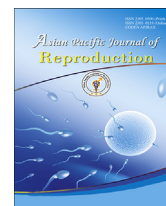




Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.netOriginal research <http://dx.doi.org/10.1016/j.apjr.2016.06.008>

Histopathological changes associated with oxidative stress induced by electromagnetic waves in rats' ovarian and uterine tissues

Ali S.H. Alchalabi^{1,2*}, Hasliza Rahim³, Erkihun Aklilu¹, Imad I. Al-Sultan¹, Abd Rahman Aziz¹, Mohd F. Malek³, Suzanna H. Ronald³, Mohd Azam Khan¹¹Faculty of Veterinary Medicine, UMK City Campus, Pengkalan Chepa, Locked Bag36, 16100 Kota Bharu, Kelantan, Malaysia²Veterinary Medicine College, Mosul University, Mosul, Iraq³School of Electrical System Engineering, University Malaysia Perlis (UniMAP), Pauh Putra, 02600 Arau, Perlis, Malaysia

ARTICLE INFO

Article history:

Received 3 Apr 2016

Received in revised form 11 Jun 2016

Accepted 12 Jun 2016

Available online xxx

Keywords:

Histopathological changes

Female sex organs

Oxidative stress

Electromagnetic radiation

ABSTRACT

Objective: To evaluate the histopathological changes in ovarian and uterine tissues associated with oxidative stress induced by electromagnetic waves.**Methods:** Thirty female Sprague Dawley of 180 g body weight and 3 months old were used in the experimental work. Animals were divided into control and two experimental groups. Experimental groups were exposed to 1800 MHz Global System for Mobile Communication radiofrequency radiation emitted by a signal generator for 2 h per day for 30 and 60 d, respectively. Following exposure, serum, ovaries and uteri were collected for biochemical and histopathological investigations.**Results:** Biochemical analysis showed alterations in oxidative stress parameters in both ovaries and uteri tissues in comparison to control group. The histopathological changes were more prominent in experimental groups, in the ovary were included vacuolation in interstitial, granulosa, luteal cells and ooplasm. Other histopathological changes are disorientation of corona radiata, disruption and thinning of the zona pellucida. Cellular nucleus changes similar to fragmentation of the nucleus indicate the start of a degeneration process at Graafian follicles as well as micronuclei formation in oocyte nucleus and in some luteal cells. Histopathological changes in uterine tissue confined to increase height of luminal epithelium cells, sever apoptosis of glandular and luminal epithelium cells, and sever eosinophils, polymorphonucleocyte lymphocytes and macrophage's infiltration in myometrium and endometrium layers. Vascular congestion points out for the existence of inflammatory response changes in the endometrium.**Conclusion:** Results executed that the potential alteration of antioxidant capacity may contribute to endometrial oxidative damage that could be related to pathogenesis and progression of endometritis.

1. Introduction

Global System for Mobile Communication (GSM) is one of the operating systems used in cellular phone's communications. Our cellular phones are emitting electromagnetic waves (EMW) which are non-ionizing radiation in its nature. This mean does not have an ability to ionize the water and macromolecules in human or animal tissues, in contrast to the ionizing radiation such as X-ray.

Logically, this is right, but now the researchers' data change this opinion due to of many studies were conducted about the non-thermal action of EMWs mediated by oxidative stress or other mediators on living tissues [1–5]. Some of the recent studies have performed to explain the role of oxidative stress as the harmful effect on living tissues induced by GSM signals [6–9]. Oxidative stress is a status result due to imbalance between oxidant agents and antioxidant system leading to production of free radicals who are responsible for oxidative damage to proteins, lipids and DNA in the body [10]. Impact of EMW on the effectiveness of the reproductive tract and its direct or indirect role in females' fertility, has contradictory outcomes in most cases and possibly due to variation in the experimental design in terms of source of EMWs, frequency, exposure duration and the specific absorption

*Corresponding author: Ali S.H. Alchalabi, Faculty of Veterinary Medicine, UMK City Campus, Pengkalan Chepa, Locked Bag36, 16100 Kota Bharu, Kelantan, Malaysia.

Tel: +60 112947731

E-mail: alisaeedchalaby@yahoo.com

Peer review under responsibility of Hainan Medical College.

rate (SAR) used. For instance, Sprague-Dawley rats exposed to low frequency 50 Hz for 18 weeks (24 h/d) exhibited a harmful effect on rats' fertility and reproduction [11]. In the experiment carried out by researchers on the impact of electromagnetic waves on young rats aged 21 d of mothers exposed during pregnancy to mobile phone for 11 h and 45 min in the standby and 15 min in speech mode to evaluate the ovarian follicle's activity, number of follicles and growth were significantly affected due to apoptosis among follicles, hyperplasia in ovarian stroma and elongation cell mitosis time was observed [12]. Another study on Wister rats which exposed to mobile phone 12 times for 10 min (calling mode) for 2 weeks and 1 month, the study suggests that mobile phone wave can increase ovarian and atresia follicles as well as changing in sex hormone's levels [13]. Devrim and his team found that exposure to mobile phone radiation "four times a day for 10 min in call position," electromagnetic radiation (EMR) emitted by mobile phones causes oxidative stress and lipid peroxidation in kidney and erythrocytes, and vitamin C can give some protection against the oxidant stress [14]. On other sides, some studies reported no adverse effects on reproduction activity and fertility in rats and mice exposed to EMWs (GSM/Wi-Fi signals) [15–18]. The correlations between mobile phone use and histopathological changes were evaluated in different organs and different electromagnetic field (EMF) setup based on the aims of the studies [19–21]. In 2007, a Turkish study concluded that female rats exposed to mobile phone frequency of 900 MHz exhibited oxidative endometrial damage which is responsible for endometrial impairment and vitamin E & C supplement reduces this damage at a tissue level [22]. An investigation of EMF (50 Hz, 0.5 mT) on epididymis and deferens duct in mice exposed for 2 months revealed low reproductive efficiency in mice due to a decrease in diameter of reproductive ducts, the height of epithelial cells and weight of the testes [23]. In a study to clarify the effect of low-frequency electromagnetic field (ELF-EMF) on fertility and heights of epithelial cells in pre-implantation stage of endometrium and fallopian tube in mice, daily 4 h for 2-week exposure to 50 Hz 0.5 mT EMF showed that ELF-EMF has the detrimental effect on a female genital tract via increasing the fallopian tube epithelial cell's height and reduction in flushed blastocyst's number [24]. Female rats whole body exposure by EMF at 50 Hz for 40 d revealed a detrimental effect on ovarian tissue via increasing fibrosis and venous congestion, and these bad effects were minor in other exposed groups received *Ocimum basilicum* at a dose 1.5 g/kg B.W. as antioxidant therapy [25]. Iranian researchers group found that in-utero exposure by ELF-EMF at 50 Hz 3 mT, 4 h/d for 21 d in rats led to induce oxidative stress and granulosa cells interspaced from the base membrane with narrow and irregular zona pellucida, vacuolization in ooplasm were observed [26]. Our study, it was aimed to evaluate the correlation between chronic exposure to 1800 MHz GSM signals "heavy use of mobile phone" induces oxidative stress and histopathological changes in ovarian and uterine tissues in adult female rats.

2. Material and methods

2.1. Animal and EMF setup

The study was approved by the scientific committee of faculty veterinary medicine of University Malaysia Kelantan (UMK) and was conducted in accordance with the UMK guidelines for animal experiments (FPV-PGSC-2014). Thirty

female Sprague Dawley type rats at an average body weight 180 g and 3 months old were used throughout the study. Estrus synchronization was done before starting the experiment, and the animals in pro-estrus phase were used throughout the experiment. The animals were distributed over the three groups (control group and the two exposure groups as whole-body exposure for 2 h/d, 7 d/week for 30 and 60 continuous days). The animals were obtained from the laboratory animal research unit of faculty of veterinary medicine (UMK). Animals were kept in plastic cages at room temperature (25 ± 1) °C and humidity (60 ± 10)% (relative humidity) with light/dark cycle 12–12 h, and tap water and standard rat pellet were provided *ad libitum*. Especial designed exposure Plexiglas box (60 cm × 40 cm × 20 cm) was used during the RF-EMR exposure time.

Whole-body exposure with 1800 MHz GSM-like frequency of mobile phone at SAR level value 0.974 W/kg was calculated using this equation:

$$\text{SAR} = (\delta/\rho)E^2$$

where (E) is the magnitude of electric field 28.156, (δ) is the conductivity 1.34 s/m and (ρ) is the mass density of the tissue-equivalent media 1090 kg/m³. The exposure setup described by previous publication [27].

2.2. Biochemical analysis

At the end of experiment, rats were anesthetized by intra-peritoneal (IP) injection of Ketamine and Xylazine combination at a dose 0.1 mL/100 g b.w. (80 mg/kg b.w. ketamine and 5 mg/kg b.w. Xylazine) and then sacrificed. Ovaries and uterus were surgically removed for biochemical and histopathological investigations. Kits were purchased from Cusabio Biotech Co., Ltd. and Abcam for biochemical analysis. Malondialdehyde (MDA) was assessed as lipid peroxidation biomarker by using of lipid peroxidation (MDA) assay kit (ab119870, Abcam, U.K.) according to the manufacturer's instructions [28]. Glutathione peroxidase (GSH-PX) activity and melatonin (MT) concentration in serum were estimated by using of rat Glutathione peroxidase ELISA kit and rat Melatonin ELISA kit (CUSABIO Rat GSH-PX ELISA Kit, CSB-E12146r, CUSABIO Melatonin ELISA Kit, CSB-E13433r, Wuhan University Science, Wuhan, Hubei province 430223 P.R. China), respectively. According to the manufacturer's instructions, estimation was performed.

2.3. Histopathological sample preparation

All rats were sacrificed in the estrus cycle. Removed ovaries and uterus were cleaned from fatty tissues and washed by normal saline. Tissue paper was used to remove the excess fluids and weighed using a digital scale with precision of 0.001 g. Uterus and ovary samples were fixed in 10% neutral buffer formalin for 48 h before starting the process of preparation for histological slides. Specimens were embedded in paraffin, and sections at 5 µm was prepared and stained with H&E. Ovarian and uterine tissue slides were examined under the light microscope for histopathological study and measurement of uterine horn layer's thickness (five slices from each sample were randomly selected) at a magnification of 4× and 40× for luminal epithelium layer height. Tissue samples were assessed at a magnification of 20× and 40× and photographed by

Olympus microscope (model: BX43F Japan). The evaluation of ovarian and uterine pathological changes was confirmed by histologist and pathologist in veterinary medicine college of UMK. Uterine layer's thickness was measured by using Cellsens Dimension software.

2.4. Statistical analysis

Data were presented as mean \pm standard deviation (mean \pm S.D.). SPSS programs V. 22 software (SPSS Inc. Chicago, IL, USA) was used to test significant differences among groups. One-way ANOVA and *LSD* test were used to evaluate the significance between groups. *P* values of less than 0.05 were considered as significant.

3. Results

3.1. Body, ovarian and uterine weight

Table 1 shows the effect of 1800 MHz GSM electromagnetic field on body and sex organ's weight of rats after 30 and 60-day irradiation by GSM signals. As observed no significant difference between the body and uterine weight of females of all groups. However, ovarian weight was significantly decreased in 60-days EMF exposure when compared with 30-days EMF exposure and control groups ($P = 0.024$).

Table 1

Effect of 1800 MHz GSM electromagnetic field on body, ovarian and uterine weight (g).

Groups	Body weight	Ovarian weight	Uterine weight
Control	293.95 \pm 14.30	0.094 \pm 0.010	0.694 \pm 0.090
30-days EMF exposure	291.60 \pm 11.80	0.106 \pm 0.010	0.700 \pm 0.100
60-days EMF exposure	297.50 \pm 10.04	0.089 \pm 0.010*	0.721 \pm 0.100
<i>P</i> value	0.588	0.024	0.773

Values are Mean \pm S.D. * $P < 0.05$ statistically significant difference vs control group.

Table 2

Effect of 1800 MHz GSM electromagnetic field on oxidative stress parameters.

Groups	Ovary		Uterus		Serum MT (pg/mL)
	GSH-PX (mIU/mg)	MDA (nmol/mg)	GSH-PX (mIU/mg)	MDA (nmol/mg)	
Control	19.74 \pm 1.06	0.92 \pm 0.12	9.88 \pm 1.20	0.69 \pm 0.15	41.32 \pm 7.06
30-days EMF exposure	18.43 \pm 1.28**	1.16 \pm 0.23**	9.15 \pm 0.89	0.90 \pm 0.14	34.99 \pm 2.52*
60-days EMF exposures	18.35 \pm 1.33**	1.12 \pm 0.26*	8.46 \pm 1.08**	1.48 \pm 0.67**	32.24 \pm 3.91*
<i>P</i> value	0.004	0.004	0.002	0.005	0.006

Values are Mean \pm S.D. ** $P < 0.01$; * $P < 0.05$ statistically significant difference vs control group.

Table 3

Effect of 1800 MHz GSM electromagnetic field on thickness of the uterine horns' layers and height of epithelial cells of lumen (μ m).

Groups	Perimetrium thickness	Myometrium thickness	Endometrium thickness	Height of epithelial cells of lumen
Control	81.67 \pm 22.13	165.70 \pm 35.34	566.28 \pm 47.72	164.19 \pm 61.63
30-days EMF exposure	77.05 \pm 30.66	181.09 \pm 40.69	542.26 \pm 202.41	220.72 \pm 41.65*
60-days EMF exposure	100.97 \pm 24.16	175.97 \pm 22.18	696.86 \pm 186.86	201.35 \pm 46.23
<i>P</i> value	0.118	0.602	0.590	0.028

Values are Mean \pm S.D. * $P < 0.05$ statistically significant difference vs control group.

3.2. Biochemical analysis

Biochemical analysis results in Table 2 showed a significant decrease in GSH-PX activity and an increase in MDA level in ovarian tissue in both EMF exposure groups compares to control group. Uterine tissue GSH-PX activity in the 60-days EMF exposure group was significantly lower than 30-days exposure and control groups. Moreover, endometrial MDA level in a 60-days EMF exposure group for was significantly higher than 30-days EMF exposure and control groups. Serum melatonin level was reduced significantly in both EMF exposure groups compare to control animals.

3.3. Measurement of thickness of the uterine layers

The statistical analysis in Table 3 did not show a significant difference in the thickness of the uterine horns' layers in terms of micrometers among the exposure and control groups. However, the height of epithelial cell's layer was significantly increased in 30-days EMF exposure group compared to epithelial cells in control group ($P < 0.028$).

3.4. Histopathological changes

3.4.1. Ovarian tissue changes

Different histologic sections of the ovaries from the female control group indicate well development of ovarian follicles, normal blood vessels and normal stroma cells. The pre-ovulatory follicle showed typical morphology of oocyte and euchromatin nucleus, which was surrounded by typical zona pellucida and several layers of granulosa cells are resting on the basement membrane. Theca interna and theca externa are clearly developed. The primary follicles with the normal oocyte surrounded by single layer of cubical granulosa cells are showed to rest on the basement membrane. Hypertrophy of corpus luteum and proliferation and differentiation of steroidogenic cells with extensive angiogenesis are evident in Figure 1.

The most evident histopathological changes in the ovaries of females EMF exposure group 30 days post exposures are

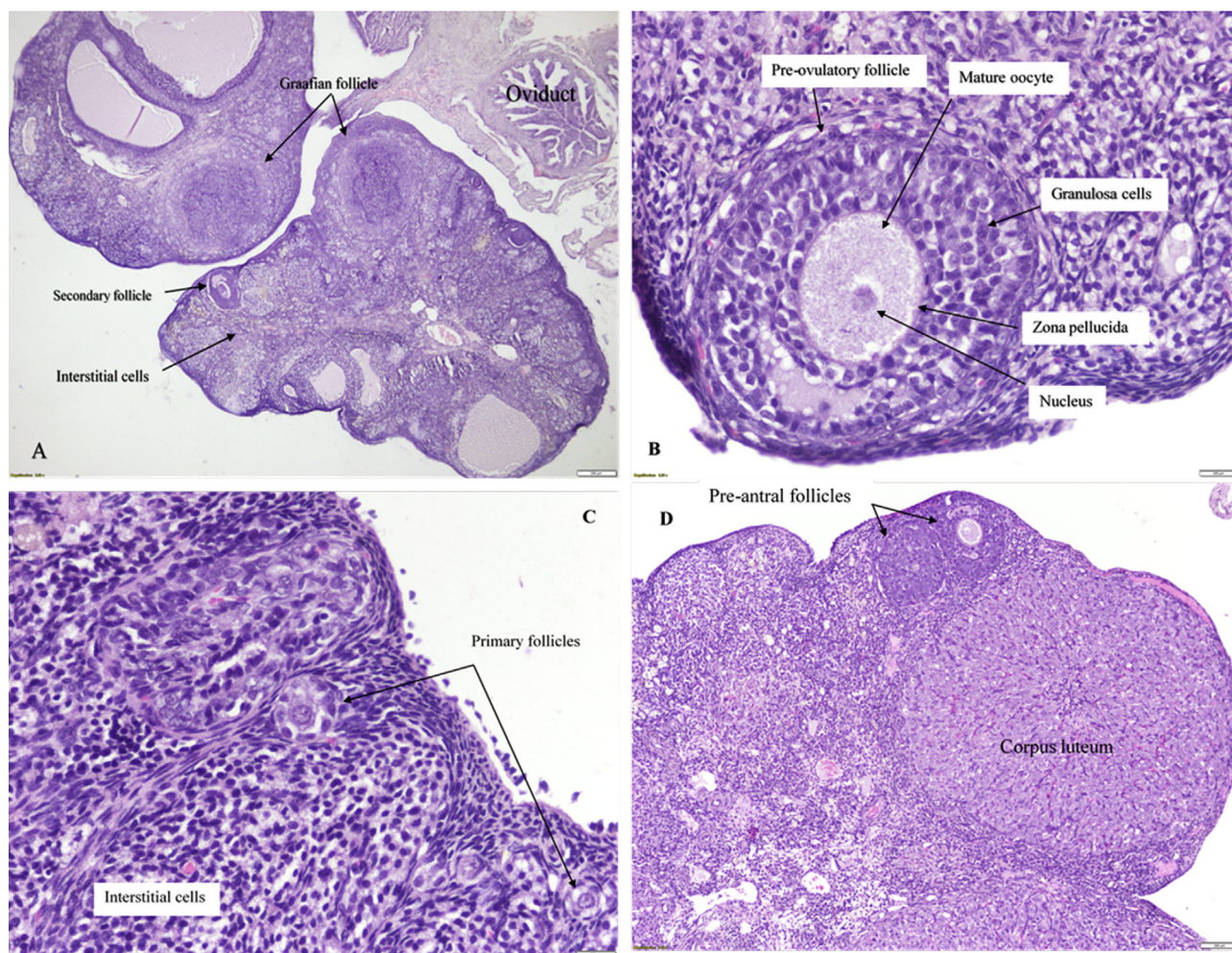


Figure 1. Ovarian sections from the female control group.

(A) Normal ovary showing well follicular development 4x. (B) Pre-ovulatory follicle with mature oocyte surrounded by granulosa cells with normal zona pellucida and theca interna and externa 40x. (C) Well developed primary follicles indicated by arrows 20x. (D) 1. Preantral follicles, 2. Corpus luteum showing normal luteal cells 10x. H&E staining.

congestion and reduction in the number of ovarian follicles. Other changes include vacuolation in luteal cells, ooplasm and in granulosa cells of pre-ovulatory follicles. Moreover, the histologic changes seen indicate a thin and irregular zona pellucida. Granulosa cells perform a strong contact with the oocyte in the complementary process of autophagy leading to cell death. Micronuclei formation and persistence of pre-ovulatory follicle oocyte and in luteal cells are an indication of DNA damage (Figure 2).

Microscopic examination of the ovarian tissue sections of females EMF exposure group 60 days post exposure showed a reduction in ovarian follicles, congestion of blood vessels, and degeneration of pre-ovulatory follicle cells and infiltration of macrophages. Vacuolation of interstitial cells and granulosa cell's layers is activated. Micronuclei formation was found in pre-ovulatory follicle's oocytes. Further changes disclose on the disorientation of corona radiata, disruption and thinning of the zona pellucida. Cellular nucleus changes similar to fragmentation of the nucleus indicate the start of the degeneration process at pre-ovulatory follicles (Figure 3).

3.4.2. Uterine tissue changes

Tissue sections from the uterus of females control group shows common uterine histology with regular columnar

epithelial cells lining the uterine lumen and glands. The mitotic figures were observed clearly in both epithelial and glandular cells along with normal histology of blood vascularity. Fibrosis, necrosis, apoptosis or any other adaptive cellular changes did not observe in the control uterine tissue sections (Figure 4).

Electromagnetic irradiation for 30 days had the significant effect on female uterine tissue. Histopathological alterations were apoptosis demonstrated in the luminal epithelial cells in a form of individual cell debris that form a halo within the luminal epithelium. Changes in glandular epithelium range from desquamation of epithelial cells to degenerative process. Infiltration of the endometrium with an abundant number of lymphocytes and polymorph nuclear cells along with the vascular congestion point out for the existence of inflammatory response in the endometrium (Figure 5).

Endometritis of acute onset was observed in of females exposed to EMF for 60 days. The cellular inflammatory reaction was composed of infiltrative neutrophils, fewer numbers of lymphocytes as well as severe diffuse eosinophil's infiltration in endometrium and perimetrium layers with apoptosis and desquamation of luminal and glandular epithelium (Figure 6).

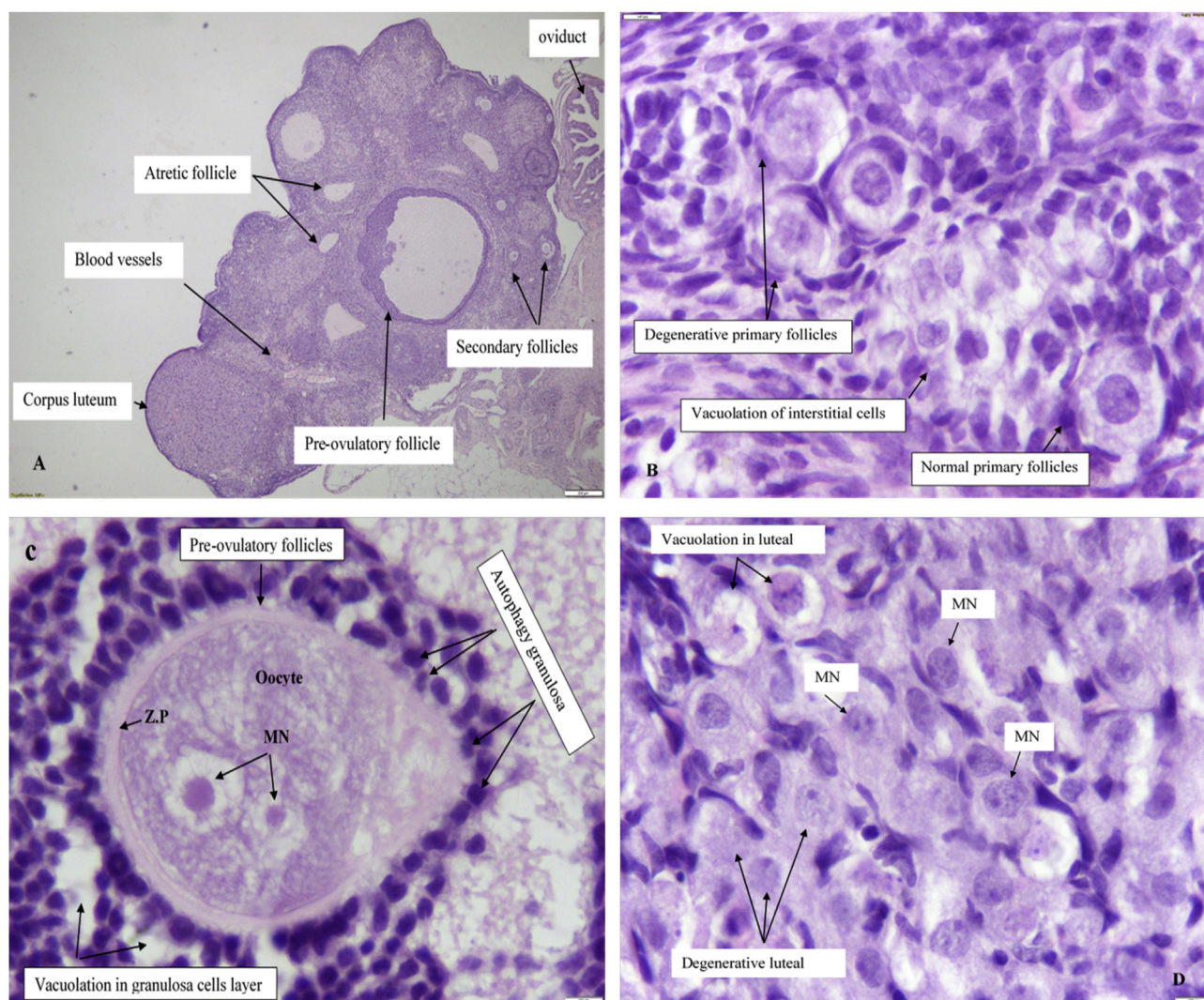


Figure 2. Ovarian sections from 30-days EMF exposure group.

(A) Ovary showing reduction in ovarian follicle's numbers with congestion of blood vessels and degeneration of interstitial cells and some follicles. (G. F) Pre-ovulatory follicle, (A. F) Atretic follicle, (C. L) Corpus Luteum, (b.v) blood vessel, (o.d) oviduct. 4×. (B) Primordial follicles 1. Degenerative oocytes with vacuolization in ooplasm. 2. Normal primordial follicle with a single layer of flattened pre-granulosa cells surrounding the oocyte 40×. (C) Pre-ovulatory follicles shows oocyte with micronucleus formation indicated by (MN) enclosed by the thin, irregular zona pellucida indicated by (Z.P). Granulosa cells were separated from each other and vacuolization in ooplasm and granulosa cell's layer. Autophagy granulosa cell is strong contact with the oocyte indicated by arrows 100×. (D) Corpus luteum showing degenerative luteal cells with vacuolation and micronucleus formation (MN) in some luteal cells indicated by arrows 100×. H&E staining.

4. Discussion

The interaction between electromagnetic waves emitted from cellular phone devices and vital organ's activity, especially the female reproductive system performance in terms of the biological role of these waves in the development of oxidative stress and pathological changes became controversial about the reality of the impact of these waves on public health.

This study proved that chronic exposure to mobile phone have no effect on body weight, but the exposure for long periods led to significantly reduced ovarian weight compared to the control group as well as induced oxidative stress represented by elevation of MDA (a lipid peroxidation biomarker) and decline in the activity of antioxidant enzyme GSH-PX in both exposure periods 30 and 60-days at the tissue level of the ovary. Sixty-day exposure group encompassed the reduction of GSH-PX enzyme activity and increased MDA level at the uterine tissue level with reduction in melatonin level. Results are consistent with

previous study [22]. One-month old infant rabbit showed an increased hepatic lipid peroxidation as a result of intrauterine exposure of their mothers to 1800 MHz for 15 min/d for 7 d [29]. Other experimental studies on pregnant and non-pregnant rabbits indicated that exposure to electromagnetic radiation resulted in oxidative damage to DNA and lipid molecules [30]. Pregnant Wister rats exposed to the extremely low-frequency electromagnetic field (ELF-EMF) showed an increase in MDA serum level and also caused an adverse effect in F1 generation ovarian follicle's development, which may affect fertility [26].

The present study showed chronic exposure to EMF 1800 MHz causes pathological changes in ovarian tissues ranging from minor to powerful changes (congestion, decreased ovarian follicle number and development, vacuolation, autophagy, apoptosis and micronuclei formation). These results are similar to previous studies [26]. The Wister rats exposed to the ELF-EMF showed that the ovarian tissue revealed separation of granulosa cells from the basement membrane and thinning,

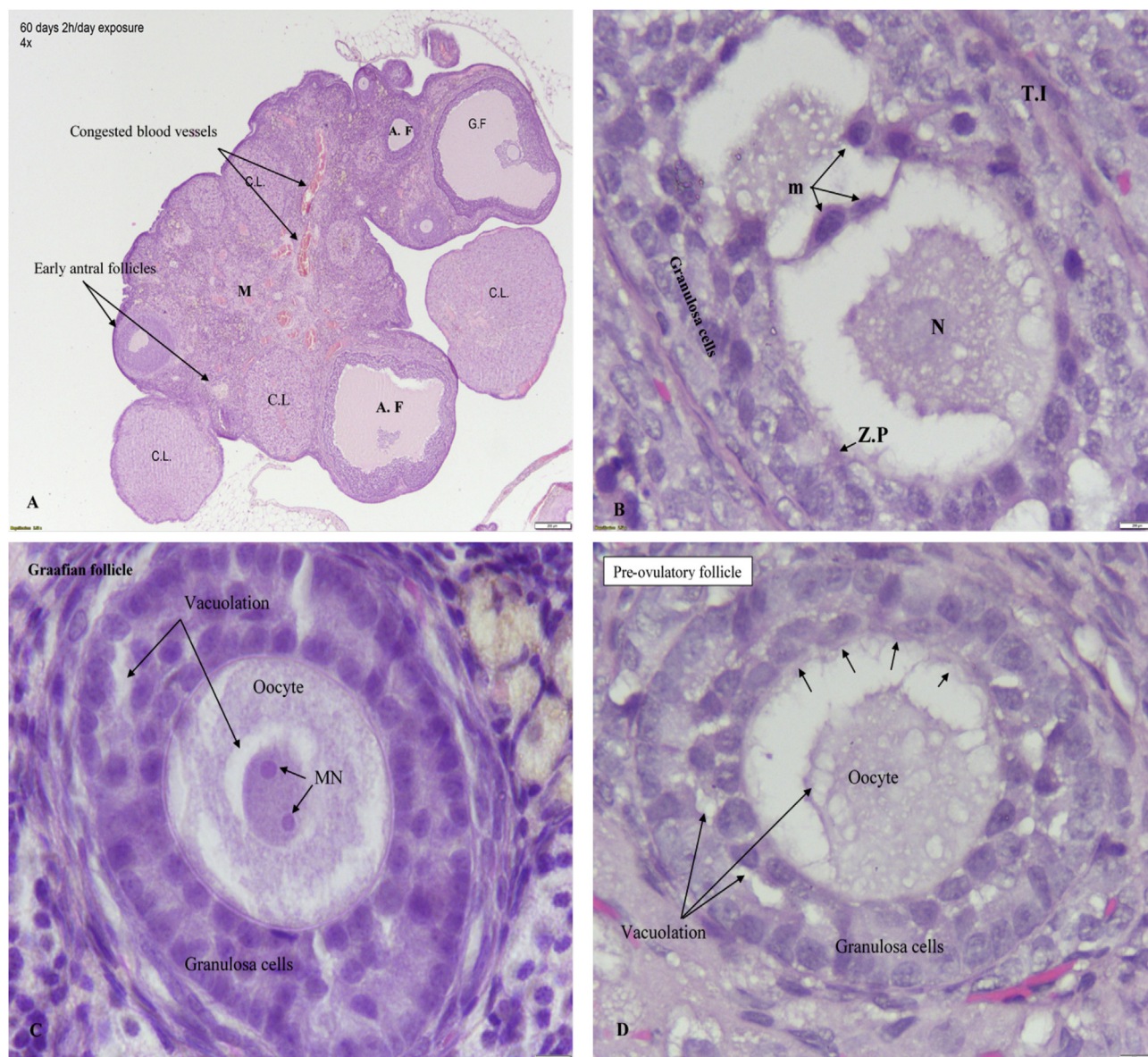


Figure 3. Ovarian sections from 60-days EMF exposure group.

(A) Ovary showing reduction in ovarian follicle's numbers with congestion in blood vessels (C. L.) corpus luteum, (3) early antral follicle, (4) atretic follicle, (M) matrix. 4×. (B) Pre-ovulatory follicle (m) macrophage strong contact with the oocyte nucleus (N), (T. I) theca interna, and degenerative oocytes with vacuolization in ooplasm and granulosa cell's layer enclosed by the thin, irregular zona pellucida indicated by (Z.P), 100×. (C) Graafian follicle with micronucleus formation (MN) and vacuolation of granulosa cell's layer and ooplasm indicated by arrows 100×. (D) Pre-ovulatory follicle showing vacuolation in ooplasm and granulosa cell's layer surrounded by disruptions and thinning of zona pellucida indicated by small arrows 100×. H&E staining.

irregular zona pellucida, ooplasm vacuolation. Another study found that mice exposed to ELF-EMF for 4 h/d during pregnancy, microscopic examination revealed that the oocyte's nests were irregularly arranged, and the primordial follicles were undeveloped as well as ooplasm vacuolation was observed. The oocyte changes and the separation of granulosa cells from neighboring cells create the main feature of atretic follicle and characteristic of zona pellucida apoptosis with theca interna vacuolation [31].

Our findings showed that EMF 1800 MHz frequency increases the oocyte degenerative changes and autophagy apoptosis in granulosa cells, which is in consistence with other study [32]. In the exposed animals, oocytes' nuclei had the irregular shape with micronuclei formation and dark chromatin condensation and had several autophagy granulocytes with different size and shape. This is in consistent with previous researches [33,34]. The production of

ova results from organized sequence of events in the folliculogenesis involving oocyte, granulosa cells and theca interna and externa cells. During folliculogenesis, the follicles not eclectic for the process of ovulation get rid of physiologically by apoptosis and/or autophagy, which is considered as another type of programmed cell death [35]. GSH-Px is an antioxidant enzyme that participates by using reduced glutathione as a hydrogen donor in the process of excluding H_2O_2 and lipid hydroperoxides and reduces peroxides to reduce the oxidative damage. The reduction in GSH-Px activity due to over consumption of reduced glutathione and reflected on the increase in MDA level that mean tissue oxidation was occurred.

Reduction of melatonin, a potent free radical's scavenger, decreased GSH-Px activity and development of oxidative damage may contribute to increase lipid peroxidation of ovarian tissue [36,37].

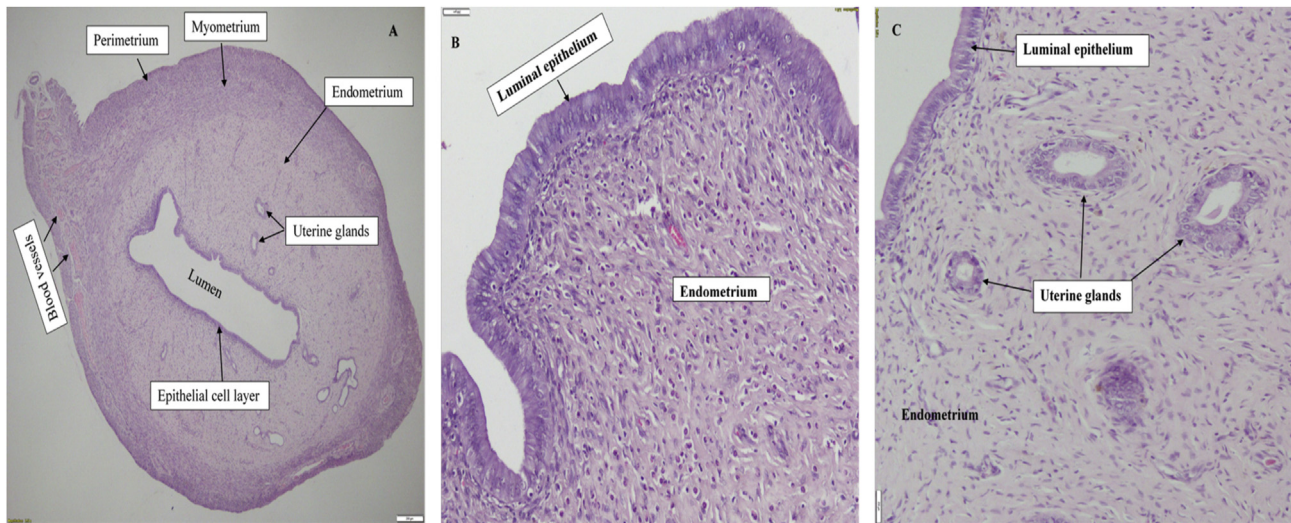


Figure 4. Uterine horn sections from the female control group.

(A) Photograph showing cross-section of uterine horn layers with normal histology 4×. (B) Endometrium with columnar epithelial cells characterized by normal oval nucleus 20×. (C) Endometrium with uterine glands having normal columnar epithelial cells with mitotic figures, which also seen in luminal epithelium 20×. H&E staining.

The mechanisms of incidence of DNA damage and follicular atresia are not well known, which can be proposed by free radical's production and leads to activation of the apoptotic process [38]. Theca interstitial cells are sensitive to free radicals' level and reduction in antioxidant triggers apoptosis and antioxidants with precisely different mechanisms of action activate a course of actions consistent with the apoptosis mechanism in ovarian mesenchyme [39]. Endometrial bio-activities may be involved oxidative stress, which induces macrophage's activation leading to excessive production of reactive oxygen and nitrogen species. These free radicals interact

with low-density lipoproteins and other proteins leading to produce MDA [40–42]. Our findings showed that MDA level in uterine tissue was elevated after 60-days of exposure to EMF, suggesting involvement of lipid peroxidation as end product of oxidative damage and accompanied by decrease antioxidant enzyme GSH-Px activity. This is in consistence with previous studies [43–45]. In our study, we found that mobile phone radiation increases the height of the uterine luminal epithelium cells, which is consistent with an experimental study on mice exposed to the extremely low-frequency field (50 Hz, 4 h/d, 6 d a week for 2 weeks). The experiment indicates an increase in

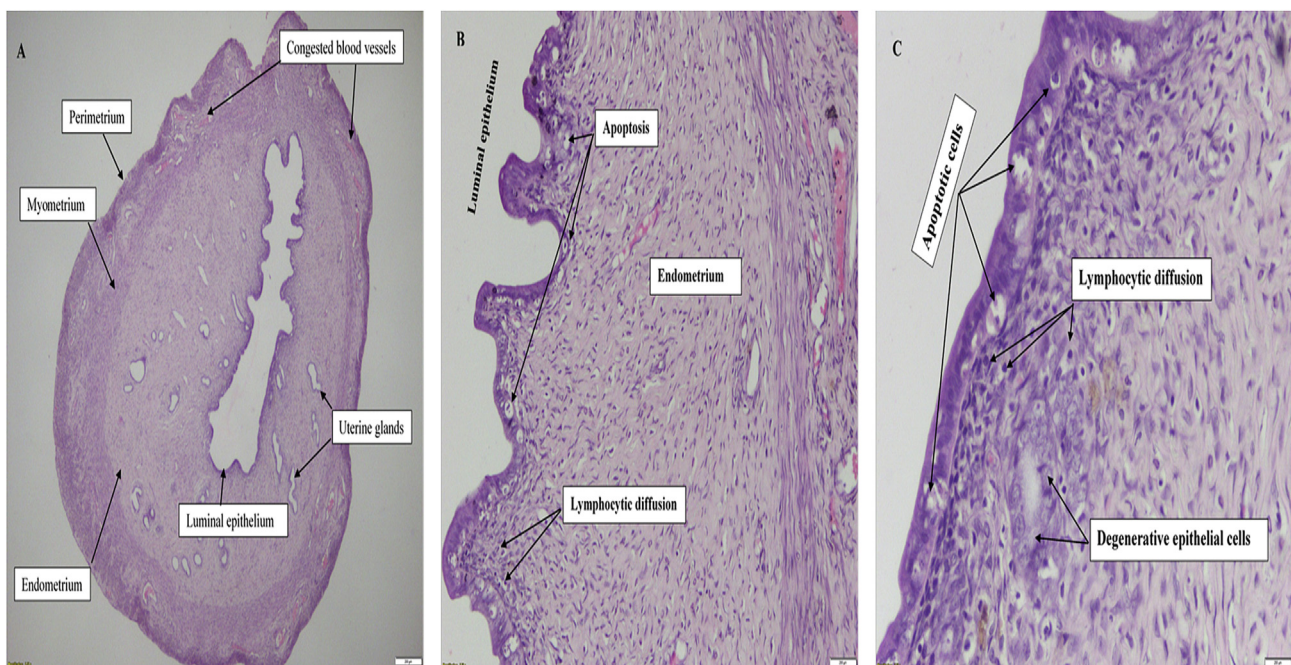


Figure 5. Uterine sections from the exposed female group for 30 days.

(A) Cross section to uterine horn showing vascular congestion with other inflammatory infiltration 4×. (B) Apoptosis in the luminal epithelium with lymphocytic infiltration 20×. (C) Moderate apoptosis in the luminal epithelium with degeneration of glandular epithelial cells and diffuse infiltration by lymphocytes and granulocytes 40×. H&E staining.

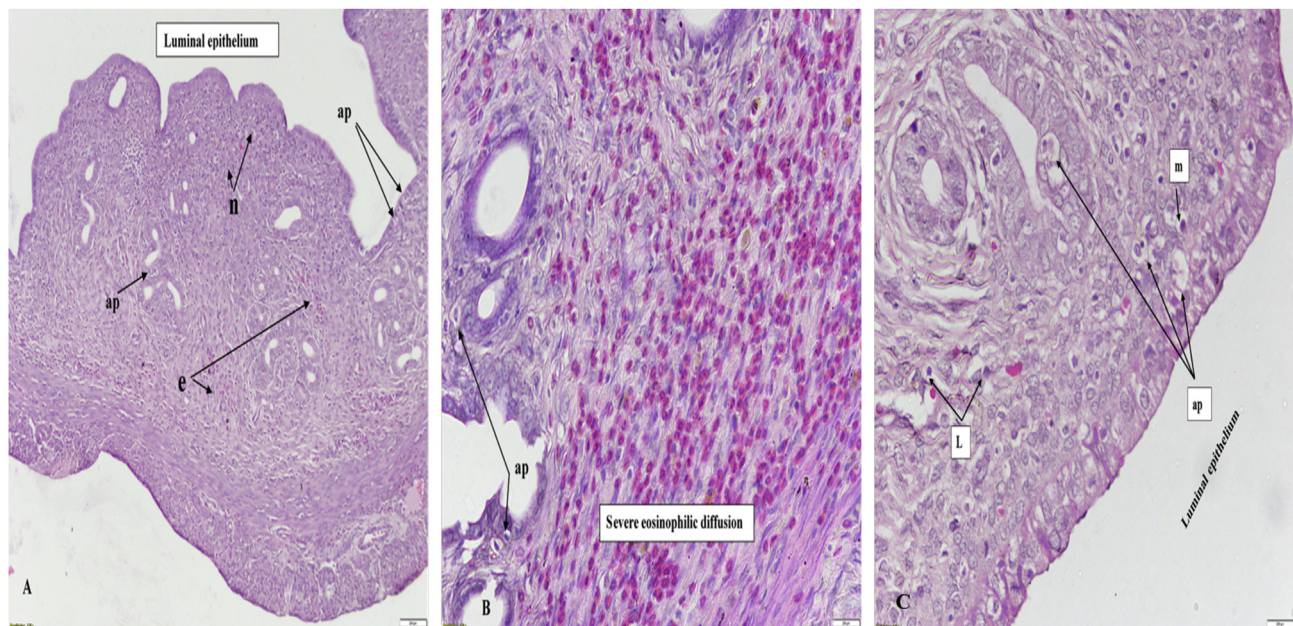


Figure 6. Uterine sections of females exposed to EMF for 60 days.

Photograph (A) shows degeneration/necrosis of luminal and gland epithelium with high rate of apoptotic cells (ap). Severe neutrophils infiltration in the endometrium (n) with severe scattered eosinophils infiltration (e) 10x. (B) Severe diffused eosinophils infiltration in endometrium and perimetrium layers with degeneration and apoptosis in glandular epithelium (ap) 40x. (C) Diffuse infiltration of the endometrium by macrophage (M) and lymphocytes (L) with inflammatory reaction and apoptosis in both glandular and luminal epithelium 40x. H&E staining.

height of luminal epithelium layer of Fallopian tubes and decrease in flushed blastocysts contributed to an imbalance in the function of the female reproductive system [24].

Previous studies found that electromagnetic fields cause a decrease in estrogen level in rats due to low level of FSH after exposure to EMF, which reduce ovarian follicle capacity to reach the developmental stage that is essential for reproductive success and cause alteration in estrogen secretion that exert many biological effects throughout the body [46,47]. But physiologically the increase in height of the luminal epithelium occurred as a result of estrogen effect on uterine tissue causing an increase in cell proliferation and size and inducing uterine growth [48–50]. This indicates presence of an unknown mechanism resulting from involving other factors that effect on luminal epithelium proliferation and growth, finally resulting in increased height of luminal epithelium cells. Therefore, further investigations to clarify the correct mechanism that contributed to the increase luminal epithelial layer growth.

The present study revealed that EMF causes cellular inflammatory reaction composed of infiltrative neutrophils, lymphocytes, severe diffuse eosinophils in endometrium and perimetrium layers with apoptosis and desquamation of luminal and glandular epithelium. This is consistent with previous results [22], which proved that the EMF induced diffuse and severe apoptosis in glandular and luminal epithelium cells and diffused eosinophils, leucocytes and lymphocyte's infiltration. Several factors were involved in endometritis, including red blood cell's damage, apoptotic endometrial cells, cellular debris and some inflammatory factors. All these factors contribute in activation of polymorphonuclear cells and macrophage, which might be stimulated by immune response or free radicals [51]. Our data suggest that the potential alteration of antioxidant capacity associated with excessive production of free radicals may contribute to endometrial oxidative damage, which could be related to the pathogenesis and progression of endometritis.

Further studies need to evaluate the correlation between other factors such as interleukins, vascular endothelial growth factor, tumor necrosis factor, granulosa cell's apoptosis and leukocyte activity and pathological changes of uterine tissue under effect of radio-frequency electromagnetic radiation emitted from mobile phone.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The project was fully supported by Faculty of Veterinary Medicine of Universiti Malaysia Kelantan. The authors are indebted to our histologist (Prof. Dr. Zahirul Islam) and research assistants at the histopathology laboratory for their help.

References

- [1] Burlaka A, Tsybulin O, Sidorik E, Lukin S, Polishuk V, Tsehmistrenko S, et al. Overproduction of free radical species in embryonal cells exposed to low intensity radiofrequency radiation. *Exp Oncol* 2013; **35**(3): 219-225.
- [2] Haghdoust S. *Biomarkers of oxidative stress and their application for assessment of individual radiosensitivity*. Stockholm, Sweden: Stockholm University; 2005.
- [3] Markkanen ARI. *Effects of electromagnetic fields on cellular responses to agents causing oxidative stress and DNA damage*. Kuopio, Finland: University of Kuopio; 2009.
- [4] Mattsson MO, Simkó M. Grouping of experimental conditions as an approach to evaluate effects of extremely low-frequency magnetic fields on oxidative response in in vitro studies. *Front Public Health* 2014; **2**.
- [5] Yakymenko I, Tsybulin O, Sidorik E, Henshel D, Kyrylenko O, Kyrylenko S. Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation. *Electromagn Biol Med* 2015 Jul;(9): 1-7.

- [6] Tök L, Nazıroğlu M, Doğan S, Kahya MC, Tök O. Effects of melatonin on Wi-Fi-induced oxidative stress in lens of rats. *Indian J Ophthalmol* 2014; **62**(1): 12-15.
- [7] Ozgur E, Kismali G, Guler G, Akcay A, Ozkurt G, Sel T, et al. Effects of prenatal and postnatal exposure to GSM-like radio-frequency on blood chemistry and oxidative stress in infant rabbits, an experimental study. *Cell Biochem Biophys* 2013; **67**(2): 743-751.
- [8] Kismali G, Ozgur E, Guler G, Akcay A, Sel T, Seyhan N. The influence of 1800 MHz GSM-like signals on blood chemistry and oxidative stress in non-pregnant and pregnant rabbits. *Int J Radiat Biol* 2012; **88**: 414-419.
- [9] Kerman M, Senol N. Oxidative stress in hippocampus induced by 900 MHz electromagnetic field emitting mobile phone: protection by melatonin. *Biomed Res* 2012; **23**(1): 147-151.
- [10] Halliwell B. Biochemistry of oxidative stress. *Biochem Soc Trans* 2007; **35**: 1147-1150.
- [11] Al-Akhras MA. Influence of 50 Hz magnetic field on sex hormones and body, uterine, and ovarian weights of adult female rats. *Electromagn Biol Med* 2008; **27**(2): 155-163.
- [12] Gul A, Çelebi H, Uğraş S. The effects of microwave emitted by cellular phones on ovarian follicles in rats. *Arch Gynecol Obstet* 2009; **280**(5): 729-733.
- [13] Vahid HJ, Khaterreh D, Esmaeal F, Maryam N, Mohammad F. The effects of mobile phone waves on the reproductive physiology in adult female rats. *Adv Environ Biol* 2012; **6**(10): 2735-2741.
- [14] Devrim E, Ergüder IB, Kılıçoğlu B, Yaykaşlı E, Cetin R, Durak I. Effects of electromagnetic radiation use on oxidant/antioxidant status and DNA turn-over enzyme activities in erythrocytes and heart, kidney, liver, and ovary tissues from rats: possible protective role of vitamin c. *Toxicol Mech Methods* 2008; **18**(9): 679-683.
- [15] Akdag MZ, Dasdag S, Uzunlar AK, Ulukaya E, Oral AY, Çelik N, et al. Can safe and long-term exposure to extremely low frequency (50 Hz) magnetic fields affect apoptosis, reproduction, and oxidative stress? *Int J Radiat Biol* 2013; **89**(12): 1053-1060.
- [16] Bayat PD, Ghanbari A, Babaei S, Khazaei M, Ghorbani R, Ayubian M. Effect of exposure to extremely low electro-magnetic field during prenatal period on mice spleen. *Indian J Exp Biol* 2011; **49**: 634-638.
- [17] Gathiram P, Kistnasamy B, Laloo U. Effects of a unique electromagnetic field system on the fertility of rats. *Arch Environ Occup Health* 2009; **64**(2): 93-100.
- [18] Poulietier de Gannes F, Billaudel B, Haro E, Taxile M, Le Montagner L, Hurtier A, et al. Rat fertility and embryo fetal development: influence of exposure to the Wi-Fi signal. *Reprod Toxicol* 2013; **36**: 1-5.
- [19] Aydogan F, Unlu I, Aydin E, Yumusak N, Devrim E, Samim EE, et al. The effect of 2100 MHz radiofrequency radiation of a 3G mobile phone on the parotid gland of rats. *Am J Otolaryngol* 2015; **36**(1): 39-46.
- [20] Ait-Aïssa S, Billaudel B, Poulietier de Gannes F, Ruffié G, Duleu S, Hurtier A, et al. In utero and early-life exposure of rats to a Wi-Fi signal: screening of immune markers in sera and gestational outcome. *Bioelectromagnetics* 2012; **33**(5): 410-420.
- [21] Misa Agustino MJ, Leiro JM, Jorge Mora MT, Rodríguez-González JA, Jorge Barreiro FJ, Ares-Pena FJ, et al. Electromagnetic fields at 2.45 GHz trigger changes in heat shock proteins 90 and 70 without altering apoptotic activity in rat thyroid gland. *Biol Open* 2012; **1**(9): 831-838.
- [22] Guney M, Ozguner F, Oral B, Karahan N, Mungan T. 900 MHz radiofrequency-induced histopathologic changes and oxidative stress in rat endometrium: protection by vitamins E and C. *Toxicol Ind Health* 2007; **23**(7): 411-420.
- [23] Rajaei F, Farokhi M, Ghasemi N, Pahlevan AA. Effects of extremely low-frequency magnetic field on mouse epididymis and deferens ducts. *Iran J Reprod Med* 2009; **7**(2): 85-89.
- [24] Rajaei F, Borhani N, Sabbagh-Ziarani F, Mashayekhi F. Effects of extremely low-frequency electromagnetic field on fertility and heights of epithelial cells in pre-implantation stage endometrium and fallopian tube in mice. *J Chin Integr Med* 2010; **8**: 56-60.
- [25] Khaki A, Khaki AA, Ezzatzadeh A, A-Ashteani H. Effect of *Ocimum basilicum* on ovary tissue histopathology after exposure to electromagnetic fields (EMF) in rats. *Afr J Pharm Pharmacol* 2013; **7**(25): 1703-1706.
- [26] Gharamaleki H, Parivar K, Rad JS, Roshangar L, Shariati M. Effects of low-frequency electromagnetic field exposure during the prenatal period on biomarkers of oxidative stress and pathology of ovarian tissue in F 1. *Int J Cur Res Rev* 2013; **5**(21): 23-29.
- [27] Alchalabi ASH, Aklilu E, Aziz AR, Malek F, Ronald SH, Khan MA. Different periods of intrauterine exposure to electromagnetic field: influence on female rats' fertility, prenatal and postnatal development. *Asian Pac J Reprod* 2015; **5**(1): 14-23.
- [28] Botsoglou NA, Fletouris DJ, Papageorgiou GE, Vassilopoulos VN, Mantis AJ, Trakatellis AG. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. *J Agric Food Chem* 1994; **42**(9): 1931-1937.
- [29] Güler G, Tomruk A, Ozgur E, Sahin D, Sepici A, Altan N, et al. The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. *Int J Radiat Biol* 2012; **88**(4): 367-373.
- [30] Guler G, Tomruk A, Ozgur E, Seyhan N. The effect of radio-frequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. *Gen Physiol Biophys* 2010; **29**(1): 59-66.
- [31] Roshangar L, Hamdi BA, Khaki AA, Rad JS, Soleimani-Rad S. Effect of low-frequency electromagnetic field exposure on oocyte differentiation and follicular development. *Adv Biomed Res* 2014; **3**: 76.
- [32] Cecconi S, Gualtieri G, Di Bartolomeo A, Giulia Troiani MGC, Canipari R. Evaluation of the effects of extremely low frequency electromagnetic fields on mammalian follicle development. *Hum Reprod* 2000; **15**(11): 2319-2325.
- [33] Kheradmand A, Roshangar L, Taati M, Sirotkin AV. Morphometrical and intracellular changes in rat ovaries following chronic administration of ghrelin. *Tissue Cell* 2009; **41**(5): 311-317.
- [34] Kowalczyk CI, Robbins L, Thomas JM, Butland BK, Saunders RD. Effects of prenatal exposure to 50 Hz magnetic fields on development in mice: I. Implantation rate and fetal development. *Bioelectromagnetics* 1994; **15**(4): 349-361.
- [35] Escobar ML, E OM, V.-N GH. In: Bailly Y, editor. *Autophagy – a double-edged sword – cell survival or death?* InTech; 2013; <http://dx.doi.org/10.5772/50855>.
- [36] Chernoff N, Rogers JM, Kavet R. A review of the literature on potential reproductive and developmental toxicity of electric and magnetic fields. *Toxicology* 1992; **74**(2-3): 91-126.
- [37] Tilly JL, Tilly KL. Inhibitors of oxidative stress mimic the ability of follicular-stimulating hormone to suppress apoptosis in cultured rat ovarian follicles. *Endocrinology* 1995; **136**: 242-252.
- [38] Hughes FM, Gorospe WC. Biochemical identification of apoptosis (programmed cell death) in granulosa cells: evidence for a potential mechanism underlying follicular atresia. *Endocrinology* 1991; **129**(5): 2415-2422.
- [39] Rzepczynska IJ, Foyouzi N, Piotrowski PC, Celik-Ozenci C, Cress A, Duleba AJ. Antioxidants induce apoptosis of rat ovarian theca-interstitial cells. *Biol Reprod* 2011; **84**(1): 162-166.
- [40] Szczepanska M. Oxidative stress may be a piece in the endometriosis puzzle. *Fertil Steril* 2003; **79**(6): 1288-1293.
- [41] Gupta S, Agarwal A, Krajcir N, Alvarez JG. Role of oxidative stress in endometriosis. *Reprod Biomed Online* 2006; **13**(1): 126-134.
- [42] Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol* 2005; **21**: 1-21.
- [43] Tkalec M, Stambuk A, Srut M, Malarić K, Klobučar GI. Oxidative and genotoxic effects of 900 MHz electromagnetic fields in the earthworm *Eisenia fetida*. *Ecotoxicol Environ Saf* 2013; **90**: 7-12.
- [44] Ibrahim NK, Gharib OA. The protective effect of antioxidants on oxidative stress in rats exposed to the 950 MHz electromagnetic field. *J Radiat Res Appl Sci* 2010; **3**(4): 1143-1155.

- [45] Avci B, Akar A, Bilgici B, Tunçel ÖK. Oxidative stress induced by 1.8 GHz radio frequency electromagnetic radiation and effects of garlic extract in rats. *Int J Radiat Biol* 2012; **88**(11): 799-805.
- [46] Hajiun B. Effects of cell phone radiation on estrogen and progesterone levels and ovarian changes in rats treated with garlic (L.) hydro-alcoholic extract. *J Herb Drugs* 2013; **4**(2): 81-88.
- [47] Al-Akhras MA, Darmani H, Elbetieha A. Influence of 50 Hz magnetic field on sex hormones and other fertility parameters of adult male rats. *Bioelectromagnetics* 2006; **27**(2): 127-131.
- [48] Davoudi M, Zavareh S, Ghorbanian MT, Hassanzadeh H. Effects of steroid hormones on uterine tissue remodeling of mouse menopause model. *J Paramed Sci* 2008; **6**(1): 65-71.
- [49] Peyghambari F, Salehnia M, Moghadam MF, Valujerdi MR, Ebrahim H. The changes in morphology and morphometrical indices of endometrium of ovariectomized mice in response to exogenous ovarian hormones. *Iran J Reprod Med* 2008; **6**(3): 125-131.
- [50] Pollard RM, Finn CA. The effect of ovariectomy at puberty on cell proliferation and differentiation in the endometrium of the aged mouse. *Biol Reprod* 1974; **10**(1): 74-77.
- [51] Barcelos Ionara, Navarro Paula. *Endometriosis and infertility: the role of oxidative stress*. INTECH Open Access Publisher; 2012, p. 399-416.