

Quantitative changes in testicular structure and function in rat exposed to mobile phone radiation

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Summary

The possible effects of the electromagnetic fields (EMF) generated by mobile phones on reproductive functions have been discussed in recent years. The aim of this study was to evaluate the effects of EMF emitted from mobile phones on the rat testis morphology and histopathology using stereological techniques. We also investigated cortisol, testosterone, FSH and LH levels. A total of thirty-two ($n = 32$) male Wistar albino rats were used in this study. Animals were randomly divided into four groups as control (C, $n = 8$), sham (Sh, $n = 8$), mobile phone speech (Sp, $n = 8$) and mobile phone standby (ST by). Morphometric measurements were made with the help of a computer-assisted stereological analysis system. The testis weight and volume were significantly lower in the EMF exposed groups. The mean volume fraction of interstitial tissue was higher, but the volume fraction of tubular tissue was lower in the EMF-exposed groups. The mean tubular and germinal tissue volume, seminiferous tubule diameter and germinal epithelium height were also lower in EMF exposed groups. The cortisol levels in the EMF-exposed groups were significantly higher. In conclusion, the EMF created by mobile phones caused morphologic and histological changes by the affecting germinal epithelium tissue negatively.

KEYWORDS

Cavalieri principle, electromagnetic field, mobile phone, stereology, testis morphology

1 | INTRODUCTION

Mobile phones are communication tools which operate on different mobile phone systems and at various carrying frequencies in line with these systems (Bhalla & Bhalla, 2010). Electromagnetic energy generated by working mobile phones penetrates living tissues and may cause degenerative effects in different biological systems. Previous studies have shown negative effects of the electromagnetic fields (EMF) created by mobile phones on neurological (Saikhedkar et al., 2014), cardiovascular (Mohamed, Ahmed, El-Kafoury, & Lasheen, 2011), endocrinal (Mortavazi et al., 2009) and developmental systems (Ye et al., 2015).

Testes are the male reproductive organs in which spermatozoon production takes place and are located at a close distance from mobile phones carried in back pocket, front pocket or on waistband. This causes testes to be exposed to EMF waves emitted by mobile phones

for long hours during the day. Therefore, the number of studies on the effect of mobile phones on reproductive functions has increased in recent years. In studies carried out on rat models, it has been reported that exposure to mobile phones results in negative histological and morphological alterations in testes (Dasdag et al., 1999; Mugunthan, Anbalagan, & Meenachi, 2014; Mugunthan, Anbalagan, Samy, Rajanarayanan, & Meenachi, 2015; Salama, Kishimoto, & Kanayama, 2010). It has been shown that exposure to mobile phones (800 MHz) leads to a significant decrease in sperm count and total percentage of motile spermatozoa (Salama et al., 2010). In another study, a significant decrease was observed in the body and testis weights, testis volumes, seminiferous tubule densities, seminiferous tubule diameters, and Sertoli and Leydig cell counts of rats exposed to EMF emitted by 2G (900–1800 MHz) and 3G (1900–2200 MHz) mobile phones (Mugunthan et al., 2015). On the other hand, there are also studies

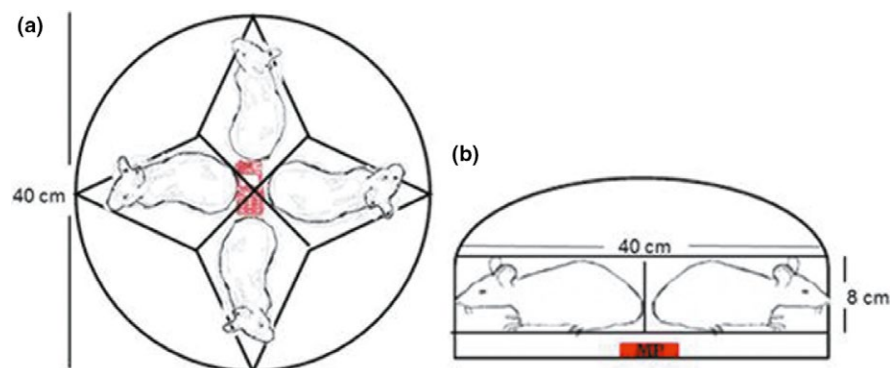


FIGURE 1 Schematic representation of the experimental set up on the (a), top view; (b), side view; and MP: mobile phone (circular plastic boxes were separated into four sections with barriers and each section was used for one rat)

reporting that mobile phone induced EMF has no negative effect on testicular parameters (Dasdag, Akdag, Ulukaya, Uzunlar, & Yegin, 2008; Dasdag et al., 2003).

Cortisol secreted by the suprarenal gland is an indicator of stress. Cortisol levels are reported to increase with exposure to EMF for a long time (Shahryar, Lotfi, Bahojb, & Karami, 2008). The increased amount of cortisol has been found to cause apoptosis of Leydig cells in testes, thus reducing testosterone levels (Gao et al., 2002). Therefore, it is important to consider the effect of cortisol in EMF studies. One of the reproductive hormones, testosterone, is secreted by Leydig cells, while two others, follicle stimulating hormone (FSH) and luteinising hormone (LH), are secreted by the adenohypophysis. There are studies in the literature examining the effects of EMF on reproductive hormones (Kim, Kim, Moon, & Shin, 2007; Nisbet, Nisbet, Akar, Cevik, & Karayigit, 2012; Ozguner et al., 2005; Salama et al., 2010). These studies have shown increasing (Nisbet et al., 2012), decreasing (Ozguner et al., 2005) and nonexistent (Salama et al., 2010) effects of EMF on testosterone levels. In testes-focused studies, it has been observed that FSH and LH hormone levels do not change in general (Kim et al., 2007; Ozguner et al., 2005). In accordance with all these findings in the literature, the hypothesis of this study is “EMF generated by mobile phones has a negative effect on testis tissue and reproductive hormones”.

The purpose of the present study was to examine the effects of EMF generated by different mobile phone modes on the testis morphology of rats using stereological techniques. Moreover, the effects of EMF on testis histopathology and on testosterone, FSH and LH levels were also evaluated.

2 | MATERIALS AND METHODS

2.1 | Experimental animals

The experimental protocol of the present study was approved by Gaziantep University Animal Experiments Local Ethics Committee decision numbered 11.2011/54-4 and dated 28.11.2011. A total of 32 ($n = 32$) male Wistar Albino rats aged 8–10 weeks and weighing 200 ± 20 g were used in the study. The animals were taken from the Experimental Animal Research Laboratory of the Faculty of Medicine at Gaziantep University. The life cycle of the rats was maintained at the same laboratory. The rats were kept under standard conditions stated by the international ethical guidelines on laboratory animals

during the experiment. All animals were kept in wire mesh cages for 12 hr at night and 12 hr in daytime, at circadian rhythms, 24–26°C ambient temperature and 45%–60% humidity. The rats were fed standard commercial rat pellets and tap water.

2.2 | Experiment protocol

In this study which was conducted using a randomised controlled experimental design, the rats were divided into four groups namely the control (C; $n = 8$), sham (S; $n = 8$), mobile phone speech (Sp; $n = 8$) and mobile phone standby (ST by; $n = 8$). In order to generate EMF, mobile phones using GSM mobile phone systems were employed. The highest SAR (specific absorption rate) of the mobile phones was 0.96 W/kg and each of these phones had a 890–915 MHz carrier frequency band, 217 Hz modulation frequency, 250 mW maximum average power and 2 W maximum peak power (Dasdag et al., 1999).

Special circular plastic experimental boxes separated into four sections with barriers and having one rat in each section were used for rats in the S, Sp and ST by groups. These experimental boxes restricted the movement of the rats while allowing respiration. One mobile phone was placed in each of the special sections located 1 cm below and in the middle of the experimental boxes (Figure 1). Our purpose of modelling the experiment in the S group was to evaluate possible stress factors that could occur in immobile rats. To this end, the rats in group S were placed in the box with their testes directed towards the mobile phone and the mobile phones were switched off (no EMF was generated). The rats were kept in this experimental box for 12 hr/day.

In order to examine the effects of EMF generated by different modes of the mobile phone, the rats in the Sp and ST by groups were placed in experimental boxes in the same way as those in the group S. Speech mode was obtained by keeping the mobile phone in the experiment box on speech mode for 2 hr/day. For the standby mode, the mobile phone was put on standby for 12 hr/day. The models of the batteries of the mobile phones were in the same standard conditions, and the batteries were fully charged before each experiment. Experimental practices were carried out between 07:00–19:00 for groups S and ST by, and 07:00–09:00 for the Sp group 7 days a week. The rats in the C group and the other groups outside the experiment period were kept in their normal life cages in a separate room without being exposed to any type of electromagnetic field. The duration of the experiment was determined as 10 weeks for all groups. The ambient temperature was

fixed at 22°C for all groups. Average rectal temperatures of the rats in groups C, S, Sp and ST by were measured with a thermometer before and after each experiment, twice a week. The body weights of the rats were measured in the day the experiment was initiated (Day 1) and in the day it was finalised (the last day of the week 10), using a digital scale with 0.01 g precision.

2.3 | Preparation of the testis tissues

At the end of the experiment (on the last day of week 10), all of the rats were anaesthetised. The anaesthetisation of rats in groups was made randomly. Ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg) were applied intraperitoneally for anaesthesia. The thoracic cages of the rats were opened through thoracotomy and blood samples were taken for hormone analysis intracardially. Later, the rats were euthanised using the exsanguination method and their left testes were removed rapidly. Each testis tissue was weighed on the scale with 0.01 g precision. The value obtained by dividing the testis weight (TW) into the body weight (BW) of the rat was multiplied by a hundred ($TW/BW \times 100$) and the gonadosomatic index (GSI) was calculated (Melo, Matta, Paula, Gomes, & Oliveira, 2010). 10% formaldehyde solution was used for fixation.

2.4 | Histological operations

Each fixated testis was cut in 2 mm thickness on a transverse plane using microtome blade. The tissues (7–8 slices) were dehydrated in graded ethanol series, cleaned with xylene and embedded in paraffin blocks with each slice facing the same direction. The prepared tissues were cut on a transverse plane at 30 µm thickness using microtome (Leica RM 2135, Leica Instruments, Nussloch, Germany) with systematic uniform random sampling (SURS). Section sampling fraction (ssf) was 1/35 and 18–22 sections were sampled from each testis. Later, the sections were stained with haematoxylin–eosin (H&E).

2.5 | Stereological and morphometric evaluation of the testes

The computer-aided stereological analysis system (Stereoinvestigator version 8.0, MicroBrightField, USA) was employed for the stereological and morphometric evaluation. With the stereological analysis system, testis volume (TV), seminiferous tubule diameter (STD), germinal epithelium height (GEH), the volume fractions (Vv) and volumes of the tunica albuginea (TAI), interstitial tissue (IT) and structures forming the seminiferous tubule were evaluated.

2.6 | Evaluation of the testis volume

The calculation of testis volumes was done using the point counting method in line with Cavalieri's principle, which is a stereological method (Gundersen et al., 1988). On the section to be evaluated, the contour of the area to be measured was drawn on a low magnification objective lens (4x). Following this, a point grid with a grid space

of 100 µm was thrown on the section. The points falling into the counters were counted automatically by the system. Testis volume was calculated automatically by the system using the formula below (Noorafsan, 2014):

$$\text{Volume (testis)} = (a/p) \times \sum P \times \text{ssf} \times t$$

In this formula; “(a/p)” stands for the area represented by each point on the point grid, while “ $\sum P$ ” stands for the number of points per area of evaluation, “t” stands for section thickness (30 µm), and “ssf” stands for section sampling fraction (1/35). In testis volume calculations, values under 5% for the coefficient of error (CE) and coefficient of variance (CV) (CE and CV < 0.05) showed that the sampling strategy was appropriate.

2.7 | Evaluation of the testis volume fractions and volumes

While evaluating the volume fractions, a square-shaped frame with 100 points (grid space=15 µm) was thrown over the section. This square moved at equal step lengths on X and Y planes on the evaluated section. Thus, the evaluated section was scanned in compliance with the SURS rule. Within the areas scanned by this square, the points corresponding to testis components were counted separately for each component (Figure 2).

The components of which the volume fractions were evaluated in the testis tissue were determined as the interstitial tissue, tubular tissue (tubular tissue=tunica propria (TP) + germinal epithelium (GE) + seminiferous tubule lumen) and tunica albuginea (Drostghoal, Sorooshnia, & Zardkaf, 2011). In the interstitial tissue; Leydig cells, connective tissue cells and fibres, blood vessels and lymphatic space were examined and all these structures were evaluated as IT (De Melo et al., 2013). Volume

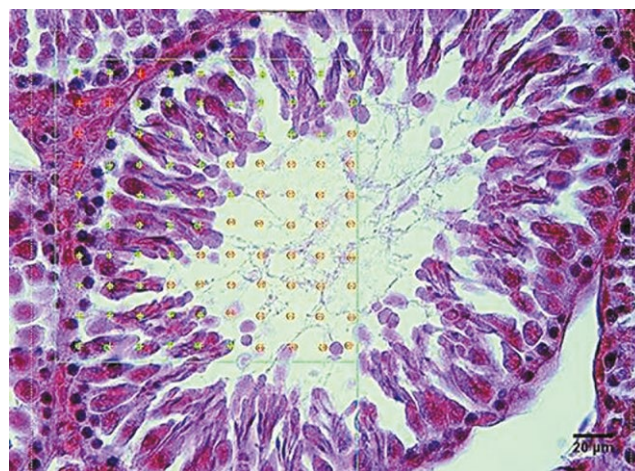


FIGURE 2 Evaluation of the volume fraction (Vv) of the testicular compartments (a square-shaped frame was thrown over the section and within the areas scanned by this square, and the points corresponding to interstitial tissue, germinal epithelium and lumen were counted separately for each component); magnification: $\times 40$

fractions of each structure were calculated automatically by the system using the formula below (Drostghoal et al., 2011):

$$Vv_{(\text{tissue/testis})} = P_n/P_t$$

" $Vv_{(\text{tissue/testis})}$ " stands for the volume fraction of the tissue component to be evaluated, " P_n " stands for the number of points corresponding to the tissue component of interest, and " P_t " stands for the number of points corresponding to the testis. The volume of each testis component was calculated by multiplying the volume fraction of the component by the testis volume (Drostghoal et al., 2011). The addition of the tunica propria, germinal epithelium and lumen volumes gave the seminiferous tubule volume. Testis parenchyma volume ($V_{\text{testis}} - V_{\text{tunica albuginea}}$) was obtained by adding the seminiferous tubule volume and the interstitial tissue volume together.

2.8 | Evaluation of the seminiferous tubule diameter and germinal epithelium height

Seminiferous tubule diameter was calculated by applying the "quick measure line" feature of the stereological analysis system in combination with the unbiased counting frame. The frame moved at equal step lengths on X and Y planes and scanned the entire section in compliance with the SURS rule. The seminiferous tubules were evaluated with the unbiased counted frame methodology (Liu, Chang, Xu, & Zhang, 2009). The tubules evaluated for STD were considered in terms of being circular or closer to a circle in shape. Two diameters (D-1 and D-2) which were perpendicular to each other and connected the outer peripheries of the seminiferous tubule were drawn and they were averaged. Measurements that gave $D-1/D-2 \geq 0.85$ ($1.0 =$ a full circle) were included in calculations (Yama & Duru, 2011). Germinal epithelium height was calculated as the distance between the tunica propria and lumen side in seminiferous tubules measured for STD. Two perpendicular measurements were taken for this (G-1 and G-2) and they were averaged (Melo et al., 2010).

2.9 | Histopathological evaluation of the testis tissue

A semi-quantitative method, "Johnsen testicular biopsy score count," was used for the evaluation of the spermatogenesis in the testis (Dasdag et al., 1999, 2003; Ozguner et al., 2005). With this scoring method, the absence or presence of the main cells in the seminiferous tubule was measured in a scale of 1–10. This study evaluated at least 50 seminiferous tubules selected randomly under a light microscope and the obtained data were averaged for each testis.

2.10 | Evaluation of the hormonal parameters

Blood samples taken intracardially were drawn into common biochemical tubes. These samples were centrifuged at 3 g for 10 min and blood serums were obtained. These serum samples were stored at -80°C before the measurements of cortisol, testosterone, FSH and LH hormone levels. Cortisol, testosterone and LH hormone levels

were evaluated with the electrochemiluminescence method on the Roche Cobas 8,000 modular analyzer. Rat FSH ELISA kit (E02F0001; BlueGene Biotech, Shanghai, China) was used to determine FSH levels.

2.11 | Statistical analysis

Kolmogorov–Smirnov test was used to check whether the continuous variables fit normal distribution. In the comparisons of more than two groups of normally distributed variables, ANOVA and LSD multiple comparison tests were conducted, whereas Kruskal–Wallis and Dunn multiple comparison tests were used for the variables that did not fit normal distribution. Differences between repeated measurements were tested using paired samples *t* test. Mean $\pm$ SD values were given as the descriptive statistics. SPSS for Windows version 22.0 package program was used for statistical analyses and $p < .05$ was accepted as the statistically significant value.

3 | RESULTS

The mean rectal temperatures of the rats in groups C, S, Sp and ST by were compared and no significant difference was observed between the values before and after the experiment ($p > .05$). Moreover, no significant difference was found among the post-experimental rectal temperature values of the study groups as well ($p > .05$). This finding indicates that the experiment was carried out under standard conditions and the experimental practices did not change central body temperatures.

3.1 | Testis and body weights of the rats and gonadosomatic index values

After the experiment, mean testis weights of the rats in the groups Sp and ST by were found to be significantly lower than the mean testis weights of the rats in groups C and S. It was found that the mean body weight of the rats in the Sp group decreased significantly in comparison with the C group. Additionally, the difference between the ST by group and the groups C and S was statistically significant. The difference between the GSI values of the experiment groups and the groups C and S was also statistically significant (Table 1).

3.2 | Testis volume values of the rats

Mean testis volumes of the rats in Sp and ST by groups (including tunica albuginea) decreased significantly in comparison with groups C and S. As compared to the control group, the rate of decrease in the testis volumes of the rats in Sp was 26.2%, while it was 32.0% in the group ST by (Table 1).

3.3 | Volume fractions and volumes of the testis components of the rats

TAI volume fractions of the rats in groups control, S, Sp and ST by were 2.26 ± 0.40 , 2.10 ± 0.08 , 2.20 ± 0.04 and 2.10 ± 0.10 respectively. No statistically significant difference was found among the

TABLE 1 The gonadosomatic index, testis and body weights, testis and testis parenchyma volumes in groups

Groups	GSI	Testis weight (g)	Body weight (g)	Testis volume (cm ³)	Testis parenchyma volume (cm ³)
C	0.55 ± 0.01	1.53 ± 0.06	276.6 ± 11.1	1.027 ± 0.071	1.005 ± 0.071
S	0.56 ± 0.07	1.49 ± 0.25	265.5 ± 10.6 ^d	0.941 ± 0.120	0.920 ± 0.117
Sp	0.49 ± 0.01**	1.27 ± 0.07**	259.8 ± 8.3 ^b	0.758 ± 0.043*	0.741 ± 0.042*
ST by	0.47 ± 0.04*	1.10 ± 0.13 ^a	232.9 ± 10.3 ^{a,c}	0.700 ± 0.094*	0.686 ± 0.093*

C, control group (n = 8); S, sham group (n = 8); Sp, speech group (n = 8); ST by, standby group (n = 8); mean ± SD.

*p < .001: compared to C and S groups, **p < .01: compared to C and S groups, ^ap < .05: compared to Sp group, ^bp < .01: compared to C group, ^cp < .001: compared to Sp group, ^dp < .05: compared to C group.

TABLE 2 The volume fractions and volumes of the testis parenchyma components in groups

Groups		IT	TP	GE	Lumen	TT
C	(Vv-%)	8.78 ± 1.42	1.73 ± 0.36	77.75 ± 2.13	11.71 ± 1.86	91.19 ± 1.43
	(V-cm ³)	0.088 ± 0.015	0.017 ± 0.003	0.782 ± 0.074	0.117 ± 0.010	0.916 ± 0.067
S	(Vv-%)	9.19 ± 1.27	1.75 ± 0.40	77.77 ± 1.95	11.31 ± 2.06	90.84 ± 1.27
	(V-cm ³)	0.084 ± 0.011	0.016 ± 0.004	0.717 ± 0.108 ^b	0.103 ± 0.015	0.837 ± 0.112 ^b
Sp	(Vv-%)	10.90 ± 1.63 ^a	1.98 ± 0.47	76.64 ± 2.42	10.43 ± 1.68	89.04 ± 1.66 ^a
	(V-cm ³)	0.081 ± 0.016	0.015 ± 0.003	0.568 ± 0.018*	0.078 ± 0.015*	0.660 ± 0.029*
ST by	(Vv-%)	12.75 ± 2.10* **	2.22 ± 0.63	75.36 ± 1.47	9.66 ± 1.26	87.23 ± 2.10* **
	(V-cm ³)	0.089 ± 0.024	0.016 ± 0.005	0.516 ± 0.062*	0.066 ± 0.007*	0.597 ± 0.070*

C, control group (n = 8); S, sham group (n = 8); Sp, speech group (n = 8); ST by, standby group (n = 8); IT, interstitial tissue; TP, tunica propria; GE, germinal epithelium; TT, tubular tissue; mean ± SD.

*p < .001: compared to C and S groups, **p < .05: compared to Sp group, ^ap < .05: compared to C and S groups, ^bp < .05: compared to C group.

groups (p > .05). TAI volumes of the rats in groups control, S, Sp and ST by were 0.023 ± 0.006, 0.020 ± 0.005, 0.017 ± 0.001 and 0.015 ± 0.001 cm³ respectively. TAI volume in the ST by group was found to decrease in comparison with both the group C and the group S (p = .001 and p = .006 respectively). TAI volumes of the rats in the Sp group decreased significantly in comparison with the group C (p = .001).

Interstitial tissue volume fractions of the rats in the Sp and ST by groups increased as compared to the groups C and S; while tubular tissue volume fractions of the rats in the Sp and ST by groups decreased in comparison with the groups C and S. The volume fractions of the IT, TT and tubular tissue subcomponents (TP, GE and seminiferous tubule lumen) are shown in Table 2.

It was found that testis parenchyma volumes (V testis–V tunica albuginea) of the rats in the Sp and ST by groups were significantly lower than the groups C and S (Table 1). The IT volumes, TT volumes and subcomponents (TP, GE and seminiferous tubule lumen) of TT volumes are given in Table 2.

3.4 | Values of seminiferous tubule diameter and germinal epithelium height

Seminiferous tubule diameter values of the rats in the Sp and ST by groups were found to be decreased in comparison with groups C and S. It was observed that the difference between the GEH values of the rats in the Sp and ST by groups and the values of those in groups C and S was statistically significant (Table 3).

3.5 | Johnsen testicular biopsy score results

Johnsen scores of the rats in the groups Sp and ST by decreased significantly in comparison with the groups C and S (Table 3).

3.6 | Morphological findings

Testis tissues of the rats were examined histopathologically under a light microscope in a qualitative manner. It was seen that germinal epithelium

TABLE 3 The seminiferous tubule diameter (STD), germinal epithelium height (GEH) and Johnsen scores in groups

Groups	STD (μm)	GEH (μm)	Johnsen scores
C	307.09 ± 2.64	86.03 ± 3.25	9.41 ± 0.28
S	292.53 ± 2.77 ^a	83.14 ± 2.24 ^d	9.07 ± 0.32
Sp	283.29 ± 2.87 ^{a,b}	76.55 ± 1.78*	8.43 ± 0.79 ^{b,d}
ST by	268.32 ± 13.26* **	73.73 ± 4.61 ^{a,c}	8.21 ± 0.89 ^{b,e}

C, control group (n = 8); S, sham group (n = 8); Sp, speech group (n = 8); ST by, standby group (n = 8); mean ± SD.

*p < .001: compared to C and S groups, **p < .001: compared to Sp group, ^ap < .001: compared to C group, ^bp < .05: compared to S group, ^cp < .05: compared to Sp group, ^dp < .05: compared to C group, ^ep < .01: compared to C group.

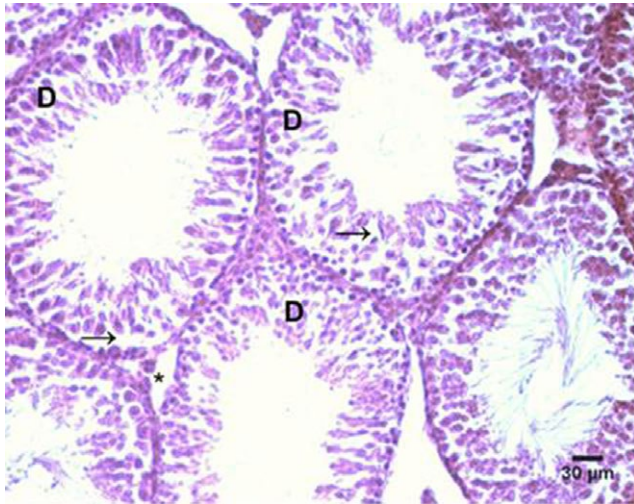


FIGURE 3 Showing the histopathological changes in the standby group (D: the degenerated seminiferous tubules, (*): the separated germinal epithelium from tunica propria, (→): the gaps were among series of spermatogenic cells); magnification: $\times 20$

tissue integrity was mostly maintained and series of spermatogenic cells were in normal order and sequence in the groups C and S.

In the Sp and ST by groups, in addition to germinal epithelium degeneration, germinal epithelium was observed to separate from tunica propria in some seminiferous tubules. Rare examples of gaps were observed among series of spermatogenic cells (Figure 3), while spermatogenic cellular masses were found in the seminiferous tubule lumen (Figure 4). It was also seen that Leydig cell groups decreased in number in the interstitial tissue while the densities of other IT components such as connective tissue and blood vessels increased.

3.7 | Hormonal values

The cortisol values of the rats in the Sp and ST by groups were increased statistically significant compared to C group. No statistically significant difference was found in the FSH, LH and testosterone hormone levels among the groups ($p > .05$) (Table 4).

4 | DISCUSSION

This study examined morphological and histopathological effects of EMF generated by mobile phones in Sp and ST by modes on rat testis. A stereological method, Cavalieri's principle (point count method), was used for the evaluations of the effects of EMF on testis volume, volume fractions and volumes of testis components. Spermatogenesis was examined using Johnsen testicular biopsy score count method. It was seen at the end of the study that exposure to mobile phones caused degeneration in germinal epithelium tissue and as a result, such parameters as testis volume, tubular tissue volume fraction and volume, germinal epithelium volume, STD, GEH and Johnsen biopsy score were affected adversely. Moreover, exposure to mobile phones was found to increase cortisol levels as well.

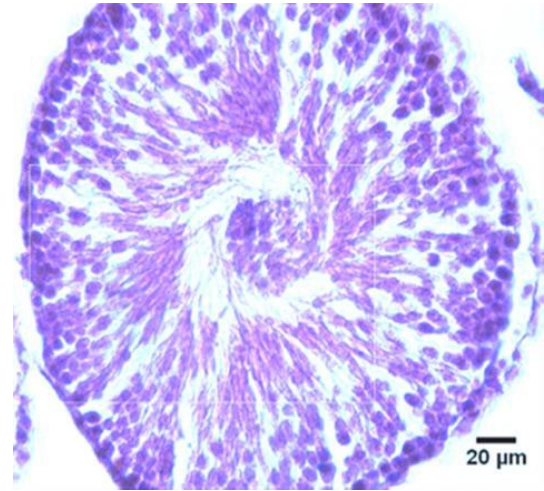


FIGURE 4 Showing the cellular masses were found in the seminiferous tubule lumen; magnification: $\times 40$

In parallel with the effects of EMF emitted by mobile phones on different systems (Mohamed et al., 2011; Mortavazi et al., 2009; Saikhedkar et al., 2014), many studies have examined its effects on male reproductive functions (Agarwal, Deepinder, Sharma, Ranga, & Li, 2008; Mugunthan et al., 2014, 2015; Veerachari & Vasan, 2012). Some of these studies have been carried out on humans and the relationship between the use of mobile phones and infertility was investigated. In a study conducted with 361 males attending an infertility clinic, Agarwal et al. (2008) found that mobile phone usage affected sperm quality negatively by decreasing the number, motility and viability of spermatozoa and this could result in infertility in males. Veerachari and Vasan (2012) reported that exposure to mobile phone generated EMF caused oxidative stress in in vitro semen samples and this had an adverse effect on sperm parameters and DNA structure.

On the other hand, since it is not possible to observe morphological and morphometric changes in testis tissue in clinical studies, some studies have examined the effects of mobile phones on animal models (Dasdag et al., 2003, 2008; Forgacs et al., 2006). Studies carried out with animal models have such advantages as reaching experimental groups at any time, keeping the experimental process under control and finalising the experiments at any moment.

No agreement has yet been reached concerning the effects of exposure to mobile phones on male reproductive functions. While the literature contains studies reporting harmful effects of mobile phones on reproductive functions (Dasdag et al., 1999; El-Bediwi, El-Kott, Saad, & Eid, 2011; Hancı et al., 2013; Nisbet et al., 2012), there are also studies (Forgacs et al., 2006; Lee et al., 2010) stating that testis functions are not affected. In these studies, there may be differences in the methodologies of generating EMF (with experimental equipment in laboratory environment or using mobile phones directly), the frequency of EMF, intensity, experiment duration, mode, total duration of implementation and the types of experimental animals used. Failure to achieve standardisation in the experimental process causes the results concerning the effects of EMF to vary.

TABLE 4 The cortisol, testosterone, FSH and LH values in the groups

Groups	Cortisol ($\mu\text{g/dl}$)	Testosterone (ng/dl)	FSH (ng/ml)	LH (IU/L)
C	4.16 ± 0.91	262.1 ± 46.2	11.33 ± 1.74	0.229 ± 0.008
S	4.39 ± 0.57	256.9 ± 42.8	10.84 ± 2.31	0.227 ± 0.010
Sp	$5.13 \pm 1.02^*$	249.2 ± 65.0	10.14 ± 1.35	0.223 ± 0.009
ST by	$5.16 \pm 0.70^*$	245.9 ± 82.6	10.96 ± 1.66	0.216 ± 0.015

C, control group ($n = 8$); S, sham group ($n = 8$); Sp, speech group ($n = 8$); ST by, standby group ($n = 8$) mean $\pm$ SD.

* $p < .05$: compared to C group.

The number of studies examining the effect of mobile phones on testis volume is quite low. In these studies, testis volume has been evaluated using the immersion method and EMF was observed to decrease testis volume (Mugunthan et al., 2014, 2015). In our study, however, testis volume, testis parenchyma volume, volume fractions and volumes of the components forming the testes were evaluated with Cavalieri's principle (point counting method). Cavalieri's principle is frequently used to calculate tissue volumes by using two-dimensional tissue samples. This method allows an objective and effective calculation of volume and volume fractions of a given tissue (Gundersen et al., 1988). In our study, testis volume, tubular tissue volume fraction and volume, and GE volume in the Sp and ST by groups were observed to decrease in comparison with the C and S groups. This finding clearly reveals the degenerative effect of the EMF generated by mobile phones on the germinal epithelium. These results obtained from the groups exposed to EMF are similar to the previous findings reached by Tenorio et al. (2011).

Seminiferous tubule diameter and GEH are important morphometric parameters showing the damage caused by EMF. Studies have shown that EMF generally reduces STD (Mugunthan et al., 2015; Salama et al., 2010; Tenorio et al., 2011). Ozguner et al. (2005) showed the negative effects of 900 MHz EMF, which they generated experimentally, on STD as well as on GEH. Hanci et al. (2013) stated that EMF (900 MHz) decreased STD and epithelium thickness in mice in the prenatal period. However, it has also been claimed that EMF had no effect on STD (Dasdag et al., 2003). In our study, STD and GEH have been sampled according to the "systematic uniform sampling principle" for the first time. As a result of our evaluations, EMF was observed to decrease STD and GEH in both experimental groups.

The degenerative effect on the germinal epithelium was reflected negatively on the Johnsen testicular biopsy scores. The Johnsen score we obtained revealed hypospermatogenesis. The related literature contains studies showing negative histopathological effects of mobile phones (Mugunthan et al., 2014, 2015). Liu et al. (2015) reported that electromagnetic radiation (900 MHz) decreased sperm count and increased the rate of apoptotic spermatozoa. Various studies have evaluated histopathological changes using qualitative methods other than the Johnsen score. These studies have revealed findings such as a decrease in the number of spermatogenic cells, breaking of these cells from the germinal epithelium (El-Bediwi et al., 2011), separation of seminiferous tubules from each other and a decrease in the number of Leydig cells (Kumar, Mehta, & Nidhi Shukla, 2015). Similar

histopathological changes have been observed in our study. In our study, the degenerative effect of EMF was higher in the ST by group than the Sp group. We associate this with the fact that testes in the ST by group absorbed more radiation.

The number of studies on the relationship of EMF with cortisol is quite limited. In a previous study, Shahryar et al. (2008) reported that long exposure (50 days) to mobile phones (950 MHz) increased serum cortisol levels. Kitaoka, Kitamura, Aoi, Shimizu, and Yoshizaki (2013) found that an extremely low-frequency (60 Hz, 3mT) magnetic field increased corticosterone (a glucocorticoid hormone produced in rats) while it did not change the ACTH levels which control corticosterone activity. In our study, EMF created a stress on the rats and serum cortisol levels increased in the experimental groups correspondingly. It has been stated that the increase in cortisol levels causes degeneration in testes and significantly decreases testosterone secretion (Phillips, Lakshmi, & Monder, 1989). Similarly in our study, the increased cortisol may have contributed to the degeneration in germinal epithelium in addition to the direct effect of EMF on morphological parameters.

Different results have been obtained in studies concerning the effects of EMF on testosterone. Salama et al. (2010) found that mobile phones (800 MHz) did not change the serum testosterone levels in rabbits. Hajioun (2014) came to a similar conclusion. On the other hand, El-Bediwi et al. (2011) stated that EMF decreases testosterone levels, whereas Nisbet et al. (2012) reported an increase. Kim et al. (2007) found that EMF (2.45 GHz) increased testosterone levels in rats but did not alter FSH/LH levels. In our study, although a decrease was observed in Leydig cell groups in the experimental groups, testosterone levels did not change in these groups. A quantitative evaluation of Leydig cells would present the relationship between EMF and testosterone more clearly.

In conclusion, it has been found in our study that EMF generated by mobile phones causes degeneration in the germinal epithelium. As a result of this degeneration, significant decreases were observed in the testis volumes, tubular tissue volumes, STD and GEH of the experimental groups. While EMF increased serum cortisol levels in the experimental group, it caused no change in gonadal hormones. All these findings are considered to be useful for studies examining the effects of EMF on reproductive functions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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