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Effects of exposure to 50 Hz, 1 Gauss magnetic field on reproductive traits in male albino rats

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Abstract

The effects of extremely low frequency magnetic field ELF-MF (50 Hz/1 Gauss) on male fertility were studied in rats. Twenty eight adult male Wistar rats were divided into 4 groups, 2 experimental groups exposed to magnetic field for 21 days and 2 control groups (sham exposed). The first exposed group was sacrificed at the end of the exposure period, and the second exposed group was kept for extra 48 days post exposure to assess the delayed effects of exposure. Significant decrease in the testes weights, sperm motility, sperm count, normal sperms and live sperms was detected in the exposed groups compared to control. Serum testosterone concentrations were significantly increased after 21 days of exposure, and then these changes were corrected after 48 days post exposure. Superoxide dismutase, catalase activities and α-tocopherol concentration were significantly decreased after 21 days of exposure, while the later became non-significant after 48 days post exposure. L-ascorbate concentration was significantly increased after 21 days of exposure, and returned to normal 48 days post exposure. Histopathological examination of the testis from the exposed rats showed a disruption in its architecture with an increase in Leydig cell number and activity, whereas 48 days post exposure, the testicular germ cell layers increased with presence of high apoptotic rates. In conclusion, prolonged exposure to 50 Hz, 1 Gauss MF is biologically toxic to the testicular functions with oxidative stress on spermatogenesis that subsequently may affect male fertility and his sexual efficiency.

Magnetic field, male fertility, testis, sperm, testosterone, antioxidants, delayed effects

With the ever-increasing use of the electric technology, electromagnetic fields - especially the extremely low frequency electromagnetic fields (ELF-EMF) - have become part of modern life. These fields are produced by all electric devices, including high energy sources like power lines and microwaves, but also found in low energy devices such as cell phones (van Deventer et al. 2005). Spontaneously with the abundance of ELF-EMF within the environment of every day life, the question has been raised as to whether or not these EMFs might be harmful to biological life (Sun et al. 2005). While there is a number of potential uses for EMF in biosciences and medicine (including semen separation and electro-ejaculation), it is important to look at the potential side effects that may be incurred by EMF exposure. Studies have shown several evidences related to EMF and their effects on physiological systems (Fadel et al. 1994 and McCaig et al. 2004). During recent decades much interest has centered on power lines and their possible effects on health including erythrocyte properties and heart functions (Fadel et al. 2003), cell stress (Tokalov and Gutziet 2004), reactive oxygen species and free radicals (Seyhan et al. 2006), male and female reproduction and fertility (Al-Akhras et al. 2006). The effects of ELF-EMF on the male reproduction are receiving heightened interest because human male fertility in the 20th century was diminished; human sperm count in 1940 was nearly two-fold compared to the sperm count in 1990 (Giwereman et al. 1993). Exposure to environmental pollutants enhances the generation of reactive oxygen species (ROS) and thus causes destructive effects on various cellular organelles like mitochondria and sperm DNA (Agarwal and Prabakaran 2005). This study was undertaken to investigate the effects of exposure to 1 Gauss magnetic field on the fertility of male albino rats and to clarify if these effects

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could be reversed or not. This was achieved by the evaluation of male fertility indicators including semen profile, testosterone concentration in serum, epididymal antioxidants, testicular histopathology and the relation of exposure to 1G magnetic field with these indicators.

Materials and Methods

Animals

Mature male Wistar rats weighing between 292-329 g (age 6 months) were used in this study. Animals were kept in a 12 h light/12 h dark cycle, at constant temperature of 25 °C, food and water were available *ad libitum*.

Exposure facility

Animals were exposed to a homogenous magnetic field generated by 4 solenoids of 270 turns each of electrically insulated 2.2 mm copper wire, wound around a copper cylindrical chamber of 55 cm external diameter as shown in (Plate VII, Fig. 1). For more details see our previous report (Fadel et al. 2003). Animals were kept in special plastic cages fixed on supports inside the irradiation chamber. Food and water were kept in special open containers fixed on the walls of the cages. Cleaning and changing water and food was done for all animals twice daily. Animals were exposed to the magnetic field in a free volume of about 2000 cm^2 at the center of the solenoids. The magnetic field was measured by means of a hand held Gauss (G)/Tesla (T) meter (Model 4048 with probe T-4048.001 by Bell Technologies, Inc., USA), and the magnetic flux density in the area where the animals were housed, was 1 ± 0.1 G. The coils were connected to a Variac fed from the mains (220 V and 50 Hz).

Experimental design

Twenty eight male Wistar rats were divided into four equal groups, namely group 1, 2, 3 and 4, of seven animals each. Groups 1 and 3 were exposed to the MF continuously for 21 days. Group 1 was sacrificed at the end of the 21st day of the exposure by cervical dislocation. Group 3 was kept for 48 days post the end of exposure period for studying the delayed effects of MF exposure (reversibility or irreversibility). This group was kept away from the MF in similar conditions to the control group for 48 days which is the length of spermatogenesis in male rats according to Hafez (1970). The control groups 2 and 4 were handled as the experimental groups 1 and 3, respectively, but without exposure to MF (sham exposed). Body weights were measured before and at the end of the experiment.

Blood samples were collected during scarifying the animals in tubes without anticoagulants then centrifuged at 1200 *g* for 15 min to separate serum alone.

The testes were excised and the epididymes were carefully removed and the testes weights were recorded.

Examination of semen profile

The cauda epididymis of one testis was excised and received in a sterilized Petri dish containing 2 ml warm normal saline of 37 °C, and then macerated to obtain the epididymal contents in a suspension that was handled exactly as the semen (Hafez 1970). Sperm motility assay was carried out after the method of Slott et al. (1991). A drop of the suspension was put on clean glass slide prewarmed to 37 °C and covered by glass cover slide, also, pre-warmed to 37 °C and examined under high power (× 40) of light microscope for evaluating individual motility of the spermatozoa. Several microscopic fields were examined to evaluate the percentage of motile spermatozoa.

Sperm abnormalities and live/dead spermatozoa were determined using the method of Filler (1993). One drop of the suspension was put on a glass slide and stained by eosin-nigrosine stain. Sperm cell concentration per ml of semen was performed according to the method of Robb et al. (1978). Semen was diluted 5 times (v/v) with normal saline previously add to it a few drops of formalin (40%) to kill the spermatozoa, and used for counting the spermatozoa by an improved Neubauer haemocytometer counting chamber.

Determination of the epididymal antioxidants

Four milliliters of physiological saline were added to 0.3 g of cauda epididymis, and then the contents were homogenized (Homogenizer MPW-120) for 5 min then the homogenate was centrifuged at 1200 *g* for 10 min to obtain the supernatant. Catalase (CAT) activity was determined according the method of Cohen et al. (1970). Superoxide dismutase (SOD) activity was determined according to the method of Packer and Glazer (1990). L-ascorbic acid concentration was determined according to the method of Kyaw (1978). Alpha-tocopherol concentration was determined by HPLC according to the method of Sobczk et al. (1999).

Measurement of serum testosterone

The serum samples used for measurement of testosterone concentration were estimated by the electro-chemiluminescence-immuno-assay (ECLIA) kits and measured by Roche Elecsys 2010 (Hitachi Ltd, Boehriger, Mannheim, Tokyo, Japan). The analytical sensitivity of these kits is 0.02 ηg/ml serum. (Elecsys Testosterone reagent kit, Cat. No.11776061-100 test).

Histopathological examination of the testes using H&E and PAS stains

The routine histological examination of the testes was done after fixation by Bouin's fixative and handled according to the method of Chapin et al. (1984) and Leblond and Clermort (1952) for H&E and PAS staining, respectively.

Statistical analysis of the data

The obtained data were analyzed using the statistical package for social science (SPSS, 8.0 software, 1997) for obtaining mean and standard error. The data were analyzed using one-way ANOVA to determine the significance of differences among groups. The values were significant at $P \leq 0.05$. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity.

Results and Discussion

The results of this experiment are recorded in Tables 1-4 and Figs 2 and 3. From Table 1, there is no significant difference in the mean body weights either between groups 1 and 2 (295.34 ± 13.67 g and 292.72 ± 12.02 g, respectively) or between groups 3 and 4 (329.05 ± 28.95 g and 324.75 ± 25.61 g, respectively) suggesting that there is no effect of MF on the apparent body weight. These results were similar to works reported by Al-Akhras et al. (2006). It is clear that the time factor in this work (21 days of exposure) was not long enough to produce a change in the body weight. The mean testes weight in group 1 was significantly decreased compared to control (2.46 ± 0.10 g vs. 3.08 ± 0.06 g). One may state that MF acts as a direct stressor which induces gonadal regression. After 48 days from exposure the testes weights in group 3 were significantly decreased than group 4 (2.82 ± 0.20 vs. 3.32± 0.13 g) indicating the late effects of MF on the testis morphology. These results are similar to that reported by Wilson et al. (1999). However, the results of the current study showed marked testicular histopathological changes represented by increased cell apoptosis and seminiferous tubular damage that may lead to a decrease in testicular weights (Plate VIII, Fig. 3).

Table 1. Effect of 50 Hz, 1 G MF exposure on body and testis weight in rats.

Groups	Body weight (g) ± SE	Testis weight (g) ± SE
1	295.34 ± 13.67[a]	2.46 ± 0.10 [a]
2	292.72 ± 12.02[a]	3.08 ± 0.06 [bc]
3	329.05 ± 28.95[b]	2.82 ± 0.20 [ab]
4	324.75 ± 25.61[b]	3.32 ± 0.13 [c]

Group 1 was exposed to MF for 21 days; group 2 is control of group 1; group 3 was kept for 48 days post the end of exposure; and group 4 is control of group 3.
*Means within the same column carrying different superscriptions are significant at $P \leq 0.05$

A significant reduction in the mean of sperm cell concentration, motile sperms and viable sperms with a significant increase in abnormal sperms in group 1 and 3 (Table 2) was found compared to the control groups. Fig. 2 showed some of the studied sperm abnormalities; either primary abnormalities (Plate VII, Fig. 2a - stunted sperm with an abnormal head shape; c, d - double tailed sperm and f - abnormal hook shape) or secondary abnormalities (Fig. 2b - bent tailed sperm with cytoplasmic droplet; e - detached head and h - detached tail). The abnormal semen profile may be a direct and late effect of MF on spermatogenesis process. These results were similar to that reported by Saito et al. (1999) and Davoudi et al. (2002). It was stated that tubulin dimmers in cells have a piezoelectric property (Tuszynski and Kurzynski 2003), and this may be a target for action of magnetic field that will affect the sperm motility and spermiogenesis resulting in sperm morphological abnormalities. Regarding sperm viability, Ayrapetyan (2006) concluded that EMF causes disturbances in sperm cell membrane mechanisms that govern ion passage especially to sodium and potassium and consequently the water content and sperm viability, these findings are similar to the present results. We can hypothesis the action of 50 Hz, 1 G MF on the moving ions and molecules in the testis during metabolism and cellular functions is the orientation of these molecules and ions from pathways to other pathways, and these interactions will occur for these moving ions and molecules with frequency 50 Hz (Fadel

Table 2. Effect of 50 Hz, 1 G MF exposure on semen profile in rats.

Indicators		Group 1	Group 2	Group 3	Group 4
Motility (%)		37.14 ± 6.44[a]	91.42 ± 0.92[b]	52.85 ± 10.62[a]	92.85 ± 1.01[b]
Alive (%)		46.10 ± 5.71[a]	96.24 ± 0.63[b]	58.68 ± 9.48[a]	96.22 ± 0.89[b]
Sp.C.C./ ml × 125 × 10^4		12.50 ± 1.44[a]	25.72 ± 1.16[b]	15.20 ± 2.28[a]	26.71 ± 1.77[b]
	Head	0.89 ± 0.51[a]	0.64 ± 0.17[a]	8.23 ± 1.76[b]	0.62 ± 0.69[a]
	Tail	42.09 ± 2.93[a]	4.22 ± 0.94[c]	26.75 ± 2.22[b]	2.15 ± 0.69[c]
Abnormalities (%)	Midpiece	2.10 ± 0.42[a]	0.37 ± 0.14[b]	1.25 ± 0.51[a]	0.35 ± 0.20[b]
	Multiple	2.16 ± 0.58[a]	0.35 ± 0.11[b]	1.99 ± 0.65[a]	0.65 ± 0.20[b]
	Total	47.24 ± 2.81[a]	5.51 ± 1.07[c]	38.15 ± 0.86[b]	3.80 ± 0.99[c]

Group 1 was exposed to MF for 21 days; group 2 is control of group 1; group 3 was kept for 48 days post the end of exposure; and group 4 is control of group 3.
Sp.C.C. is the sperm cell concentration multiplied for 125 × 10^4.
*Means within the same column carrying different superscriptions are significant at $P \leq 0.05$.

Table 3. Effect of 50 Hz, 1G MF on serum testosterone and epididymal antioxidants.

Groups	Serum testosterone (ng/ml) ± SE	Catalase ± SE (µmol H_2O_2/min/ml)	SOD (eU) ± SE	L-ascorbate (mg/dl) ± SE	α-tocopherol (mg/l) ± SE
1	5.29 ± 1.50[a]	254.28 ± 2.65[a]	0.51 ± 0.02 [a]	0.63 ± 0.01[a]	29.87 ± 7.99[a]
2	0.99 ± 0.18[b]	369.74 ± 1.53[b]	1.03 ± 0.05[b]	0.52 ± 0.01[b]	91.61 ±4.32[b]
3	0.77 ± 0.12[b]	370.19 ± 1.98 [b]	1.04 ± 0.02[b]	0.48 ± 0.04[b]	102.45 ± 4.39[b]
4	0.68 ± 0.10[b]	383.84 ±1.45 [c]	1.27 ± 0.02[c]	0.47 ± 0.03[b]	106.07 ± 5.51[b]

Group 1 was exposed to MF for 21 days; group 2 is control of group 1; group 3 was kept for 48 days post the end of exposure; and group 4 is control of group 3.
*Means within the same column carrying different superscriptions are significant at $P \leq 0.05$.

1998) i.e. at resonance frequency. Prolonged exposure of the testes to 50 Hz, 1 G MF results in permanent changes in cellular functions and structure. Based on this understanding we can explain all histological deterioration in the sperms and testicular tissue architecture (Plate VIII, Fig. 3).

From Table 3, serum testosterone concentration in group 1 was directly increased by direct exposure to 50 Hz MF (5.29 ± 1.50 ηg/ml vs. 0.99 ± 0.18 ηg/ml in the control group) while there was no significant difference between groups 3 and 4. This result is consistent with the increase in Leydig cell proliferation and activity that recorded in the present study and may becaused by the direct effect of MF on Leydig cells and steroidogenesis.

Imbalance in the activity and concentration of the antioxidants (Table 3) in group 1, represented as decrease in catalase and SOD activities, increase in L-ascorbate concentration and decrease in α-tocopherol concentration, indicated the presence of oxidative stress, i.e. the increase in the reactive oxygen species (ROS), which will disturb the cellular metabolism. Apoptosis and decrease in cellular numbers in the seminiferous tubules were found that may be due to the direct effect of MF or to the oxidative stress found in this study. However, in group 3 concentrations of vitamin C and E showed no significant difference between control groups but catalase and SOD activities still decreased. Animals after 48 days post-exposure began to regain their antioxidative equilibrium but to some extent they failed to return to normal. In conclusion, prolonged exposure to 50 Hz, 1 G MF is biologically toxic and direct exposure of the testes to such magnetic field should be avoided. We may advise users of devices such as laptops not to put the equipment on their knees while using it, to avoid exposure of the testes to electromagnetic fields that also affect the testosterone concentration which plays an important role in male fertility and sexual efficiency.

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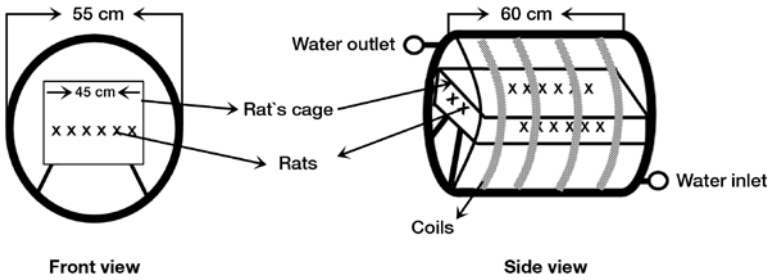

Fig. 1. A diagram of the magnetic exposure system. It is composed of 4 solenoids producing a 0.1 mT homogeneous magnetic field at their central axis using a sinusoidal current of 50 Hz. Animals were exposed continuously as a group in a plastic cage on the shelf within the solenoid.

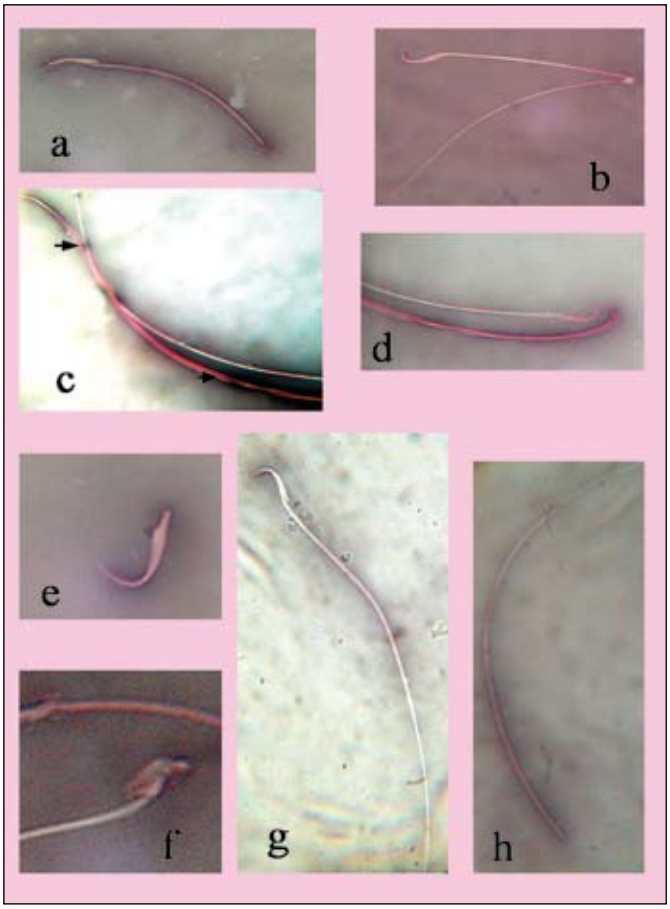

Fig. 2. A plate showing different forms of epididymal sperm abnormalities recorded in groups 1 and 3. a - Stunted sperm with abnormal head shape, b - Bent tail with cytoplasmic droplet (arrow), c - d - Double tailed sperm (arrows), e - Detached head, f -Abnormal hook shape g - Normal sperm, h - Detached tail.

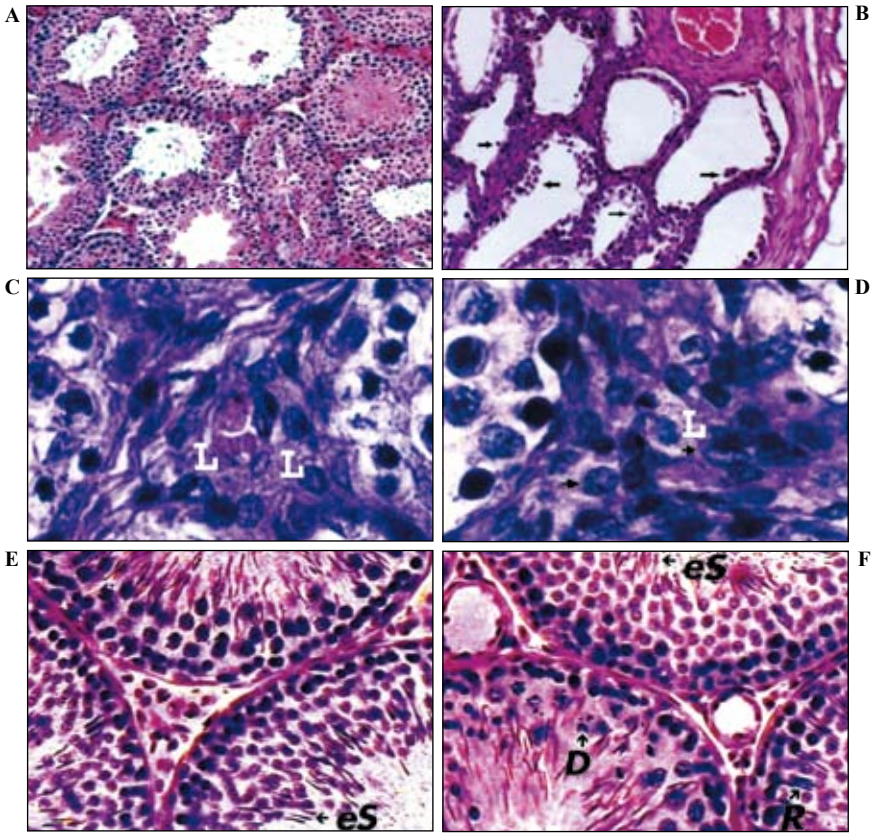

Fig. 3. Photomicrographs of sections in mature rat testes. A; Group 2 showing normal view of seminiferous tubules, spermatozoa in the tubular lumen and normal Leydig cell number, × 40. H&E stain. B; Group 1 showing degeneration, distortion of tubular architecture and tubular dilation. Sloughing and pyknosis of germ cells (arrows), absence of spermatids and vasodilation of interstitial blood vessels, × 40. H&E stain. C; Group 2 showing PAS reactivity in Leydig cells (L), Sertoli cells (SC), × 100. PAS stain. D; Group 1 showing more PAS reaction in cytoplasm of Leydig cells (L) and a higher increase of the numbers of Leydig cells (arrows), × 100. PAS stain. E; Group 4 showing normal arrange of seminiferous tubules layers, abundance of the elongated spermatids (eS) and normal number of sperms, × 100. PAS stain. F; Group 3 showing primary spermatocytes in division (D), late elongated spermatids (eS), retained late elongated spermatids which were malformed and pyknotic (R). Few number of spermatozoa and detached pyknotic round spermatids in the lumen, × 100. PAS stain.