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The effects of an electromagnetic field on the boundary tissue of the seminiferous tubules of the rat: a light and transmission electron microscope study

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Human beings are unavoidably exposed to ambient electromagnetic fields (EMF) generated from various electrical devices and from power transmission lines. Controversy exists about the effects of EMF on various organs. One of the critical issues is that EMF may adversely affect the reproductive system. In order to examine this 30 rat pups were exposed to 50 Hz EMF (non-ionising radiation) during in utero *development (approximately 3 weeks) and postnatal life (5 weeks). Groups of exposed rats were subsequently left in an environment free of EMF in order to observe recovery, if any, from the changes induced by EMF on the boundary tissue of the seminiferous tubules. The materials were processed and observed under a light and a transmission electron microscope. In the experimental rats boundary tissue was found disrupted at various layers. This tissue showed infoldings, which were perhaps due to the loss of collagen and reticular fibrils from the inner and outer non-cellular layers. The outer non-cellular layer, which was thinner than that of the control, was stripped away from the myoid cell layer in multiple regions, giving a "blister-like" appearance. The myoid cells showed fewer polyribosomes, pinocytotic vesicles and glycogen granules. Most mitochondria were found to lack cristae. The connections between individual myoid cells were apparently lost. There were signs of recovery in the boundary tissue following withdrawal from EMF exposure. These results suggest that EMF exposure may cause profound changes in the boundary tissue of the seminiferous tubules. Therefore exposure to EMF may result in pathological changes that lead to subfertility and infertility.*

Key words: electromagnetic fields, testes, boundary tissue, seminiferous tubules, rats, electron microscopy

INTRODUCTION

In an industrialised society ambient electromagnetic fields (EMF) are encountered everywhere and cause unavoidable exposure. With the increased use of power lines and modern electrical devices concern about the public health hazards of chronic

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exposure to EMF has gained more attention. The transport and use of electricity generates both electrical and magnetic fields with a wide spectrum of frequencies, intensities and waveforms. The power frequency for electrical transmission and distribution for domestic services is 60 Hz in North America and 50 Hz in Europe. Accordingly, most mammalian reproductive research has focused on these frequencies because of their ubiquitous presence in the environment [2, 22]. One critical issue is whether EMF may potentially affect the reproductive system.

It has been shown that exposure to EMF adversely affects spermatogenic, Sertoli and Leydig cells [5, 8, 12, 15, 23]. Magnetic fields of 50 Hz may induce cytotoxic and cytostatic changes in the differentiating spermatogonia of mice [2]. Little is known about the effect of EMF on the cytoarchitecture of the boundary tissue of the seminiferous tubules that perform a number of crucial functions, including the mechanical support and transport of nutrients for the spermatozoa [3, 16, 26] and sperm discharge by maintaining pressure on the tubules [13]. Earlier studies have been made of the transitory effects of EMF on the testes and no study has revealed, to date, the possibility of recovery from the potentially harmful effects of EMF exposure after an exposure-free time. Furthermore, the gonadal effects of EMF at the ultrastructural level have only infrequently been studied.

The aim of this study was to investigate the possible effects of exposure to 50 Hz EMF (non-ionising radiation) on the cytoarchitecture of the boundary tissue of seminiferous tubules during both the prenatal and postnatal periods. A group of exposed rats was subsequently left in an unexposed situation in order to observe the recovery, if any, from the changes brought about by EMF exposure. The result of this study is of potential use, as exposure to electromagnetic waves is ubiquitous; a large portion of the world's population is constantly exposed to a variety of this radiation as a result of professional, residential, medical, industrial or other uses.

MATERIAL AND METHODS

Animals and maintenance

A total of 40 male and 40 female Wistar rats (of approximately 15 weeks old) were used for the study. Rats of the same sex were housed together (5 per cage) and kept in quarantine for one week to rule out any disease. Rats were fed on compact food in the form of granules and water. This food consisted of all the essential ingredients, including vitamins and minerals. The environmental conditions (temperature and humidity) in all the animal holding areas were continuously monitored. Temperature was maintained in the range of 20–30°C and relative humidity was monitored at 35–60%. Fluorescent light was provided on a 12 h light/dark cycle and kept turned on from 8 a.m. till 8 p.m. Lights were located at a distance of three metres from the cages so that these did not interfere with EMF of the experimental design.

EMF-producing system

The equipment was based on the Helmholtz coil, which works following Fleming's right hand rule. This produced an alternate current of 50 Hz, creating an EMF of 80 G. The intensity of the EMF could be controlled by a transformer. The equipment had two main parts. In the first there were two copper coils placed one above the other and separated by a distance of 50 cm. Between the coils (the exposure area) there was a cylindrical wooden vessel, the interior of which had a chamber for holding the cages of the experimental animals. The second part was the transformer, which checked the input and output voltage with a voltmeter and the current with an ampere meter. To prevent increases in temperature inside the chamber a fan was utilised as necessary. Five cages at a time were placed within the chamber with seven or eight rats per cage.

EMF exposure

Rats were selected at random into breeding pairs. Each of the 40 breeding pairs was contained in a separate cage in order to allow monogamous mating. Females were observed for signs of pregnancy (e.g. vaginal plugging on the next day). Of the 40 breeding female rats 30 were selected randomly for exposure to EMF as the experimental group and 10 as the control group (unexposed). The male rats used for breeding were subsequently returned to the animal house.

A total of 172 pups, which received EMF exposure *in utero* (i.u.), were delivered by the experimental female rats (with a gestation period of approximately 3 weeks). In the control group 83 pups were born to 10 control female rats. From both experimental and control groups, 30 pups each were chosen randomly (Table 1). The experimental pups were exposed to EMF until 5 weeks of postnatal (p.n.) age. At the end of this period 15 pups from both groups were sacrificed (termed experimental group 1 and control group 1). The remainder were left unexposed

Table. 1. Experimental design to show duration of EMF exposure

Exposed animals				Control animals		
Group	**Sex**	**No.**	**Duration of exposure**	**Group**	**Sex**	**No.**
EG — 1	Male	15	3 weeks i.u. + 5 weeks p.n.	CG — 1	Male	15
EG — 2	Male	15	3 weeks i.u. + 5 weeks p.n. + 8-weeks period without exposure	CG — 2	Male	15

i.u. — intrauterine; p.n. — postnatal; EG — experimental group; CG — control group

for another 8 weeks in a normal environment and then sacrificed (termed experimental group 2 and control group 2).

Tissue fixation

At the termination of the stipulated exposure period as laid down in the experimental design the rats were anaesthetised with chloroform and 10% formalin was then injected through the inferior vena cava. The testes were removed and fixed in formalin for light microscopy. Haematoxylin and eosin were used to stain the 6 mm thick histological sections.

Transmission electron microscopy

For transmission electron microscopy (TEM) the tissue samples were cut into pieces (2 × 2 mm) and fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 6–8 h at 4°C. They were washed and postfixed in 2% OsO_4 for 1 h at 4°C. The tissue was dehydrated through ascending grades of ethanol and embedded in araldite CY212. Semithin sections (1 μm) were cut and stained with toluidine blue. Ultrathin sections (60–70 nm) were cut, mounted onto copper grids and stained with uranyl acetate and alkaline lead citrate. Sections were observed under a Philips CM10 transmission electron microscope.

Data analysis

All data were expressed as means ± SD. All statistical analyses were performed using SPSS software, version 12, on the basis of the ANOVA test. Post-hoc tests were performed using the LSD test. A P value less than 0.01 was considered significant.

RESULTS

Light microscopy

Control group 1 (5 weeks of p.n. age). A number of round, oval or hexagonal seminiferous tubules could be seen in the transverse sections with connective tissue separating them. The boundary tissue or lamina propria consisted of outer and inner layers of collagen fibres. Between these two layers there was a single layer of flat cells with elongated nuclei, the myoid cells, similar to the peritubular contractile cells in humans. In the seminiferous tubules somatic (Sertoli) and germ cell lineage were seen. The latter were spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa (Fig. 1A). The interstitium showed Leydig cells, sections of small blood vessels and lymphatics.

Control group 2 (13 weeks of p.n. age). The observations made for this group did not differ from those for control group 1.

Experimental group 1 (EMF exposure 8 weeks; 3 weeks i.u. + 5 weeks p.n.). This group showed multiple features of disruption of the normal architecture of the seminiferous tubules. The tubules had an irregular shape (Fig. 1B). The boundary tissue was

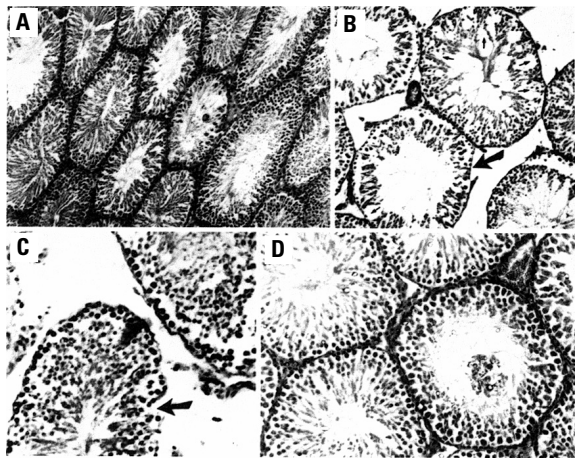

Figure 1. Light micrographs of seminiferous tubules of control group (**A**), experimental group 1 (**B**, **C**) and experimental group 2 (**D**) rats. Arrows show disruption of boundary tissue in experimental group 1 (B, C). Small arrows in Figure 1B indicate abnormal intercellular spaces. Seminiferous tubules and boundary tissue appear almost normal in recovery, experimental group 2 (D). Magnifications: × 240 (A), × 320 (B), × 480 (C, D).

Table. 2. Effects of 60 Hz EMF on boundary tissue

Pinocytic vesicle	Mitochondria (dilated/dense)	Blistering	Breaks	Group
1.60 ± 0.82^{d}	0.73 ± 0.04^{c}	4.60 ± 1.39^{b}	0.89 ± 0.21^{a}	EG — 1
4.00 ± 0.05^{d}	0.29 ± 0.08^{c}	1.40 ± 0.82^{b}	0.40 ± 0.05^{a}	EG — 2
7.00 ± 2.05	0.12 ± 0.11	0.40 ± 0.50	0.11 ± 0.07	CG — 1, 2

EG — experimental group; CG — control groups. The number of boundary tissue breaks per seminal tubule, blistering around single myoid cell, ratio of dilated/dense mitochondria and number of pinocytic vesicles within myoid cells have been shown; ^{a,b,c,d} — comparison between groups show significant statistical differences with p value < 0.01

thinner with breaks in its continuity seen at multiple locations (Figs. 1B, C; Table. 2). The sparse interstitial tissue had wide spaces between the tubules (Fig. 1A as opposed to Fig. 1B). Within the tubules small spaces could be seen as a result of separation of the cells of the spermatogenic lineage. These spaces were oriented circumferentially and radially (Fig. 1B, small arrows). The radially oriented spaces gave it a "frothy" appearance.

Experimental group 2 [EMF exposure 8 weeks (3 weeks. i.u. + 5 weeks p.n.) + unexposed 8 weeks]. The seminiferous tubules appeared almost normal in shape (ovoid or hexagonal), as the large spaces between the tubules disappeared (Fig. 1D). The boundary tissue appeared thicker and unfragmented. The nuclei of the spermatogonia and primary spermatocytes were more euchromatic. Between the spermatogenic cells frequent small spaces (smaller compared to those of experimental group 1) were present, separating the cells arranged in rows (Fig. 1D).

Transmission electron microscopic findings

Control group 1 and 2 (5 weeks and 13 weeks of p.n. age respectively). The four principle layers of boundary tissue were clearly delineated (Figs. 2A, B, 3A, B). The innermost layer was acellular and corresponded to the basal lamina seen by light microscopy (LM). This layer was composed of two dense regions separated by a relatively clear zone (Fig. 2A). The inner dense region was adjacent to the plasma membrane of Sertoli and spermatogonial cells. The next layer, the inner cellular layer, was composed of thick and elongated myoid cells (Figs. 2A, B). The cytoplasm of these cells showed the existence of abundant

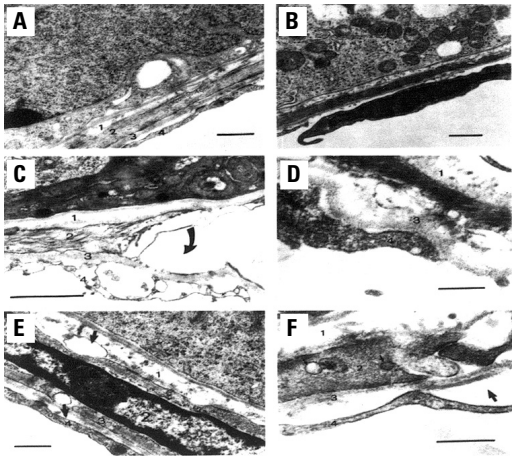

Figure 2. Electron micrographs of boundary tissue in control and experimental groups. **A**, **B**. From control groups showing four layers of boundary tissue (1 — inner acellular layer; 2 — inner cellular layer; 3 — outer acellular layer; 4 — outer cellular layer). **C**, **D**. From experimental group 1. There are ruptures and spaces (curve) in layers 2–4. Note that myoid-to-myoid connection is broken (indicated by arrows). There is infolding of layer 3 (D). **E**, **F**. From experimental group 2. There is recovery of the normal architecture of the boundary tissue layers, except for a few small spaces (arrows). Pinocytotic vesicles (arrows) reappeared in myoid cells (F). Magnification bars: 1 μm (Fig. A, B) and 0.5 μm (C, D, E, F).

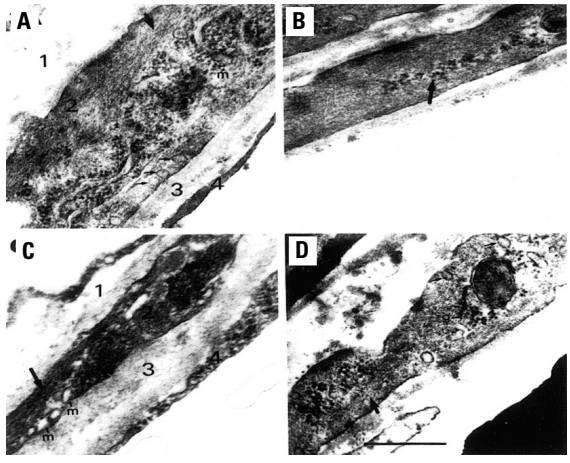

Figure 3. Electron micrographs of boundary tissue of control and experimental groups. **A**, **B**. From control group, showing actin filaments in bundles (A, large arrow), abundant pinocytotic vesicles (small arrows) and mitochondria (m) in myoid cells of layer 2. **B**, **C**. From experimental group 1. There is condensation of actin filaments (arrow) in thin myoid cells. Mitochondria (m) without cristae are indicated. **D**. From experimental group 2. There is a reappearance of less condensed actin filaments (large arrow), pinocytotic vesicles (small arrows) and glycogen granules (g). See Figure 2 for labelling of the various layers of boundary tissue. Magnification bars: 0.5 μm.

glycogen granules, polyribosomes attached to the endoplasmic reticulum (ER) cisternae, numerous actin filaments aggregated into bundles and mitochondria with transverse cristae. Abundant pinocytotic vesicles were observed (Figs. 3A, B; Table. 2). The third layer was acellular and thinner than the innermost one and was composed of collagen and reticular fibrils (Figs. 2A, 3A). The fourth layer, the outermost cellular layer, was thin and composed of lymphatic and endothelial cells, which formed an extensive system of peritubular lymphatic sinusoids. The cell surface was irregular in some regions with an outer lymphatic space present.

Experimental group 1 (EMF exposure 8 weeks: 3 weeks i.u. + 5 weeks p.n.). The boundary tissue of the seminiferous tubules showed spaces and ruptures in its various layers (Figs. 2C, D, 3C). The clear zone of the innermost acellular layer was pale and irregular in thickness with indistinct fibrils. It showed a fragmentation of collagen and reticular fibril bundles and in some areas no fibrils were visible (Figs. 2C, 3C). The myoid cells present in the second layer were thinner and their nuclei were denser than those of the control group (Figs. 2D, 3C). The mitochondria were without cristae and their matrix appeared electron-opaque (Figs. 3C; Table. 2). There were few ER glycogen granules and tubules (Fig. 3C). The actin filaments were less condensed (Figs. 2D, 3C). Pinocytotic vesicles were fewer than in the controls (Figs. 2C, D, 3C; Table. 2). There was a decrease in the abundance of collagen fibrils in the outer acellular layer, which was thinner than that of the controls. This layer was stripped away from the myoid cell layer in many areas (Figs. 2C, D). The outermost endothelial cells also showed separation from the third layer (Fig. 2C). This was thrown into deep folds was thrown into deep folds at several places (Fig. 2D). Its outer surface had a "blister like" appearance (Figs. 2C, D; Table. 2).

Experimental group 2 [EMF exposure 8 weeks (3 weeks i.u. + 5 weeks p.n.) + unexposed 8 weeks]. The innermost layer of the basal lamina appeared normal. The clear zone of this layer was thin, pale, and more regular compared to experimental group 1 (Fig. 2E, $p < 0.01$). Its dark thick outer region had regularly oriented collagen and reticular fibrils and showed no discontinuity. The myoid cells had elongated nuclei with scanty cytoplasm and were wider and less electron-dense than those of experimental group 1 (Figs. 2E, F, 3D; Table 2). There were only small areas of separation between these cells and the inner layers when compared to the same in experimental group 1 (Figs. 2E, 3D). The actin filaments appeared uncondensed. A large number of pinocytotic vesicles were seen in their cytoplasm (Fig. 3D, Table 2). Many tubules of ER and mitochondria with cristae were observed in these cells ($p < 0.01$ vs. experimental group 1). The outer acellular layer appeared thinner ($p < 0.01$ vs. control) with a few cleft-like spaces. The outermost cellular layer was normal (Fig. 2F), the cells being thicker and aligned in a normal fashion (Figs. 2E, F). They adhered to the outer layer of collagen fibrils, thereby abolishing the space (blistering) between the third and fourth layers (Figs. 2E, 3D; Table 2). The blistering here was less frequent than that of experimental group 1 ($p < 0.01$).

DISCUSSION

The potential of EMF adversely affecting the health of the human population is an issue which continues to receive a great deal of attention in both public and scientific forums. The harmful effects of EMF ionising radiations (e.g. X-rays and gamma rays) have previously been demonstrated on gonadal tissues [1, 4, 15, 17, 21]. In this study we have shown the effects of EMF (non-ionising radiation) on the boundary tissues of seminiferous tubules investigated by light and transmission electron microscopy.

McGivren et al. [19] revealed that low-frequency intermittent EMF exposure during the critical prenatal period for neurobehavioural sex differentiation can demasculinise male scent-seeking behaviour and increase the weight of accessory sex organs in adulthood. Lundesberg et al. [18] found no association between occupationally related categories of EMF exposure and male subfertility as evaluated by sperm morphology, motility and concentration. Shafik [23] and his colleagues revealed that polyester underwear (creating an electrostatic potential) significantly impairs spermatogenesis and causes testicular degeneration. In contrast to this, Chung et al. [3] showed that exposure to EMF (from 60 Hz up to 500 mT), both prenatally and postnatally, did not alter offspring spermatogenesis in the rat. Elbetieha et al. [6] also demonstrated that exposure to EMF (50 Hz, 25 mT for 90 days) had no significant effect on the weight of the testes or the number of implantation sites and viable foetuses.

In this study the boundary tissue of the seminiferous tubules was found disrupted at several places and revealed large spaces in the connective tissue layers as seen in LM [16]. TEM studies revealed that the inner non-cellular layer did not show the normal three laminations (consisting of two dense laminae on either side and a homogenous intervening zone). The homogenous zone was paler, with fragmented fibrils

indicating destruction of the middle area. The nuclei of the myoid cells were highly electron-dense, a sign of inactivation brought about by the transition from euchromatic to heterochromatic states that are metabolically inert. Myoid cells were thinner in experimental group 1, which was probably due to condensation of actin filaments. The loss of mitochondria cristae in these cells suggests that they had undergone degeneration. This may have a direct consequence on various physiological activities of these cells. The adjacent myoid cells showed a loss of connectivity. These cells, which are modified smooth muscle cells, probably maintain a certain pressure in order to facilitate sperm discharge [13]. Hence exposure to EMF may potentially alter the characteristics of seminal fluid as a result of impairment of myoid cells within the boundary tissue of seminiferous tubules.

The outer acellular layer was thinner with a number of gaps. These spaces were seen among the collagen and reticular fibrils of the third layer and between this layer and the endothelium of the lymphatic vessels. The infoldings of boundary tissue were most likely due to loss of collagen and reticular fibrils. The irregular gaps and formation of blisters (infolding of boundary tissue) with a break in the endothelium of the lymphatics could be responsible for lack of lymph drainage and the resultant oedema, which was evident from the frothy spaces among the seminiferous epithelial cells under LM. Similar changes in the acellular layers of the boundary tissue of seminiferous tubules were documented in rats subjected to X-ray exposure (ionising radiation) [9]. These findings are similar to the changes that occur in the seminiferous tubules in ageing males (such as thickening of the boundary tissue, a decrease in spermatogenic cell lineage and abnormal extracellular spaces) [10, 11]. Thus it can be postulated that EMF (non-ionising radiation) causes premature ageing of the seminiferous tubules in rats.

Yamamoto et al. [26] opined that vesicular bodies found in the inner and outer cellular layers of the boundary tissue represent the transfer of substances to and from the tubules. The damage to myoid cells affects the mechanical support of the seminiferous tubules. It has been postulated that the contractile myoid cells govern the rate of entry of substances passing in and out of the seminiferous tubules and serve as a barrier to the penetration of substances into the germinal epithelium [3]. This damage to the second defence line of the blood-testis barrier might induce harmful changes in the spermatogenic cells. The absence of pinocytotic vesicles in myoid cells of experimental group 1 indicates that these cells had a decreased rate of endocytosis. This may lead to restriction in the availability of various substances and hormones that are transported from the interstitial tissue to the lumen of the seminiferous tubules for spermatogenesis and sperm maturation, which may result in a decreased rate of spermatogenesis and sperm viability [20]. A reduction in the number and in the content of ER suggests a decreased rate of protein synthesis in the myoid cells following EMF exposure.

The aetiopathogenesis of the morphological changes

TEM studies revealed that EMF exposure resulted in the destruction of boundary tissue in experimental group 1 animals, as evidenced by its thinning, infolding and the separation of boundary tissue layers from each other (the so-called "blister like" appearance) and loss of collagen and reticular fibrils. These cytological changes are likely to affect the process of spermatogenesis. Myoid cells were flat and thin with the presence of non-cristae mitochondria, condensation of actin filaments, an absence of pinocytotic vesicles and a decrease of glycogen content, ribosomes and ER systems. These cytological changes may potentially impair the process of spermatogenesis and sperm maturation.

The boundary tissue of rats in experimental group 2 showed no signs of destruction as seen under LM. TEM studies showed signs of recovery in all layers of the boundary tissue, although a few small spaces were seen (Fig. 2E). The inner acellular layer contained three different zones (two dense areas and one clear intervening area). Myoid cells were close to each other, with only minimal spaces to be seen and large spaces and blisters having mostly disappeared.

The present study demonstrated the harmful effects of EMF on the boundary tissue of the seminiferous tubules in rats. At the molecular level EMF produces biological stress and free radicals, which can make the susceptible animal population prone to congenital malformations, tissue and cell damage or death [14, 24, 25]. Free radicals released can cause oxidative damage at the cellular level, interfering with protein synthesis. These elements also play an important role in acute inflammation, endothelial destruction, increased vascular permeability and exudation of plasma, resulting in tissue oedema. It has been postulated that short-term exposure to EMF produces high levels of oxidative stress as a result of its effect on the immune response [27], and long-term exposure to EMF may be linked to even higher levels of oxidative stress [7].

In conclusion, the results of this study indicated that exposure to EMF had a deleterious effect on the boundary tissue of the seminiferous tubules. It can be concluded that exposure to EMF could result in pathological changes that may lead to male subfertility and infertility. It is therefore suggested that exposure to EMF should be avoided and that an opportunity for exposure-free time should be given to make a possible recovery.

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