RESEARCH ARTICLE

Dalia FA. Abou-Zaid

THE EFFECT OF ARTIFICIALLY GENERATED ELECTROMAGNETIC FIELDS ON THE HISTOPATHOLOGY OF THE OVARIIES AND GONADOTROPHIC HORMONE LEVELS OF FEMALE MICE

ABSTRACT:
Exposure to artificially generated electromagnetic fields (EMFs) is a common occurrence for a large number of individuals, its biological consequences, still largely unknown, are being studied in experimental animals and in humans. The present work aimed to investigate the possible harmful effects of EMF flux on the ovaries and gonadotrophic hormones (HL and FSH levels) of female mice. Adult female mice were exposed to visible light (Cool-white Fluorescent lamps) with intensity 77 mW/cm² on the area of 72.5 cm² and frequency of electromagnetic radiation between 3.9 x 10¹⁴ Hz and 7.5 x 10¹⁴ Hz W/cm² (from violet to red colours) for 8 hours/day for 3, 6, and 12 days. Histological observations of the ovaries of exposed mice showed varying degrees of degenerating follicles; the ovarian tissues showed congested blood vessels, haemorrhage, and great reduction in the number of the corpora lutea and Graafian follicles. Plasma FSH and LH levels showed a significant decline in treated groups. The damage was proportional with the exposure periods; 12 days of exposure resulted in female mice ovaries without mature Graafian follicles and suppression of FSH and LH hormones. In conclusion, besides the puzzling question regarding the impaired physiological processes, the negative role exerted by man-made EMFs on the reproductive potential of females exposed to such fields is a problem that needs to be addressed.

KEY WORDS:
Electromagnetic fields, gonadotrophic hormones, histopathology, ovary.

INTRODUCTION:
The continuous increase in the use of devices producing electromagnetic fields (EMFs) in various branches of the national economy has led to growing of the number of persons exposed-enormously. Devices producing EMFs such as mobile phones, computers, power lines, Fluorescent lamps, and domestic wiring are now in common use (Jeong et al., 2005). Both theoretical deliberations and laboratory studies indicate that EMFs affect the human body by inducing a number of physical phenomena represented as biological changes or even disorders in organic functions (Zmyslon et al., 1996). These magnetic fields easily penetrate buildings and people. Because power-frequency electric fields do not penetrate the body, it is generally assumed that any biologic effect from residential exposure to power-frequency fields must be due the magnetic component of the field, or to the electric fields and currents that these magnetic fields induce in the body (Moulder, 2000). The world health organization (WHO) tackled seriously the concerns raised by reports about possible hazards from exposure to EMFs. Cancer, reproductive effects, changes in behaviour, memory loss, Parkinson, and Alzheimer's diseases have been suggested to result from exposure to EMFs (WHO, 1984).

In the last decades, the widespread use of electric devices and telecommunication equipments increased the electromagnetic radiation in our environment from 0.0 Hz up to 300 GHz. In this frequency range the non-ionizing radiation has too week photon energy to break the atomic bonds (Stavroulakis, 2003).

Ambient Electric (E) or Magnetic Fields (MFs) span a wide spectrum of frequencies, intensities, and waveforms. The research conducted on possible associations between E/MF exposure and reproductive system has, however, focused on two frequency ranges: extremely low frequency (ELF) and very low frequency (VLF). The ELF range includes E/MFs with frequencies less than 300 Hz. The power frequency for electric transmission, distribution and domestic service is 60 Hz in USA and 50 Hz in Egypt and Europe.
Accordingly, much of the mammalian reproductive research has focused on these frequencies because of their ubiquitous presence in the environment (Chernoff et al., 1992).

The transport and use of electricity generate both electric and magnetic fields (E/MFs). Several reviews on the biological effects of E/MFs have been written in recent years (Tenforde, 1979; Adey, 1981 & 1996; O’Connor and Lovely, 1988; Nair et al., 1989; Cecconi et al., 2000; Abou-Zaid et al., 2006; Forgács et al., 2006). An area, which has been studied by a number of workers, is that of the potential of these fields to adversely affect reproduction. The majority of studies that have focused upon the potential reproductive toxicity of E/MFs have specifically dealt with the potential of these agents to affect prenatal development.

Gantt et al. (1979) indicated that Cool-white fluorescent light induces crosslinks in DNA when proliferating cells are exposed at 37°C for 20 h to 4.6 J/m2/s in culture medium supplemented with fetal bovine serum. They added that increased light intensity increases DNA crosslinks. From these results, they conclude that the mechanism of light-induced crosslinks differs from that of light-induced chromatid breaks and that the major lesion observed is protein-DNA cross-linkage rather than DNA strand breaks.

Bracken (1988), and Kavet et al. (1992) investigated the power-frequency electric and magnetic fields in the environment and stated that the magnitude of environmental 60-Hz electric and magnetic fields varies over a wide range, as the order-of-magnitude estimates residential background was approximately about (EF: 1-10 V/m; MF: 0.1-5 mG); near household appliances (EF: 10-100 V/m; MF: 10-1000 mG); edge of transmission right of way (EF: 100-1000 V/m; MF: 10-100 mG); electrical occupation (EF: 3000-s3000 V/m; MF: 10-100 mG).

There is very little information in the literature regarding the possible harmful effects of ELF-EMF on the reproductive system. One report by Denegre et al. (1998) demonstrates that exposure to a very strong static magnetic field (1T) can alter normal cleavage planes of Xenopus embryos, thus suggesting a direct action on the microtubules of the mitotic apparatus. At present, the only report on mammalian oocytes, by Mailhes et al. (1997), demonstrated that electromagnetic fields enhance chemically-induced hyperploidy in mouse oocytes. However, no other data concerning the role played by ELF-EMF on important aspects of mammalian oogenesis are available.

Cecconi et al. (2000) studied the effect of ELF-EMFs on in vitro mouse pre-antral follicle development and reported that ELF-EMFs severely impaired antrum formation at the frequencies of 33 and 50 Hz. The authors added that the follicles with failed antrum formation showed lower oestradiol release and granulosa cell DNA synthesis, but these effects were not related to granulosa cell apoptosis.

It must be noted that the field ranges listed above are rough approximations and are not at all interchangeable with exposure within each scenario. Similar to the situation with various air pollutants integrated E/MFs exposure depends on an individual’s activity, which may include proximity to a variety of field sources in and out of the home.

The main goal of the present study was to evaluate the possible effect of the exposure to isothermal non-ionizing electromagnetic fields, represented by the residential Cool-white fluorescent lights, on the histological structure of the ovaries, as well as the levels of the gonadotrophic hormones (FSH and LH) in female mice.

**MATERIAL AND METHODS:**

Adult female BALB/c mice (19 – 21 g) purchased from Tudor Bilharzias animal house, Cairo, Egypt, were used in the present study. Animals were housed in a standard animal facility under controlled temperature 28 ± 2°C and 50 ± 10 % humidity with free access of standard chow and water ad libitum.

Linear Source Lamps (LSL) were designed as square plate with a reflected mirror, fixed on it eight Cool-white Fluorescent lights (60 cm length) at distance > 0.4 m to irradiate electromagnetic flux. Photometer was used for measuring the intensity of electromagnetic flux (El-Bardie, 2003). Mice cages were prepared from a polymer material, which is not dielectric material.

Forty mice were divided randomly into 4 groups, 10 animals each. Three experimental groups (G1, G2, and G3) were exposed to visible light (Cool-white Fluorescent lamps) with intensity 77 mW/cm² on the area of 72.5 cm² and frequency of electromagnetic radiation between 3.9 x 10¹⁴ Hz and 7.5 x 10¹⁴ Hz W/cm² (from violet to red colours) for 8 hours/day for 3, 6, and 12 days, respectively. The fourth shame exposed group served as control (C). The experiments were repeated three times. Weights of animals were recorded daily and the percentages of survival were calculated.

After the end of the exposure periods, both control and experimental animals were sacrificed by cervical dislocation and the ovaries were excised and processed for routine histology. The ovaries were removed into saline and then were fixed in Bouin’s fluid, dehydrated in ascending series of ethanol, cleared, embedded in paraffin wax, and serially sectioned at 5-7 μm. Serial

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sections were stained with Ehrlich’s haematoxylin and counter stained in eosin for general histology.

Blood samples were collected, immediately after death from the inferior vena cava of each animal and the plasma was separated and stored at -20º C for subsequent hormone assays. LH and FSH in frozen plasma of mice were measured with chemiluminescence assays (Chiron Diagnostics ACS 180) in First Lab Clinical Laboratories.

RESULTS:
Percentage of survival:
No mortalities were recorded in the control group through the experimental period. The percentages of survival of G1, G2, and G3 were 96.66, 100, and 86.66, respectively (Fig. 1).

Histological observations of the ovaries:
The ovaries of a normal female mouse are somewhat flattened, ovoid bodies, suspended on either side of the uterus. Histologically, the ovary consists of three distinct regions: an outer cortex and a central medulla, and an inner hilum. Directly underneath the surface epithelium, the connective tissue forms the tunica albuginea.

In sections of the ovary, the central deeper medulla and an outer cortex are blended without distinct demarcation. The medulla is composed of a framework of loose connective tissue and the cortex consists of a compact, richly cellular connective tissue.

The cortex of the ovary of adult mouse contains a large number of growing and degenerated (atretic) follicles. The most abundant and smallest follicles are the primordial follicles (Fig. 3).

The follicular cells divide and enlarge to form the granulosa cells as a complete layer of cubical cells. A basal lamina separates these cells from the rest of the ovary. As the oocyte increases in size, an acidophilic extracellular material begins to accumulate between the oocyte and the granulosa cells. This material forms a thick uniform layer around the oocyte called the zona pellucida. The stromal cells in the connective tissue surrounding the granulosa layer become organized into a distinct layer; the theca interna. The next stage of development of the follicle is the Graafian follicle (antral follicle). As the antrum enlarges, it separates the cells lining the wall of the follicle, the mural granulosa, from those surrounding the oocyte (the cumulus oophrous) leaving only a stalk of cells connecting the two groups. The layer of cumulus cells immediately adjacent to the zona pellucida is often called the corona radiata (Fig. 4).
Fig. 4. Photomicrograph of a section of the ovary of control mouse showing developing Graafian follicle showing; TI: theca interna, G: granulosa cells, ZP: zona pellucida, A: antrum, BV: blood vessel, and O: oocyte. × 380

The mature Graafian follicle contains a large oocyte with a large nucleus and prominent nucleoli. This primary oocyte is still in arrested prophase of the first meiotic division. A very thick zona pellucida separates the oocyte from the innermost layer of the granulosa cells, the corona radiata, except for areas where the long slender processes of the granulosa cells penetrate to the oocyte.

Not all the ovarian primary follicles and growing follicles reach maturation. Follicles may degenerate at various stages of development. This process is called “atresia” and the degenerating follicles are called atretic follicles.

**The ovaries of exposed females:**

The ovaries of the G₁ and G₂ groups (female mice exposed for 3 and 6 days, respectively) showed varying degrees of degenerating follicles. Some of the degenerative follicles revealed partial loss of the oocytes while others had atrophied ones. The ovarian tissues showed congested blood vessels, besides haemorrhage that could be seen everywhere throughout the ovarian tissues. In general, more destructive signs were detected in the ovarian tissues of females of G₂ than in those of G₁ (Fig. 5). In general examination of the ovaries of G₁ and G₂ mice showed a great reduction in the number of the corpora lutea and Graafian follicles.

In females of G₂, the ovary revealed follicular atresia of the majority of ovarian follicles characterized by swollen and lyases of oocytes and degenerated granulosa cells with pyknotized nuclei (Figs 6).

Fig. 5. Photomicrograph of a section of the ovary of treated mouse (G₁) showing; DF: varying degrees of degenerating follicles, CB: congested blood vessel, besides H: haemorrhage. × 250.

Fig. 6. Photomicrograph of a section of the ovary of treated mouse (G₂) showing; FA: follicular atresia of the majority of ovarian follicles and DG: degenerated granulosa cells with pyknotized nuclei. × 200.

The ovarian tissues of all females belonging to G₃ showed a complete destruction of the follicles. All of the ovarian follicles and granulosa cells exhibited a severe degeneration and some follicles became atrophied. Haemorrhage was detected everywhere in the ovarian tissues.

The ovary of G₃ mice contained numerous abnormal corpora lutea with degenerated granulose lutein. The ovarian stroma showed widespread distribution of vacuolated structures and many of the ovarian follicles were devoid of oocytes (Figs 8 & 9). In general, the females of G₃ became dry mothers.

Fig. 7.

Fig. 8.

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Figs 7, 8, & 9. Photomicrograph of a section of the ovary of treated mouse (G3) showing: DF: complete destruction of the follicle, DG: all of the ovarian follicles and granulosa cells exhibited a severe degeneration, AF: atrophied follicles, DO: follicles devoid of oocytes and CB: congested blood vessels. Haemorrhage was detected everywhere. × 170, × 250, and × 200, respectively.

Gonadotropic hormone levels:

Plasma FSH and LH levels of control mice were 158.7 ± 13.6 and 32.8 ± 4.7 ng/ml, respectively. These levels were declined significantly to 122.3 ± 7.9 and 25.6 ± 4.7 ng/ml in 3 days exposure group and to 62.1 ± 8.7 and 13.2 ± 3.6 in 6 days exposure group, respectively. However both hormones were suppressed in 12 days exposure groups (Fig. 12).

Fig. 12. Plasma FSH and LH levels in control and different experimental groups.

DISCUSSION:

In the present investigation, exposure of female mice to artificial visible light (Cool-white fluorescent light) with intensity 77 mW/cm² on the area of 72.5 cm² and frequency of electromagnetic radiation between 3.9 x 10¹⁴ Hz and 7.5 x 10¹⁴ Hz W/cm² for 8 hours/day for 3, 6, and 12 days, showed varying degrees of degenerating follicles. The damage was proportional with the exposure periods; 12 days of exposure resulted in female mice ovaries without mature Graafian follicles.

Hirao and Yanagimachi (1978) reported that short wavelength visible light (less than 470-480 nm) emitted from ordinary light sources is detrimental to unfertilized hamster ova in that prolonged exposure to the light disturbs the completion of normal meiosis after the ova are penetrated by spermatozoa. The fluorescent light is more harmful than the light from incandescent lamps.

The dramatic decrease in FSH and LH levels caused by EMFs exposure in the present study seems to agree with the significant decreases in cell proliferation rate (Kwee and Raskmark, 1998; Velizarov et al., 1999).

Although there isn’t any established biochemical explanation for the biological effects of EMFs, we suggest that an explanation of the present results, can be though in correlation with other laboratory studies which have showed that electromagnetic fields, alter the proliferation rate of cells, as well as the rate of DNA, RNA, and protein synthesis (Fitzsimmons et al., 1992; Schimmelpfeng and Dertinger, 1993; Kwee and Raskmark, 1995 & 1998).

In the present work, sections of the ovary of the treated groups showed many of the ovarian follicles were devoid of oocytes and others had atrophied ones. Moreover, the histological sections revealed follicular atresia of the majority of ovarian follicles characterized by swollen and lysed of oocytes and degenerated granulosa cells with pyknotized nuclei. These results confirm those of Cecconi et al. (2000) who stated that ELF-EMF exposure might impair mammalian female reproductive potentially by reducing the capacity of the follicles to reach a developmental stage that is an essential prerequisite for reproductive success. Moreover, animals exposed to the EMFs could suffer a deterioration of health, changes in behavior, and changes in reproductive success (Doherty and Grubb, 1996; Fernie et al., 2000).

The data presented suggest that ELF-EMF exposure has a detrimental effect on the levels of gonadotrophic hormones (FSH and LH) of female mice and this may cause a detrimental effect on the somatic cells of Graafian follicles (granulosa and theca cells).

Since a significant decrease in oestradiol production and granulosa cell proliferation has been related to granulosa cell apoptosis (Kaipia and Hsueh, 1997; Drummond and Findlay, 1999), an intriguing explanation for the current results may be that ELF-EMFs are capable of inducing granulosa cell apoptosis. Indeed, data reported in the literature show that ELF-EMF exposure affects this process in various somatic cell types (Flipo et al., 1998; Ismael et al., 1998; Fanellia et al., 1999).
The potential mechanisms through which ELF-EMF promote these effects are not yet known. In other mammalian cell systems, weak fields interact with cells by altering free calcium concentrations, membrane-dependent signal transduction pathways, as well as activities of key protein kinases (Campbell-Beachler et al., 1998; Jahreis et al., 1998; Kristupaitis et al., 1998; Löschinger et al., 1998; Tuinstra et al., 1998). Thus, it may be postulated that ELF-EMFs alter the follicle proliferative/differentiative programme by negatively affecting an, as yet undefined, regulatory mechanism in ovarian somatic cells. This possibility is further sustained by the fact that theca and granulosa cells play a central role in enhancing follicle development (McGee et al., 1997) by modulating the action of gonadotrophic hormones and the production of apocrine/paracrine factors (Spears et al., 1998).

Animals are very sensitive to electrochemical complexes that communicate with their environment through electrical impulses. Ionic currents and electric potential differences exist throughout the cellular membranes and corporal fluids (Heredia-Rojas et al., 2003). The intrinsic electromagnetic fields from the biological structures are characterized by certain specific frequencies that can be interfered with by the electromagnetic radiation, through induction and causing modification in their biological responses (Hyland, 2000 & 2001).

Research has shown such effects on the living organisms at molecular (Daniells et al., 1998) and cellular levels (Dutta et al., 1989) on immune processes (Obukhan, 1998), in DNA (Sarkar et al., 1994), on the nervous, cardiac, endocrine, and reproductive systems (Altpeter et al., 1995; Grigor’ev et al., 1996; Kolodynski and Kolodynska, 1996; Belousova and Kargina-Terent’eva, 1999; Dasdag et al., 1999; Petrides, 2000; Nikolaevich et al., 2001), modification of sleep and alteration of the cerebral electric response (Mann and Roschke, 1996), increase of the arterial pressure and changes in the heart rhythm (Szmigielski et al., 1998).

There is little information in the literature regarding the possible harmful effects of ELE-EMFs on the reproductive system. In mammals, the application of lower frequency fields (50 Hz, 1-100 mT) can affect the proliferative/differentiative capacity of mouse spermatogonia (Furuya et al., 1998), but does not induce clastogenic effects on human sperm chromosomes (Tateno et al., 1998). Mailhes et al. (1997) demonstrated that electromagnetic fields enhance chemically induced hyperploidy in mouse oocytes.

The results of the present study showed that the ovarian tissues of the exposed mice exhibited congested blood vessels, besides haemorrhage that could be seen everywhere throughout the ovarian tissues.

Ferrara et al. (1998) reported that the cyclical growth of blood vessels associated with the development of ovarian corpus luteum is essential for progesterone release and thus for implantation and maintenance of pregnancy. Phillips et al. (1990) and Kos (1995) demonstrated that vascular endothelial growth factor is essential for luteal angiogenesis leading to corpus luteum development. High vascularization seems to be necessary to provide luteal cells with large amounts of cholesterol needed for progesterone synthesis and for the delivery of progesterone to the circulation (Smith et al., 1994).

Although the most well-known function of vascular endothelial growth factor is its angiogenic activity, it plays more than a simple angiogenic role in the female reproductive system as it is involved in a number of key events in the course of the ovulatory cycle. The modulation of vascular endothelial growth factor expression varies in different reproductive tissues, probably related to its various functions at these different sites (Lam and Haines, 2005).

Bekhite (2005) suggests that the exposure of pregnant mice to MF resulted in suppression of vascular endothelial growth factor (VEGF), which plays a significant role in the function and structure of the ovary through inhibition of corpus luteum development.

In conclusion, besides the puzzling question regarding the impaired physiological processes, the possible negative role exerted by EMFs on the reproductive potential of women chronically exposed to such fields is a problem that needs to be addressed. Minimal exposure to the light or the use of appropriate filters is recommended.

REFERENCES:

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