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Effect of electromagnetic field on the sperm characteristics and histopathological status of testis in rats*)

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Summary

The aim of the study was to investigate the effects of electromagnetic fields ELF-EMFs generated by 170 kV (50 Hz) high power lines on the epididymal sperm characteristics, biochemical parameters testosterone levels and histopathology of testis. The experimental group consisted of 28 adult male rats placed in an cottage 7.5 m far from transfer lines transferring 170 kV (50 Hz) energy. They were randomly divided into four groups of 7 rats' each. Group 1, 2 and 3 were exposed continuously (24 hr) to ELF-EMF (48.21 ± 1.58 mG) for 1, 2 and 3 months, respectively. The rats of group three served as the control and were placed in laboratory conditions without a magnetic field. Insignificant ($p > 0.05$) decreases were determined among the groups in terms of reproductive organ weights, testes dimensions and epididymal sperm concentration and sperm motility, and an insignificant increment was observed in abnormal sperm rates in relation to the varying periods of exposure to the ELF-EMF. Although marked reductions ($p < 0.001$) were observed among the groups in relation to plasma and testis catalase activity, depending on exposure time, no significant differences were found in terms of glutathione and malondialdehyde levels. In the light of Johnsen's testicular biopsy score the mean scores of groups 1, 2, 3 and control were determined as 9.24 ± 0.08, 8.02 ± 0.12, 6.98 ± 0.11 and 9.88 ± 0.07, respectively. Histopathology examinations of testis revealed a deceleration of spermatogenesis and degeneration of germ cell order in relation to exposure time.

Keywords: Electromagnetic fields, testis, rat, sperm characteristics

Extremely low frequency (50 and 60 Hz) ELF-EMFs are associated with the production, transmission, and use of electricity; thus the potential for human exposure is very high (29). Therefore, the possible adverse effects of EMF on reproductive and developmental outcome have been extensively studied in both experiments involving animals and humans over the past several decades (5). However, limited data have been published about these potential adverse effects (19, 25). Moreover, there have been conflicting findings regarding the alteration of spermatogenic and reproductive functions. A number of studies showed that exposure to EMF did not induce any adverse effects on spermatogenesis and reproductive capacity in experimental animals and human (14, 25). In contrast, some studies conducted by other investigators showed clear damage to spermatogenesis (2, 8, 24, 29). Therefore, more careful and detailed studies need to be carried out to determine whether EMF exposure can induce adverse effects on spermatogenesis and reproductive capacity.

Power frequency fields, 60 Hz in USA, Canada and South America, and 50 Hz in Europe and elsewhere, are extremely low in electromagnetic frequency terms. Such fields are described as ELF-EMF, and there is essentially no propagation of energy at power frequencies, although there are induced currents in conducting objects nearby. At 50 Hz the wavelength of the electromagnetic field is 6000 km. At practical distances from power lines, within what is termed the near field, the electric and magnetic fields are essentially separate entities and should be treated as such. Thus, the terms electric and magnetic fields will be used here to describe power line fields (13).

Epidemiological and laboratory reports suggest that children exposed to EMFs from power lines are at greater risk of developing leukemia, and that adults exposed to EMFs at work run a higher risk of leukemia and brain cancer (13, 28).

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The results of animal study on ELF electric fields are rather consistent and do not suggest adverse effects on development (6). On the other hand, another recent study (15) have showed that exposure to ELF electromagnetic fields had adverse effects on reproduction in male mice, including reduced testicular weights, decreased sperm counts and sperm motility. EMF stimulation may result in Leydig cell proliferation, increase in testosterone level and testis weight, but decrease in germ cell population (23).

The aim of the study was to investigate the possible adverse effects of 50 Hz electromagnetic fields on spermatogenesis, testosterone levels and testis morphology associated with biochemical parameters of adult male Wistar rats.

Material and methods

Animals. In the present study, a total of 28 Wistar male rats, aged 4-6 months, and weighed 250-300 g were used. The rats in the experiment group (n = 21) were placed in an experiment cottage (2 × 2 × 1.85 m) 7.5 m away from a 170 kV energy transfer line (fig. 1). Groups were exposed continuously to 50 Hz field from the electromagnetic field (EMF) for 1-3 months. To determine the intensity of magnetic flow and the distribution character, measurements were made in the cottage the animals were put and in the same height. The animals in the control group and were kept under the same conditions without the magnetic field.

The rats were housed in cages specially designed to minimize field perturburance. The size of the cages was similar to that of commercially available rat breeding cages (33 × 13 × 15 cm). The walls of the cages consisted of Perspex, and feeding pens and water bottles were mounted outside the cages. The rat had free access to maintenance food and water. Commercially obtained cork flakes were used as bedding material. The cages were washed once a week. Animals were maintained under standard laboratory conditions on a 12 h light – 12 h dark cycle in a temperature-controlled room at 21-22°C. The investigation was made with the permission of the Ethical Committee on Animal Experiments of the University of Firat.

Measurements of electric and electromagnetic fields. The ELF-EMFs values that were measured in the experiment barn which had a 7.5 meters vertical distance to 170 kV (50 Hz) high frequency line and which was 10 meters away from the transformer station (ELF; 1.66 ± 0.01 kV/m, EMF; 48.21 ± 1.58 mG). ELF-EMFs measurements were taken twice a day for four months and the average values of power and frequency were calculated. Table 2 presents the statistics for power voltage, and electrical and electromagnetically field values measured at 170 kV high-frequency. Electromagnetic field was measured by Gauss meter (hand type gauss meter/F.W.BEL Model/4080 Frequency Range/ 25-1000 H Accuracy/< ± %2 Measurement Type/True RMS). As the frequency of the present system and the transformer center is 50 Hz, intensity of the magnetic flow was measured by the device in the 50 Hz caliber.

The ELF-EMFs measurements were also taken in the laboratories housing the control group and the average values were calculated. The average value of the electrical field

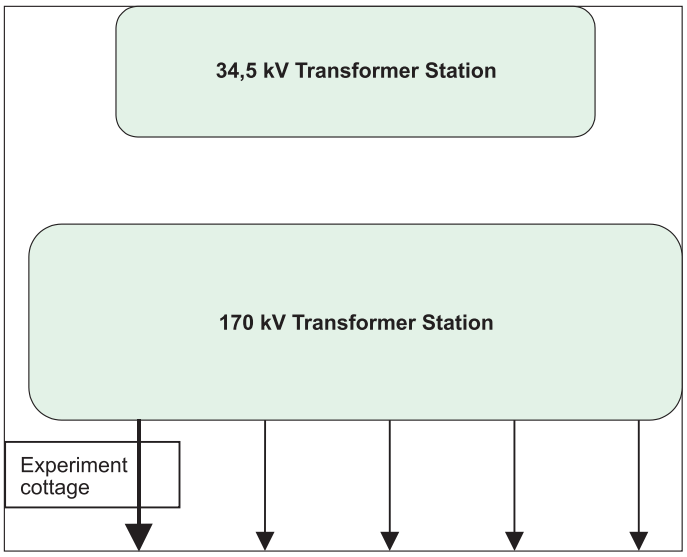

Fig. 1. Non-scaled plan of the 170 kV transformer center. 170 kV high voltage power lines

(ELF) was 0.75 ± 0.05 V/m whereas the value of the electromagnetic field (EMF) was calculated to be 0.48 ± 0.05 mG.

Sample collection. Rats were sacrificed by cervical dislocation under light ether anesthesia. Blood samples were collected and centrifuged at 3000 rpm for 10 min. The testes, epididymes, seminal vesicles and prostate were removed, cleared of the adhering connective tissue and measurement of testis weight, length, thickness, epididymal, seminal vesicle and prostatic weight were evaluated along with epididymal sperm concentration, sperm motility and sperm morphology. One of the testes was fixed in 10% formalin for histopathological examinations. Blood and other testis samples were stored at –20°C until biochemical analyses.

Epididymal sperm concentration, motility and abnormal sperm rate. Spermatozoa in the epididymis were counted by a modified method of Yokoi et al. (30). Briefly, the epididymis was minced with anatomical scissors in 5 ml of physiological saline, placed in a rocker for 10 min., and incubated at room temperature for 2 min. The supernatant fluid was diluted 1 : 100 with a solution containing 5 g sodium bicarbonate, 1 ml formalin (35%) and 25 mg eosin per 100 ml of distilled water. Total sperm number was determined with a hemocytometer. Approximately 10^{-1} of the diluted sperm suspension was transferred to each counting chamber and was allowed to stand for 5 min. for counting under a light microscope at 200 × magnifications.

The percentage of progressive motility was evaluated using a light microscope with heater table with an earlier method described by Sönmez et al. (26). For this process a slide was placed on microscope stage and, allowed to warm to a temperature of 37°C by heater stage. Several droplets of Tris buffer solution (Tris – hydroxymetyl aminomethane 3.63 g, glucose 0.50 g, citric acid 1.99 g and distilled water 100 mL) were then dropped on the slide, and a very small droplet of fluid obtained from left cauda epididymis with a pipette was dropped on the Tris buffer solution and mixed by a coverslip. The percentage of motility was evaluated visually at a magnification of 400 ×. Motility estimations were performed on three droplets and five different fields per drop in each sample. The mean value of five successive estimations was used as the final motility score.

The described method by Atessahin et al. (4) was used for determination of the percentage of morphologically abnormal spermatozoa. Briefly, several droplets of Tris buffer solution was dropped on a clean, dry and pre-warmed slide, a very small droplet of fluid obtained from left cauda epididymis with a pipette and, two droplets of Indian ink stain were dropped on the Tris buffer solution and mixed by a cover-slip for one min. A thin film of the stained sample was then drawn out along another pre-warmed slide. Immediately the slide was left to dry in a clean, dry and dust-free environment. After preparation the slide was viewed under a light microscope at 400 × magnification. A total of 400 sperm cells were examined on each slide, and the head, tail and total abnormalities of spermatozoa were expressed as %.

Histological analysis of the testis. Formalin-fixed testis was embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (H&E) for evaluation by light microscopy. In order to determination of gene related with apoptose, samples were examined under light microscope after dying with Bax and Bcl-2 immunahistochemically. Histological analysis of testis tissue including Johnsen's testicular biopsy score (17) was performed for control and exposed groups. All cross sectioned tubules were evaluated systematically, and each was given a score from 1 to 10 following Johnsen's criteria. Twenty five tubules were evaluated for each animal.

Biochemical assay. The testes tissue was homogenized in Teflon-glass homogenizer with a buffer containing 1,5% potassium chloride to obtain 1 : 10 (w/v) whole homogenate. Concentrations of malondialdehyde (MDA), as proceeding of lipid peroxidation (LPO), were measured in homogenate and plasma. Then homogenates were centrifuged at 18.000 × g (+4°C) for 30 min. to determine reduce glutathione (GSH) levels. MDA concentrations were assayed according to a modified method of Ohkawa et al. (22) based on the reaction with thiobarbituric acid, and were expressed as nmol/ml for plasma, nmol/g protein for testes tissue. Plasma and tissue GSH concentrations were measured by a kinetic assay using a dithionitrobenzoic acid recycling method described by Ellman (10) and were expressed as µmol/ml for plasma, µmol/g protein for testes tissue. The method of Goth (12) was used for the determination of catalase activity in plasma. The yellow complex of molybdate and hydrogen peroxide was measured at 405 nm against blank using a spectrophotometer. The values of CAT activity were expressed as ku/L for plasma, ku/g protein. Protein concentrations were measured according to Lowry et al. (21). The testicular tissue catalase activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi (1), and was expressed as k/g protein, where k is the first-order rate constant.

Hormone assay. Testosterone was determined by DRG testosterone EIA-1559 (DRG Instruments GmbH, Germany.)

Statistical analyses. All values were presented as mean ± SEM (Standard Error of Means). A p-value = 0.05 was considered statistically significant. Data were analyzed by One-way analyses of variance (ANOVA) and post-hoc Tukey--HSD test being used the SPSS/PC computer program (version 12.0) to determine the differences among all groups in the whole parameters.

Results and discussion

Table 1 demonstrates the effect of 50 Hz exposure on organ weights of male rats. Table 2 shows the effects on testosterone levels and Johnsen testicular biopsy score, epididymal sperm concentration, sperm

Tab. 1. Testes, epididymis, accessory glands weights and testes dimensions in control and exposed rats

Examing organs		Control	Exposure Time (month)		
			1	2	3
Testes Weight (g)	Right	1.362 ± 0.101	1.322 ± 0.042	1.312 ± 0.072	1.256 ± 0.068
	Left	1.398 ± 0.100	1.354 ± 0.051	1.322 ± 0.073	1.270 ± 0.064
Testes Length (cm)	Right	1.974 ± 0.039	1.967 ± 0.044	1.970 ± 0.044	1.905 ± 0.023
	Left	1.990 ± 0.036	1.985 ± 0.042	1.995 ± 0.029	1.940 ± 0.028
Testes Thickness (cm)	Right	1.132 ± 0.008	1.071 ± 0.017	1.085 ± 0.029	1.087 ± 0.021
	Left	1.128 ± 0.006	1.104 ± 0.022	1.095 ± 0.031	1.075 ± 0.025
Epididymis Weight (g)	Right	0.474 ± 0.017	0.471 ± 0.033	0.505 ± 0.041	0.488 ± 0.031
	Left	0.510 ± 0.046	0.501 ± 0.031	0.480 ± 0.019	0.481 ± 0.025
Seminal Vesicles (g)		0.924 ± 0.126	0.870 ± 0.041	0.854 ± 0.198	0.850 ± 0.110
Prostate (g)		0.571 ± 0.116	0.536 ± 0.063	0.434 ± 0.029	0.451 ± 0.036

Tab. 2. Testosterone level, Johnsen's testicular biopsy score and epididymal sperm characteristics of control and exposed rats

		Control	Exposure time (month)		
			1	2	3
Testosterone (ng/ml)		1.96 ± 0.27^{a}	1.52 ± 0.13^{ab}	1.06 ± 0.50^{ab}	0.97 ± 0.50^{b}
Johnsen's biopsy score (1-10)		9.88 ± 0.07^{A}	9.24 ± 0.08^{B}	8.02 ± 0.12^{C}	6.98 ± 0.11^{D}
Germinal cell thickness		62.27 ± 1.22^{a}	56.13 ± 1.34^{b}	49.47 ± 1.40^{c}	44.44 ± 1.20^{d}
Diameters of Tubulus seminiferus		254.67 ± 1.63	251.07 ± 1.49	251.06 ± 1.58	249.73 ± 1.42
Epididymal Sperm Concentration (million/g)		381.73 ± 59.55	364.61 ± 30.00	308.06 ± 42.06	305.64 ± 62.48
Sperm Motility (%)		60.66 ± 2.83	54.95 ± 3.44	53.43 ± 1.99	50.82 ± 3.15
Abnormal Sperm Rate (%)	Head	5.43 ± 1.92	5.94 ± 1.26	7.37 ± 1.69	8.40 ± 1.64
	Tail	6.73 ± 1.09	8.26 ± 1.52	6.76 ± 1.27	7.94 ± 1.18
	Total	12.16 ± 2.96	14.22 ± 2.24	14.13 ± 3.05	16.34 ± 2.70

Explanations: The differences among values bearing different lower cases – a, b, c, d at $p < 0.05$ and A, B, C, D at $p < 0.01$ in the same line are statistically significant

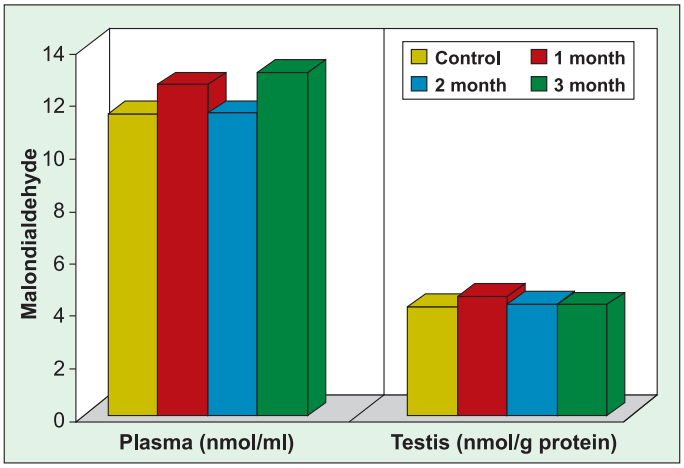

Fig. 2. The plasma and testis malondialdehyde (MDA) levels

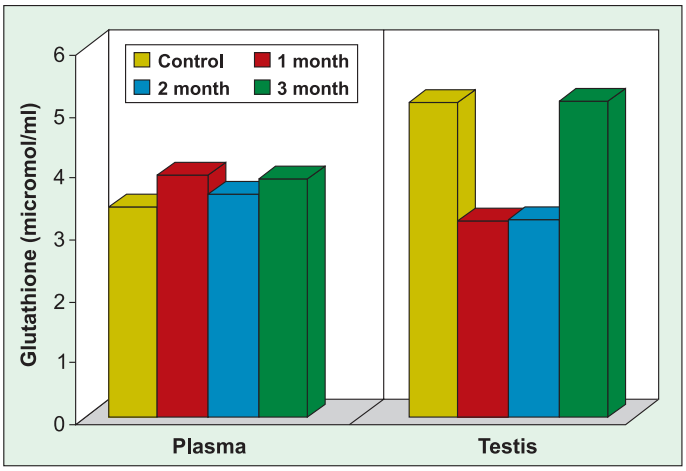

Fig.3. The plasma and testis glutathione (GSH) levels

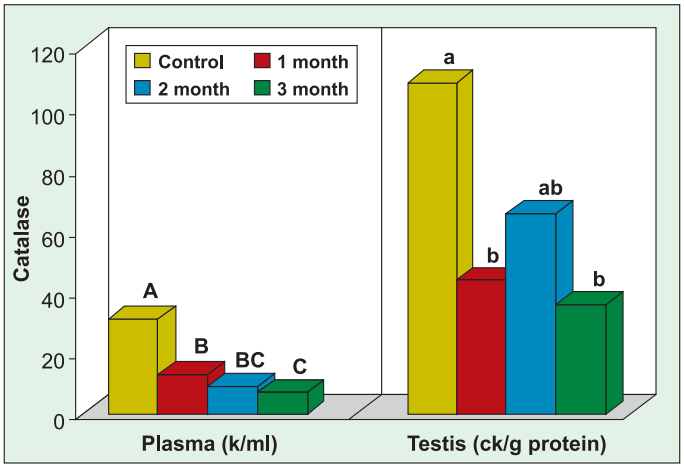

Fig. 4. The plasma and testis catalase (CAT) activities. The istatitical differences among bars bearing different uppercases (A, B, C; $p < 0.001$)

motility and abnormal sperm rate. 50 Hz magnetic fields had no significant effect on epididymal sperm concentration and sperm motility, insignificant increment was observed in abnormal sperm rate depending on different period of exposure to the ELF-EMFs. The level of testosterone ($p < 0.05$) showed a significant decreases during the 3 month exposure period. The decrease in germinal cell thickness with Johnsen's testicular biopsy score of Tubulus seminiferus were statistically important ($p < 0.05$), whereas the diameters of Tubulus seminiferus measured by means of a micrometer were not statistically important ($p > 0.05$).

The plasma and testis malondialdehyde (MDA), glutathione (GSH) levels and catalase (CAT) activities are presented in fig. 2, fig. 3, fig. 4, respectively. Although marked reduces ($p < 0.001$) were observed among the groups in point of plasma and testis catalase activities depending on exposure time, no significant differences were found in terms of glutathione and malondialdehyde levels. The most reduced plasma and testis catalase activities were observed in 3 months exposure groups compared to the other groups ($p < 0.001$).

In histopathological evaluation of animals that were exposed for one month, it was observed that the testis tissue was nearly normal except that there were some spatial disorders in the germinal cell distribution and a reduction of cell count. There was also a maturation arrest in spermatogenesis (fig. 5).

The maturation arrest in spermatogenesis and disorder in germinal cell distribution was more pronounced in animals that were exposed for two months (fig. 6) than one month exposed animals. In animals that were

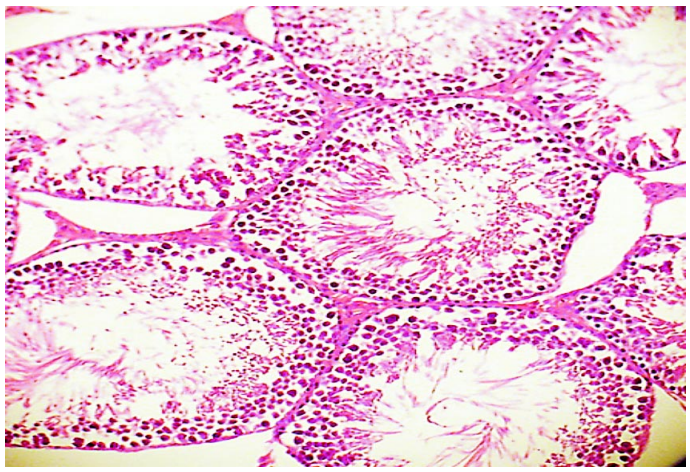

Fig. 5. Spermatogenesis arrest, light disorders in germinal cell distribution (1st month) (H-E, × 20)

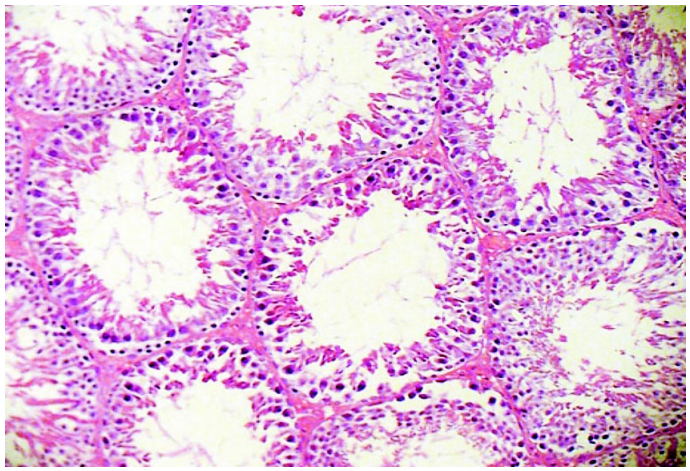

Fig. 6. Spermatogenesis arrest, medium disorders in germinal cell distribution (2nd month) (H-E, × 20)

exposed for three months there were even more maturation arrest in spermatogenesis and the germinal cells disappeared (fig. 7). In addition, a reduction in leyding cell population linked to exposure time was also observed. After Bcl-2 and Bax immunohistochemical staining, Bax staining of spermatocytes in all groups was observed, strongest in two month exposed animals (fig. 8). One month and three months exposed groups followed. Staining of cells were completely intracytoplasmic. Bcl-2 staining was generally weak with strongest seen in 2 month exposed group. There was nearly no staining in one month group whereas a faint staining was observed in three month group (fig. 9). Generally spermatocytes were stained with Bcl-2.

The main objective of this work was to investigate the effects of ELF magnetic fields on spermatogenesis, testosterone levels and testis morphology associated with biochemical parameters of adult male rats. Al-Akhras et al. (2) reported that exposure of adult male rats to 50 Hz 25 μT magnetic fields for a period of 18 consecutive weeks caused a significant reduction in the weights of seminal vesicles and prostate.

Exposure of adult male rats to 50 Hz magnetic fields for 90 days had significant effects on fertility of females impregnated by the exposed males in terms of the number of pregnant females, was reduced and the number of resorbed fetuses was decreased (2). In multigeneration reproductive toxicity study showed by Ryan (25) that continuous exposure of rats to 60 Hz magnetic fields have no significant adverse effects on adult reproductive capacity, developing fetus and neonatal development in rats. Heredia-Rojas (14), reported that 60 Hz and 2 mT magnetic field exposure did not affect meiotic chromosomes and morphological characteristic of male germ cells in mice. On the contrary, De Vita et al. (8) reported that exposure to 50 Hz and 1.7 mT for 4 h caused a significant decrease of spermatid level. Other authors (7) reported that exposure of 60 Hz magnetic fields for continuously did not produce any detectable alterations in offspring spermatogenesis and fertility. In this study the results of sperm motility observed in the three exposure groups did not differ from the control values. There was no obvious difference in the incidence if abnormal sperm between the groups.

Reactive oxygen species (ROS) and oxidative damage to biomolecules as a mechanism of chemical and physical environmental agents may contribute to male infertility by reducing sperm function (4). The teratogenic effects of chemical and physical environmental agents are often related to their ability to damage DNA. The possibility that magnetic fields induce genotoxic effects was determined (16). In the present investigation, significant decreases in testis and plasma catalase activities were observed in 3 months exposure groups.

Lee et al. (19) reported that continuous exposure to EMF for 8 weeks caused an increased incidence of

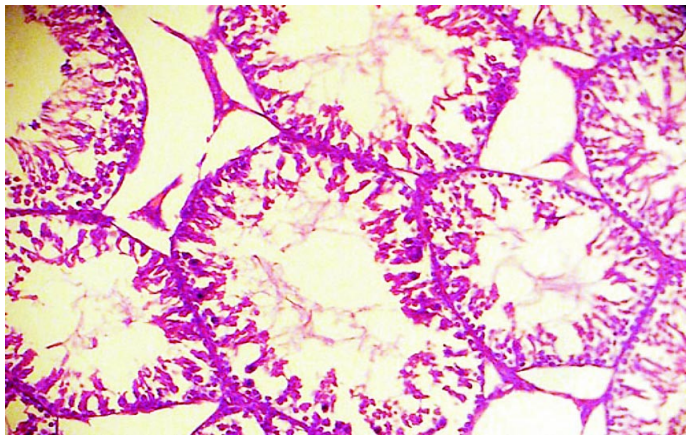

Fig. 7. Spermatogenesis arrest, strong disorders in germinal cell distribution (3rd month) (H-E, × 20)

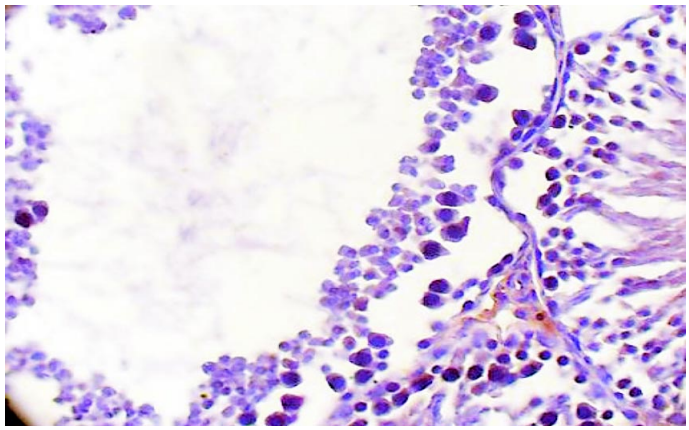

Fig. 8. Positive immunohistochemical Bax staining in testis spermatogenesis cell (Bax, × 40)

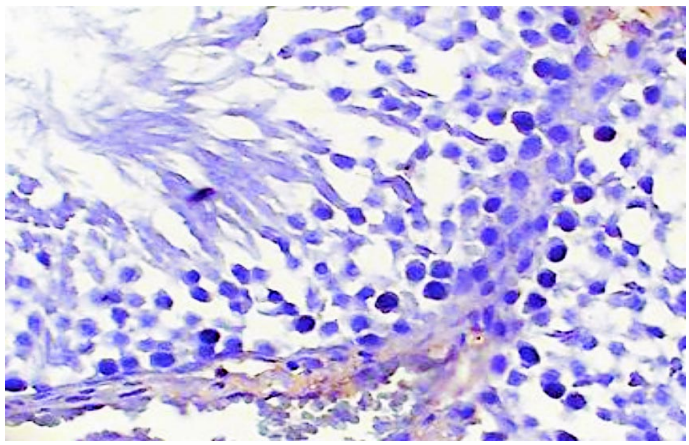

Fig. 9. Positive immunohistochemical Bcl-2 staining in testis spermatogenesis cells (Bcl-2, × 40)

testicular germ cell death and this finding resulted from an increased incidence of germ cell apoptosis in mice. In the present study, the decreases in germ cells and testosterone level and spermatogenesis hesitation have also been determined. The results presented here in show that exposure to ELF EMFs did not caused a significant reduction in testicular sperm count in the exposed groups. Such reduction may be caused by a direct effect of ELF magnetic field on testicular Leydig cells, causing a decrease in testosterone pro-

duction. This could cause to spoil the histological structure of testis (3).

It is well known that Bcl-2 and Bax proteins, which are gene products related with apoptose, are normally present in spermatogenetic cells. In some studies (27), it was found to change in the levels of Bcl-2 and Bax when spermatogenesis effected and so apoptose increased. In the present study, apoptose increased for a time period and then decreased with spoiling of spermatogenesis. It was found that Bax (proapoptotic) level increased during first month and reached maximum in second month and then decreased to minimum level in third month again. Bax positive cells counted the highest in group exposed to ELF-EMF field during 2 months and it was the lowest in group exposed to ELF-EMF field during 3 months. However, Bcl-2 level was the lowest in group exposed to ELF-EMF field during 2 months and it was the hishest in group exposed to ELF-EMF field during 3 months. Dying with Bcl-2 showed only in a month group.

The balance between Bax and Bcl-2 regulate the apoptose or living of cells (27). In the present study, at least in the beginning it was found to increase in Bax level for getting opoptose of spoiled cells with th effect of magnetic field. Then Bcl-level increased to be protected healthy germ cells and apoptose was stoped mainly. Later Bax level decreased rapidly, whereas Bcl-2 level decreased very slowly. This result confirmed the results of preceding studies.

In conclusion, adverse effects on cell proliferation, decrease in testestorone level and germ cell population were determined on rats exposed to chronic 50 Hz magnetic field. But no effect on sperm motility and abnormal sperm level were found.

References

1. *Aebi H.*: Catalase, [in:] Methods in Enzymatic Analysis. Ed HU Bergmeyer. New York: Academic Press 1983, 276-286.
2. *Al-Akhras M. A., Elbetieha A., Hasan M. K., Al-Omari I., Darmani H., Albiss B.*: Effects of extremely low frequency magnetic field on fertility of adult male and female rats. Bioelectromagnetics 2001, 22, 340-344.
3. *Amman R. P.*: Use of animal model for detecting specific alterations in reproduction. Fundam. Appl. Toxicol. 1982, 2, 13-26.
4. *Atessahin A., Karahan I., Turk G., Gur S., Yılmaz S., Ceribası A. O.*: Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and lipid peroxidation in rats. Reprod. Toxicol. 2006, 21, 42-47.
5. *Brent R. L.*: Reproductive and teratologic effects of low-frequency electromagnetic fields: a review of in vivo and in vitro studies using animal models. Teratology 1999, 59, 261-286.
6. *Chung M. K., Kim J. C., Myung S. H., Lee D. I.*: Developmental toxicity evaluation of ELF magnetic fields in Sprague-Dawley rats. Bioelectromagnetics 2003, 24, 231-240.
7. *Chung M. K., Lee S. J., Kim Y. B., Park S. C., Shin D. H., Kim S. H., Kim J. C.*: Evaluation of spermatogenesis and fertility in F1 male rats after in utero and neonatal exposure to extremely low frequency electromagnetic fields. Asian. J. Androl. 2005, 7 (2), 189-194.
8. *De Vita R., Cavallo D., Raganella L., Eleuteri P., Grollino M. G., Calugi A.*: Effects of 50 Hz magnetic fields on mouse spermatogenesis monitored by flow cytometric analysis. Bioelectromagnetics 1995, 16, 330-334.
9. *Ebling F.*: Hormonal control of sebaceous glands in experimental animals, [in:] Mantagra W., Ellis R., Silver A. F. O., (ed.): Advances in the Biology of Skin, vol. 4. Pergaman Press. Oxford 1963, 2000-2219.
10. *Ellman G.*: Tissue sulphydryl groups. Arch. Biochem. Biophys. 1959, 82, 70-77.
11. *Furuya H., Aikawa H., Hagino T., Yoshida T., Sakabe K.*: Flow cytometric analysis of the effects of 50 Hz magnetic fields on mouse spermatogenesis. Nippon. Eiseigaku. Zasshi. 1998, 53, 420-425.
12. *Goth L.*: A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta. 1991, 196, 143-151.
13. *Henshaw D. L.*: Does our electricity distribution system pose a serious risk to public health. Medical Hypotheses. 2002, 59 (1), 39-51.
14. *Heredia-Rojas J. A., Caballero D. E., Rodriguez A. O., Ramos G., Rodriguez L. E.*: Lack of alterations on meiotic chromosomes and morphological characteristics of male germ cells in mice exposed to a 60 Hz and 2.0 mT magnetic field. Bioelectromagnetics 2004, 25, 63-68.
15. *Hong R., Liu Y., Yu Y. M., Hu K., Weng E. Q.*: Effects of extremely low frequency electromagnetic fields on male reproduction in mice. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 2003, 21, 342-345.
16. *Huuskonen H., Lindbohm M. L., Juutilainen J.*: Teratogenic and reproductive effectts of low frequency magnetic fields. Mutation Res. 998, 410, 167-183.
17. *Johnsen S. G.*: Testicular biopsy score count a method for registration of spermatogenesis in human normal values and results in 335 hypogonadal males. Hormones 1970, 1, 2-25.
18. *Kowalczuk C. I., Robbins L., Thomas J. M., Saunders R. D.*: Dominant lethal studies in male mice after exposure to a 50 Hz magnetic field. Mutat. Res. 1995, 328, 229-237.
19. *Lee J. S., Ahn S. S., Jung K. C., Kim Y. W., Lee S. K.*: Effects of 60 Hz electromagnetic field exposure on testicular germ cell apoptosis in mice. Asian. J. Androl. 2004, 6, 29-34.
20. *Lin R. S., Lee W. C.*: Risk of childhood leukemia in areas passed by high power lines. Rev. Environ. Health. 1994, 10, 97-103.
21. *Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J.*: Protein measurement with folin phenol reagent. J. Biol. Chem. 1951, 193, 265-275.
22. *Ohkawa H., Ohishi N., Yagi K.*: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979, 95, 351-358.
23. *Ozguner I. F., Dindar H., Yagmurlu A., Savas C., Gokcora I. H., Yucesan S.*: The effect of electromagnetic field on undescended testis after orchiopexy. Int. Urol. Nephrol. 2002, 33, 87-93.
24. *Ramadan L. A., Abd-Allah A. R., Aly H. A., Saad-el-Din A. A.*: Testicular toxicity effects of magnetic field exposure and prophylactic role of coenzyme Q10 and L-carnitine in mice. Pharmacol. Res. 2002, 46, 363-370.
25. *Ryan B. M., Symanski R. R., Pomeranz L. E., Johnson T. R., Gauger J. R., McCormick D. L.*: Multigeneration reproductive toxicity assessment of 60-Hz magnetic fields using a continuous breeding protocol in rats. Teratology 1999, 59, 156-162.
26. *Sönmez M., Turk G., Yuce A.*: The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. Theriogenology 2005, 63, 2063-2072.
27. *Taylor M. F., Woolveride I., Metcalfe A. D., Streuli C. H., Hickman J. A., Morris I. D.*: Leyding cell apoptosis in the rat testes after administration of the cytotoxin ethane dimethanesulphonate: role of the Bcl-2 family members. J. Endocrinol. 1998, 157, 317-326.
28. *Wartenberg D.*: Residential EMF exposure and childhood leukemia: Meta-analysis and population attributable risk. Bioelectromagnetics 2001, 22, 86--104.
29. World Health Organization (WHO). Anon: Environmental Health Criteria: Magnetic fields. Geneva 1987, 69.
30. *Yokoi K., Uthus E. O., Nielsen F. H.*: Nickel deficiency diminishes sperm quantity and movement in rats. Biol. Trace. Elem. Res. 2003, 93, 141-153.

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