 6 groups of 4 couples each and treated as follows.

The first group was exposed to EMF of 1 kV/10 cm, second group to 2 kV/10 cm, and the third group to 3 kV/10 cm. The remaining 3 groups served as untreated controls of the first, second, and the third group, respectively. Twenty four plastic cages (26 x 20 x 11 cm) with wire metal cage tops were employed, and contain a couple of mice each, respectively. The cages of experimental groups with mice in side, were then put on negative terminal plate (120 x 34 cm) of a pair of parallel aluminium plate electrodes. The cages with mice inside were perpendicular to positive electrode plate (120 x 34 cm) at a distance of 10 cm. Subsequently, the electrodes were connected to an alternating current (AC) power supply. All mice belonging to experimental and groups were allowed to mate, gestate, and deliver the 1st generation. The untreated controls received no treatment to EMF and allowed to produce offspring. During the study, all mice were housed in a room having a temperature of 26^{O}C and a light - dark cycle of 12 : 12 hours.

At maturity, randomly selected individuals of mice from the 1st generation were similarly allowed to mate, gestate, deliver and rear their offspring of the 2nd generation while being continuously exposed to EMF. Randomly selected individuals from the 2nd generation were then mated to produced the 3rd generation. Finally, from the 3rd generation were allowed to produce the 4th generation. A parallel procedure was followed for the control groups, wherein 4 generations were produced in the ambient electric field. Pregnant females mice were placed in individual cages and remained with their offspring until weaning at about 4 weeks after birth.

The number of offspring and the sex ratio were noted 24 hours after birth. The number and type of anomalies were also noted 60 days after birth. The data were analyzed by 2 statistical tests. Analysis of variance was used to test for significant differences in the number of offspring between experimental groups compared to its control groups, whereas the contingency test was used to evaluate differences in the sex ratio of the litters.

RESULTS

Number of offspring

All female mice from the 1st up to 4th generations became pregnant after being mated during continuously exposured to EMF of 1 kV, 2 kV, and 3 kV, respectively. The mean number of offspring of all generations belonging to experimental groups were reduced. Analysis of variance showed a significant decrease ($P < 0,05$) in the number of offspring of experimental groups compared to untreated controls. However, there were no significant differences in the

number of offspring between the experimental groups (Table 1).

Mortality rate were noted during 7 - 35 days after birth. A large mortality rate were found in all generations, even in males or females offspring, particularly in the third and fourth generations which were treated to EMF of 2 kV and 3 kV.

Table 1. The effects of electromagnetic field on four successive generations of mice

Treatment	A	B	C	D	E
1 kV					
1st generation					
Exper. group	4	4	39	9,75	10,25 % (4)
Control group	4	4	59	14,75	3,38 % (2)
2nd generation					
Exper. group	4	4	36	9	13,88 % (5)
Control group	4	4	57	14,25	1,75 % (1)
3rd generation					
Exper. group	4	4	41	10,25	17,07 % (7)
Control group	4	4	58	14,50	3,44 % (2)
4th generation					
Exper. group	4	4	32	8	31,25 % (10)
Control group	4	4	60	15	0 % (0)
2 kV					
1st generation					
Exper. group	4	4	38	9,50	10,52 % (4)
Control group	4	4	51	12,75	0 % (0)
2nd generation					
Exper. group	4	4	24	6	16,66 % (4)
Control group	4	4	52	13	3,88 % (2)
3rd generation					
Exper. group	4	4	26	6,5	69,23 % (18)
Control group	4	4	53	13,25	5,66 % (3)
4th generation					
Exper. group	4	4	26	6,5	38,46 % (10)
Control group	4	4	51	12,75	7,84 % (4)
3 kV					
1st generation					
Exper. group	4	4	31	7,75	22,58 % (7)
Control	4	4	50	12,50	0 % (0)
2nd generation					
Exper. group	4	4	50	12,50	26 % (13)
Control group	4	4	54	13,50	3,70 % (2)
3rd generation					
Exper. group	4	3	22	7,33	50 % (11)
Control group	4	4	52	13,00	9,62 % (5)
4th generation					
Exper. group	4	4	27	6,75	37,03 % (10)
Control group	4	4	49	12,75	0 % (0)

A = number of females mated; B = number of pregnancies; C = number of pups delivered; D = average litter size; E = mortality during 8 - 35 days postpartum (the number is given in parenthesis).

The sex ratio of offspring

The sex ratios of offspring in the experimental groups were unaffected by the different treatments of EMF. With a 6 x 2 contingency table it was clear that there was no significant difference ($P > 0,2$) in the sex ratio between the various experimental groups or control groups as determined by the test (Table 2).

Number of congenital anomalies

Congenital anomalies were also noted in both sexes of the offspring sired by parents of each generation exposed to EMF of 1 kV, 2 kV, and 3 kV, respectively. However, no congenital anomalies were noted in offspring of untreated controls. Several types of anomalies were obtained, such as micropthalmy, white eyes (Figure 1) and dwarf mice (Figure 2). Other interesting result of our investigation is the occurrence of tumors in both sexes of the offspring and caused of death after 3 to 4 months of old (Figure 3).

Table 2. The effect of electromagnetic field on the sex ratio of the offspring of four successive generations of mice

Treatment	1 kV			2 kV			3 kV		
	T	M	F	T	M	F	T	M	F
1st generation									
Exper. group	39	18	21	38	16	22	31	14	17
Control group	59	27	32	51	23	28	50	25	25
2nd generation									
Exper. group	36	17	19	24	10	14	50	23	27
Control group	57	30	27	52	28	24	50	21	29
3rd generation									
Exper. group	41	21	20	26	14	12	22	10	12
Control group	58	26	32	53	24	29	52	30	22
4th generation									
Exper. group	32	16	16	26	11	15	27	15	12
Control group	60	31	29	51	20	31	49	22	27

T = number of offspring; M = male; F = female

Table 3. Number of tumors and anomalies found in the offspring of four successive generations of mice exposed to electromagnetic field

Treatment	1 kV		2 kV		3 kV	
	Tm	An	Tm	An	Tm	An
1st generation						
Exper. group	0	2	0	2	1	3
Control group	0	0	0	0	0	0
2nd generation						
Exper. group	0	2	4	2	2	5
Control group	0	0	0	0	0	0
3rd generation						
Exper. group	2	0	4	3	2	8
Control group	0	0	0	0	0	0
4th generation						
Exper. group	0	2	2	2	4	8
Control group	0	0	0	0	0	0

Tm = number of tumors ; An = number of anomalies

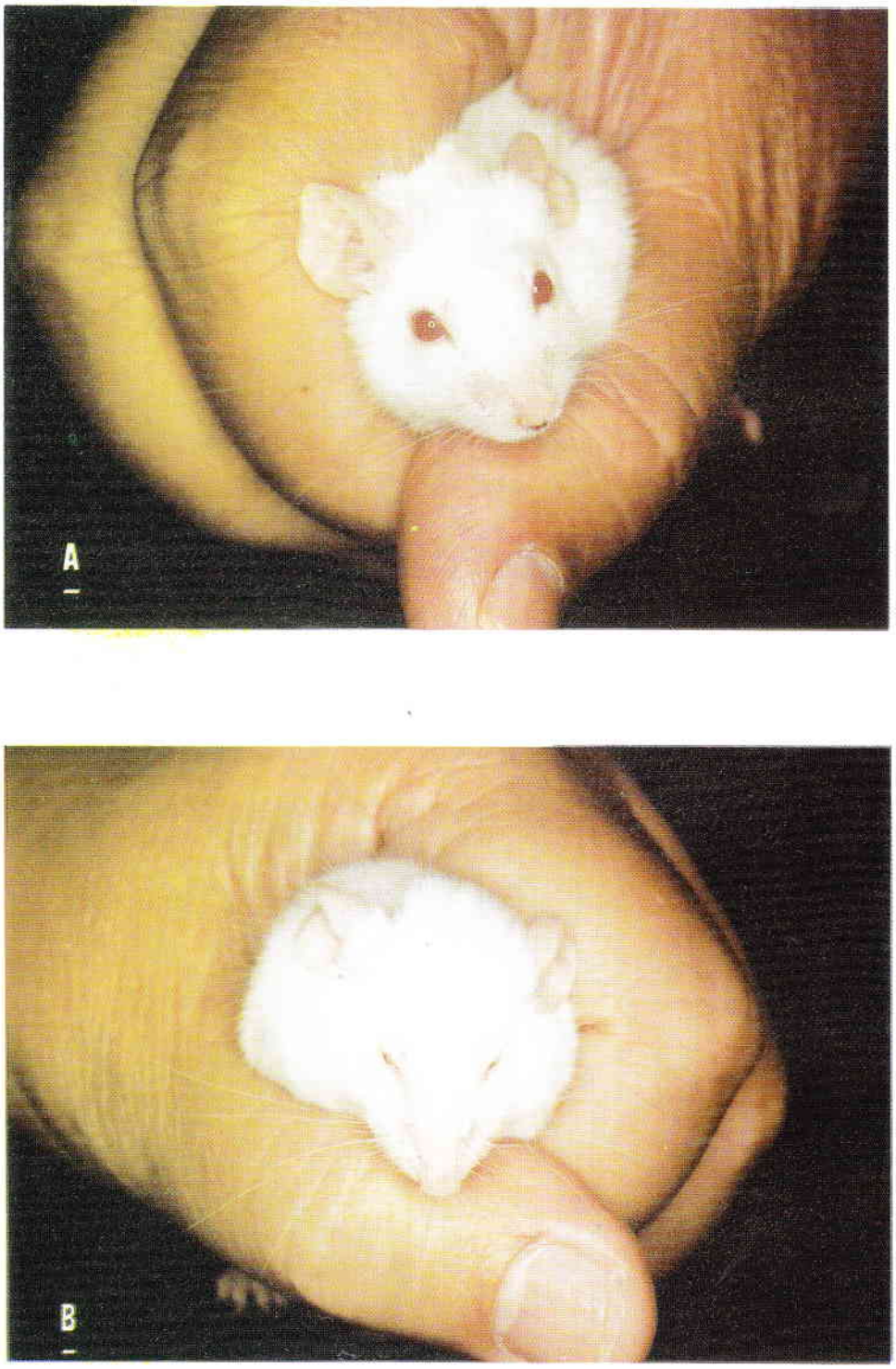

Figure 1. Normal mouse offspring with normal eye (A) and anomalous mouse offspring with microphtalmy (B)

Figure 2. Anomalous mouse offspring showing a dwarf mouse (A) and a normal mouse offspring (B) of the same age

Figure 3. Male mouse offspring showing tumors on the left fore legg and shoulder, with white colour eye

DISCUSSION

The present results show that although all female mice became pregnant after being mated to males which also exposed to EMF, the litter sizes were reduced significantly as compared to untreated controls, while litter size did not vary significantly between experimental groups. The same result have also been shown earlier for offspring in rats after treatment with electrostatic field.[2,3] It seems the effect of EMF on fertility was not immediately apparent; probably because the postgonial cells (primary and secondary spermatocytes, spermatids, and spermatozoa) are relatively resistant to the lethal effects of EMF. However, after exposure to moderate or even high doses, there is an initial fertile period followed by decreased fertility.[8] Therefore, the reduction in litter size (Table 1), is probably associated with a decrease in the number of spermatogenic cells. Electromagnetic fields can affects in several biological functions, including hormone levels, alterations in the binding of ions to cell membrane, and the modification of biochemical processes inside the cell, such as RNA tranccription and protein synthesis of enzymes[9] which are needed in spermatogenesis or oogenesis.

It has been reported previously, that one effect of electric fields is to increase the frequency of sex-linked recessive lethal mutation in female *Drosophila melanogaster*. Electrostatic field also has an effect on the frequency of non-disjunction of the X-chromosome.[10] This will cause the male to female ratio to be less than unity. The data of our present investigation show, that the sex ratio of offspring in the experimental groups were not significantly different, which implies that the number of male and female offspring was unaffected by the different treatments (Table 2).

Several types of anomalies in Rodents have been reported as a result of exposure to X-rays.[11] Micropthalmy and dwarf mice were also found in our present study as an effect of EMF, while the other types of anomalies were not similar. We suggest that the anomalies were the result of changes in the genetic material of the sperm or ova during spermatogenesis or oogenesis i.e. a mutagenic effect.

An interesting evidence of this investigation is the occurrence of tumors found in both sexes of the offspring and caused of death (Table 3). The same result was also reported in rats after exposure to an electrostatic field.[2] Researchers at Bettelle Pasific Dorthwest Laboratory in Richland, Washington, have come close to showing a direct EMF-cancer link in rats. They have found that EMF suppress levels of the hormone melatonin, something that other researchers have shown makes female rats suseptible to chemically induced mammary tumors.[5,9] In connection with the hormone melatonin shown in rats whose pineal glands have been surgically removed are more likely to develop tumors than rats with intact pineal glands. However, after being given melatonin injections the rats were no more likely to develop tumors than the controls.[9] At the level of human epidemiology, 50 studies have examined the possible correlation of electromagnetic fields exposures with adult and childhood cancers. The studies suggest that electromagnetic fields might be cancer promoters but unlikely to be cancer initiators.[12]

In conclusion, the results of this investigation suggest that continuous exposure to electromagnetic field in four successive generations of mice, results in reduced fertility with no change in the sex ratio, but effective in inducing congenital anomalies and tumors in both sexes of the offspring. In connection with these fenomena, we also suggest that the primary interaction between electromagnetic field and the living cells is more takes place at the molecullar level than the celullar level. This interaction may then cause biological effects on cells, tissues, and organs, so that finally there are consequences for the whole organism.

REFERENCES

1. Krueger MF, Giarola AJ and Bradley JW. Effects of electromagnetic fields on fecundity in the chicken. Ann NY Acad Sci 1975, 247: 391-3.
2. Soeradi O, Tadjudin MK. Congenital anomalies in the offspring of rats aftar exposure of the testes to an electrostatic field. Int J Androl 1986, 9: 1 - 9.
3. Yurnadi. Pengaruh pemejanan medan elektrostatik terhadap fekunditas dan rasio seks mencit *(Mus musculus)* strain Swiss Webster BPMSOH pada dua generasi. Tesis Magister Program Studi Biomedik Program pascasarjana UI, 1999.
4. Tomenius L. 50 Hz electromagnetic environment and the incidence of childhood tumors in Stockholm county. Bioelectromagnetics 1986, 7: 197- 207.
5. Stevens RG, Davis C, Thomas DB, Anderson LE, Wilson BW. Electric power, pineal function, and the risk of breast cancer. FASEB J 1992, 6: 853-60.
6. Sari P. Pemajanan medan elektrostatik pada mencit *(Mus musculus)* strain Swiss Webster dan pengaruhnya terhadap kromosom serta proliferasi limfosit. Tesis Magister Program Studi Biomedik Program Pascasarjana UI, 1998.
7. Skyberg K, Lise Hensteen I and Vistnes AI. Chromosomal aberrations in lymphocytes of employees in transformer

and generator production exposed to electromagnetic fields and mineral oil. Bioelectromagnetics 2001, 22: 150-60.
8. Dalrymple GV, Gaulden ME. Medical Radiation Biology. Philadelphia, Saunders WB 1973, p
9. Pool R. Electromagnetic Fields: The Biological Evidence. Science 1990, 249: 1378- 81
10. Portnov FG, Shakarins VF, Maiore DJ. Study of mutagenic effect of static electric fields in female *Drosophila melanigaste*. Genetika 1975, 11: 177-81.
11. Griem ML. The effects of radiation on the fetus, In: Dalrymple DV, Gaulden ME, Koll-morgen GM, Vogel HH (eds). Medical Radiation Biology. Philadelphia, Saunders WB, p. 97-9.
12. Hendee WR, Boteler JC. The question of health effects from exposure to electromagnetic fields. Health Phys 1994, 66: 127-36.