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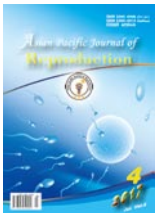

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Effects of long-term exposure to radiofrequency radiations emitted by mobile Jammers on reproduction parameters in rats

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ABSTRACT

Objective: To evaluate the effect of long-term exposure to radiofrequency radiations emitted from mobile jammers onrat's reproductive parameters. **Methods:** Eighty male rats were divided into neonatal group (n=20), immature group (n=30) and mature group (n=30). Neonatal rats were randomly divided into two subgroups. Immature and mature rats were randomly divided into three subgroups. Experimental rats were fixed in the restraining tube and were exposed to radiofrequency radiations emitted from mobile jammers at a distance of 100 cm, for 30 d (7 h/d). Sham group rats were also fixed in restraining tube but without radiations. The control group was allowed to move freely without being exposed to radiation. At the end of the experiment, weight of testes, sperm quality, histological testicular sections and serum testosterone levels were evaluated. **Results:** Long-term exposure to radiofrequency from mobile jammers could adversely affect neonatal rat fertility, but it did not have significant effects on male mature and immature rat's reproduction parameters. However, restraint stress induced by immobilizing them for a long-period could adversely affect male mature rat's reproductive parameters, but it had no significant effect on male immature rat's reproduction parameters. **Conclusion:** The effects of EMF exposure to be various based on the life stage.

1. Introduction

Mobile phone jammers produce radio frequency (RF) which is similar to a mobile phone, usually used to block mobile phone calls, text messages and Wi-Fi internet communications. In some countries, using mobile jammers is legal and they are used in universities to prevent students from cheating or sharing information. in theaters, offices, conference rooms, cinemas, and restaurants to limit the interference caused by the mobile phone users. In most countries, including the U.S., there are strict policies and laws against cell phone jamming devices that prohibit people from possession, selling or operating these devices[1,2].

In modern society, human cannot avoid electromagnetic fields (EMFs) during household and occupational activities, but should be conscious of its biological hazards. Some studies have suggested that exposure to EMF such as mobile phones or wireless internet-connected laptops can have an adverse effect on reproduction and fertilizing potential of spermatozoa[3-7].

Infertility could be the result of a combination of factors such as some diseases, genetic problems and life style, or having a job that is in constant exposure to radiation or chemicals, *etc*. Male infertility is usually caused by problems that affect either sperm generation or sperm motility.

This study aimed toevaluate the effect of long-term exposure to RF radiations emitted from common mobile jammers on testosterone level, semen quality and stereological parameters of testis in male neonatal immature and mature rats.

2. Materials and methods

2.1. Animal treatment

This experimental study was approved by the Ethics Committee of

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Shiraz University of Medical Sciences. Eighty male Sprague-Dawley ratswere purchased from the Laboratory Animal Center of Shiraz University of Medical Sciences, Shiraz, Iran. They were housed in room temperature 22-25 ℃, 12-h light/dark. Standard lab feed and water was available to animals' *ad-libitum*. Seven days prior to the study the rats were adapted to lab condition.

Eighty male rats were randomly divided into neonatal (2-5 d, *n*=20), immature (4-6 wk, weighted 80-120 g, *n*=30), and mature (2-3 mo, weighted 180-240 g, *n*=30) groups. Neonatal rats were randomly divided into two subgroups of experimental and control.In addition, immature rats were randomly divided into three subgroups, and the same formula was applied to mature rat group of experimental, sham and control groups. Rats in the experimental group were fixed in a rodent restraint device and were exposed to RF radiations emitted from mobile jammer at a distance of 100 cm, for 30 d (7 h/day). Sham group rats (off condition) were also fixed in a rodent restraint device similar to our experimental group. The control group rats were allowed to move freely in the cages without being exposed to any radiation.

2.2. Mobile jammer

In this study, jammer (MB06-Mobile Blocker) was used to work in four different frequency range (global system for mobile communications (GSM), digital cellular service, code division multiple access, third-generation). It was capable of blocking mobile communications within the ranged up to 40 meters[1].

2.3. Testosterone hormone measurement

At the end of the experiment, all the subjects were anaesthetized by ether. Blood samples were collected through cardiac puncture, and stored in tubes without anticoagulants and were allowed to clot. After 15 min of centrifugation (2 000 r/min, room temperature) to obtain the serum, the serum was stored at -20 ℃ until analysis. Serum testosterone level was evaluated by radio immunoassay technique (DIA source Immuno Assays, S.A.). The kits were purchased from Isotops Ltd, Budapest, Hungary.

2.4. Epididymis sperm preparation and sperm quality evaluation

To collect and investigate mature rats semen samples, the epididymis tail was immediately separated and placed in a petri dish containing with 1 mL pre-warmed phosphate buffered solution. Then they were transferred to an incubator at 37 ℃ and gently swirled the petri dish for 10 min to facilitate the release of sperm. To evaluate spermatozoa motility, semen samples were assessed under light microscopy in randomly 10 selected fields with a 40 × magnification. The sperm motility was assessed based on WHO criteria into three categories, progressive motile, sluggish and immotile sperms. The number of both progressive motile and sluggish sperms was considered as motile. The mean sperm counts were determined via microscopic examination. The semen samples were diluted at 1:10 and then a drop was transferred to the Improved Neubauer hemocytometer chamber and covered with a cover glass. Then, sperms were counted in one of the large squares within the central counting area of the chamber. Furthermore, sperm viability was evaluated by using eosin-nigrosin staining. Fraction of each sperm suspensions were mixed with an equal volume of 0.5% eosin-nigrosin solution and smeared on a glass microscope slide. Finally, they were assessed with light microscopy in randomly 10 selected microscopic fields for the percentage of vital (unstained) and dead (stained) spermatozoa.

2.5. Morphometric analysis

A digital weighing scale (Acculab ALC210.4) was used to weigh the right testes of mature rats. To evaluate morphometric analysis, right testes of all the groups were separated and fixed in (10%) fresh bufferedformalin. Every testis was sampled for five vertical sections from the equatorial regions. Ethanol and xylene were used for dehydration step. After that, each sample was routinely embedded in paraffin wax, sectioned at thicknesses of 5 μm and stained with hematoxylin and eosin (H&E). Finally our indices were evaluated by a light microscope[10].

Spermatids were monitored and calculated in ten circular-transverse sections of testicular tubules. Total, lumen and cellular diameters (μm), lumen, cellular and cross sectional area ($\times 10^4$ μm^2), number of tubules (per 0.5 $mm^2 \times 0.5$ mm^2), and numerical density were determined in 10 circular transverse sections in different region of testis[8-10].

The mean seminiferous tubule diameter (D) was derived by taking the average of two diameters, D1 and D2 at right angles. Cross-sectional area (Ac) of the seminiferous tubules was determined using the equation $Ac= \pi (D/2)^2$, where π is equal to 3.14 and D, the mean diameter of seminiferous tubules. The number of profiles of seminiferous tubules per unit area (NA) (Figure 1) was determined using the unbiased counting frame proposed by Gundersen[11].

Numerical density (Nv) of seminiferous tubules was the number of profiles per unit volume and it used the modified Floderus equation: Nv=NA/(D+T), where NA is the number of profiles per unit area, D is the mean diameter of the seminiferous tubule and T, the mean thickness of the section (μm)[12]. The number of spermatids in 10 tubules per testis of allthe groups was calculated.

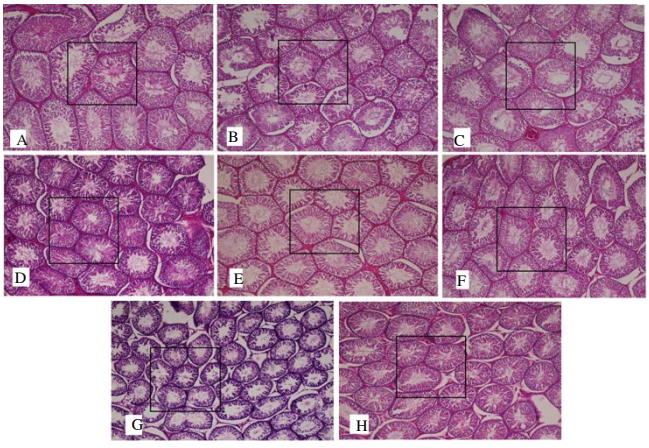

Figure 1. Profiles of seminiferous tubules in mature rats.
A: experimental, B: sham, and C: control groups. Immature rats D: experimental, E: sham, and F: control groups. Neonatal rats G: experimental and H: sham groups. H&E staining, × 40 magnification.

2.6. Statistical analysis

Data were expressed as mean±SEM for all parameters in the graphs (GraphPad Prism version 5.01 for Windows, Graph Pad software Inc., San Diego, CA, USA). Mann-Whitney rank sum test and *t*-test (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois)were used to detect the differences between the control and RF radiations exposed rats in the neonatal group. Differences between the control , sham and radiofrequency radiations exposed rats in mature and immature groups were determined by one-way analysis of variance (ANOVA), and Kruskal Wallis followed by Tukey HSD test. A P-value less than 0.05 were considered as significant difference.

3. Results

Statistical analyses showed that there were no significant differences in weight gain (g) after the exposure of the neonatal, immature and mature rats to jammer radiation (Figure 2A, B and C). Moreover, the mature rat testis weight (g) was evaluated to be less in the experimental group than in the sham and the control groups (*P*=0.008, *P*=0.001, respectively) (Figure 2D).

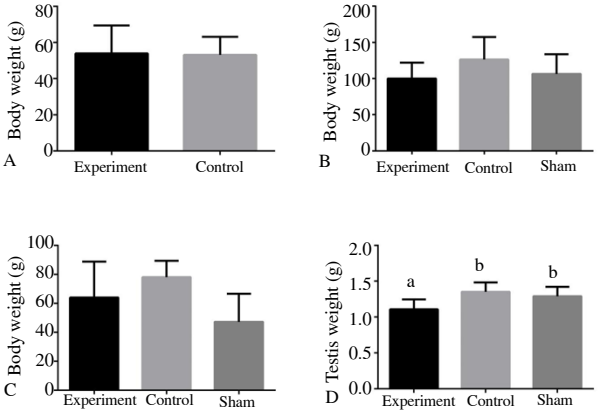

Figure 2. Mean±SE of weight gain (g) during the experiment period between groups.

A: neonatal, B: immature, and C: mature rats, D: testis weight (g) between mature rat subgroups.

Testes in all the groups showed tubules with thin basement membrane and tunica propria. Moreover, normal germinal epithelium showedan array of spermatogonia, spermatocytes, with groups of spermatids, and mature spermatozoa (Figure 3).

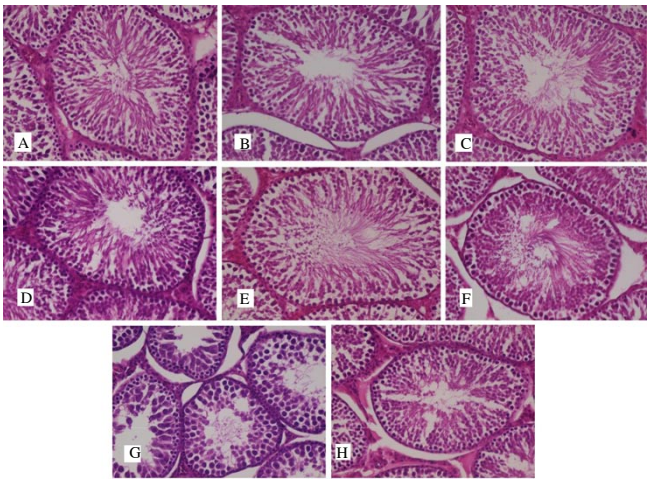

Figure 3. H&E staining of epididymis from seminiferous tubules in mature rats.

A: experimental, B: sham, and C: control groups. Immature rats D: experimental, E: sham, and F: control groups. Neonatal rats G: experimental and H: sham groups. × 200 magnification.

In neonatal rats, the luminal diameter (μm), germinal epithelium thickness (μm), total diameter (μm) of the seminiferous tubules showed a significant reduction compared with those in control animals (*P*=0.001). Besides, luminal area ($\times 10^4$ μm^2) and the germinal epithelium area ($\times 10^4$ μm^2) of the seminiferous tubules along with the total area of the tubules in cross sectional ($\times 10^4$ μm^2) also reduced when the neonatal rats exposed to the jammer radiation compared with those in control rat testis (*P*=0.001). Statistical analyses also revealed that after treated the neonate rats with jammer radiation, the number of spermatids were less compared with the control group (*P*=0.001) (Figure 4A, B and C) (Figure 5A, B and C) (Figure 6C). However, the number of seminiferous tubules per unit area (per 0.5 mm^2 × 0.5 mm^2) of the testis and numerical density in the experimental group of neonatal rats were more than in the control group (*P*=0.001) (Figure 6A and B).

In the experimental and sham groups of immature rats, lumen diameter (mm) and area ($\times 10^4$ mm^2) of the seminiferous tubules were less than the control group (*P*=0.001) (Figure 4D) (Figure 5D). Moreover, germinal epithelium thickness (μm) and area ($\times 10^4$ mm^2) of the seminiferous tubules in the experimental and sham groups showed a significant increase compared with those in the control group (*P*=0.001) (Figure 4E) (Figure 5E). In addition, numerical density (Figure 6E) and the number of the spermatids in the seminiferous tubules was the same (*P*>0.05) (Figure 6F).

In mature rats, none of the evaluated parameters showed significant difference between experimental and control testis. However, the experimental groups revealed a significant difference with sham groups in parameters such as the germinal epithelium thickness (μm) and area ($\times 10^4$ μm^2), the total diameter, numerical density and the number of the seminiferous tubules in cross sections (*P*=0.015, *P*=0.02, *P*=0.009, *P*=0.042, *P*=0.009, respectively) (Figure 4H, 5H,

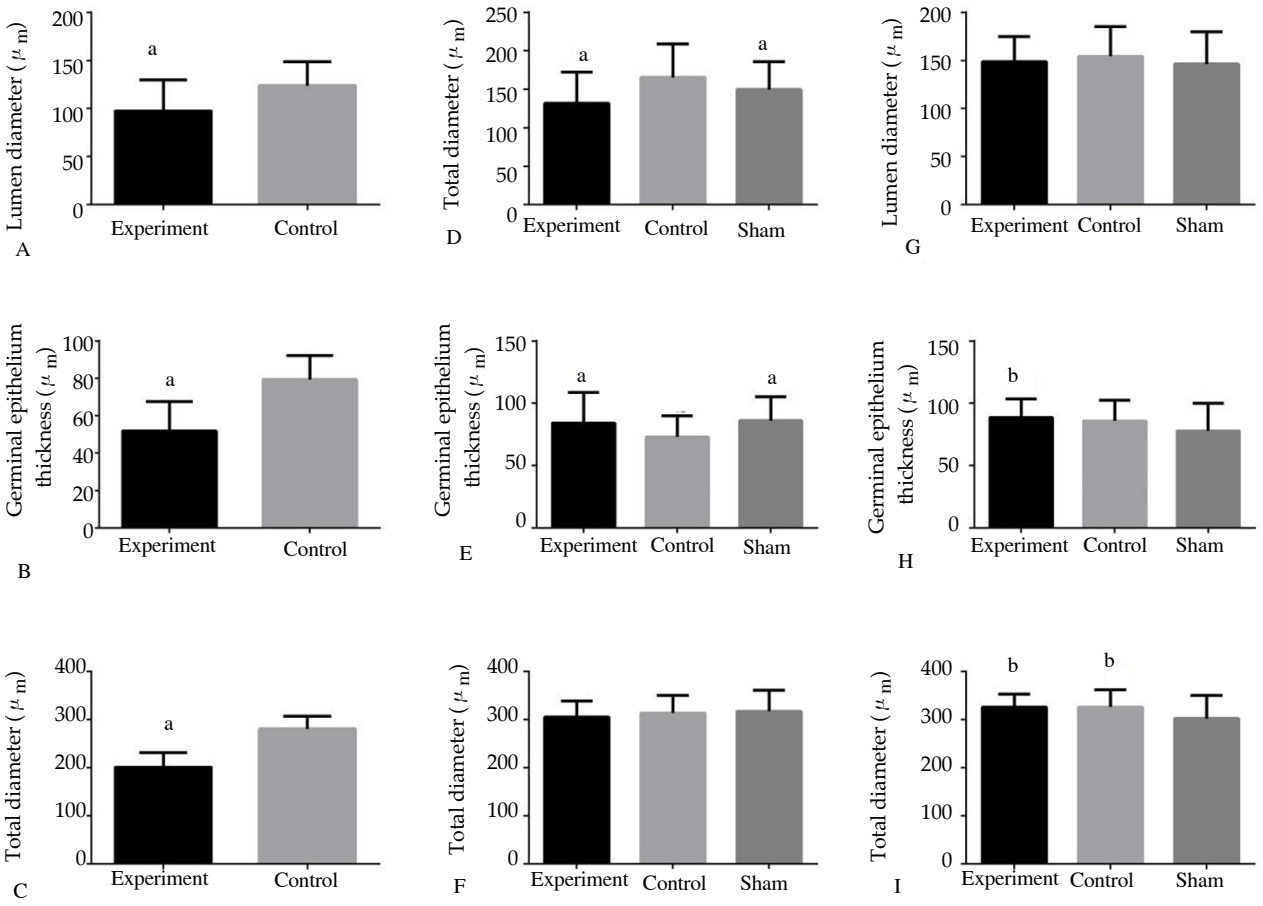

Figure 4. Mean±SE for the morphometric indices of seminiferous tubules.
Lumen diameter (μm) in A: neonatal, D: immature and G: mature rats. Cellular diameter (μm) in B: neonatal, E: immature, H: mature rats, and the total diameter (μm) in C: neonatal, F: immature, I :mature rats.a represents significant differences with control group (P<0.05); b represents significant differences with sham group (P<0.05).

4I and 5I, respectively). Moreover, germinal epithelium thickness and area and also the total diameter, the number of seminiferous tubules per unit area (per 0.5 mm^2 × 0.5 mm^2) and numerical density was more in the sham than the control group (P=0.001, P=0.003, P=0.001, P=0.001, P=0.004) (Figure 6G and H). However, there were no significant differences in the spermatids number of seminiferous tubules between subgroups (P<0.05) (Figure 6I).

Statistical analyses revealed that the most sperm parameters including count, motility and viability was similar in mature experimental, sham and control groups; however, there were some exceptions. The sperm motility showed a significant reduction in sham compared with control group (P=0.008). Also, it was revealed that the sperm viability decreased significantly in experimental group compared with sham (P=0.01) but not with control (Figure 7A and C).

The jammer radiation-treated neonatal rats showed a significant lower level of testosterone compared with control group (P=0.035) (Figure 8A). In immature rats, the experimental conditions had no impact on the level of testosterone (P=0.05) (Figure 8B). Moreover, in the mature rats, testosterone level of both experimental and control subgroups was more than of sham group (P=0.002 and P=0.001, respectively) (Figure 8C).

4. Discussion

In the current study, neonatal rats' long-term exposure to RF radiations emitted from a common mobile jammer decreased seminiferous tubules diameter, spermatids number of the seminiferous tubules,andtestosterone level, whilst, the number of seminiferous tubules per unit area (per 0.5 mm^2 × 0.5 mm^2) of testis increased.

In immature rats, long-term exposure to RF radiations emitted from acommon mobile jammer or stressed by being restrainedhad no significant effect on the diameter and spermatids number of the seminiferous tubules and testosterone level.

In mature rats, there were no significant differences in the diameter of seminiferous tubules, testosterone level and sperm quality in the exposed rats when compared with the control group. However, long-term restraint without being exposed to radiations decreased the diameter of the seminiferous tubules, percentage of motile sperm and testosterone level. Furthermore, the sperm viability and number of seminiferous tubules per unit area (per 0.5 mm^2 × 0.5 mm^2) of testis increased.

In vitro and *in vivo* studies, reported the effects of EMF exposure to

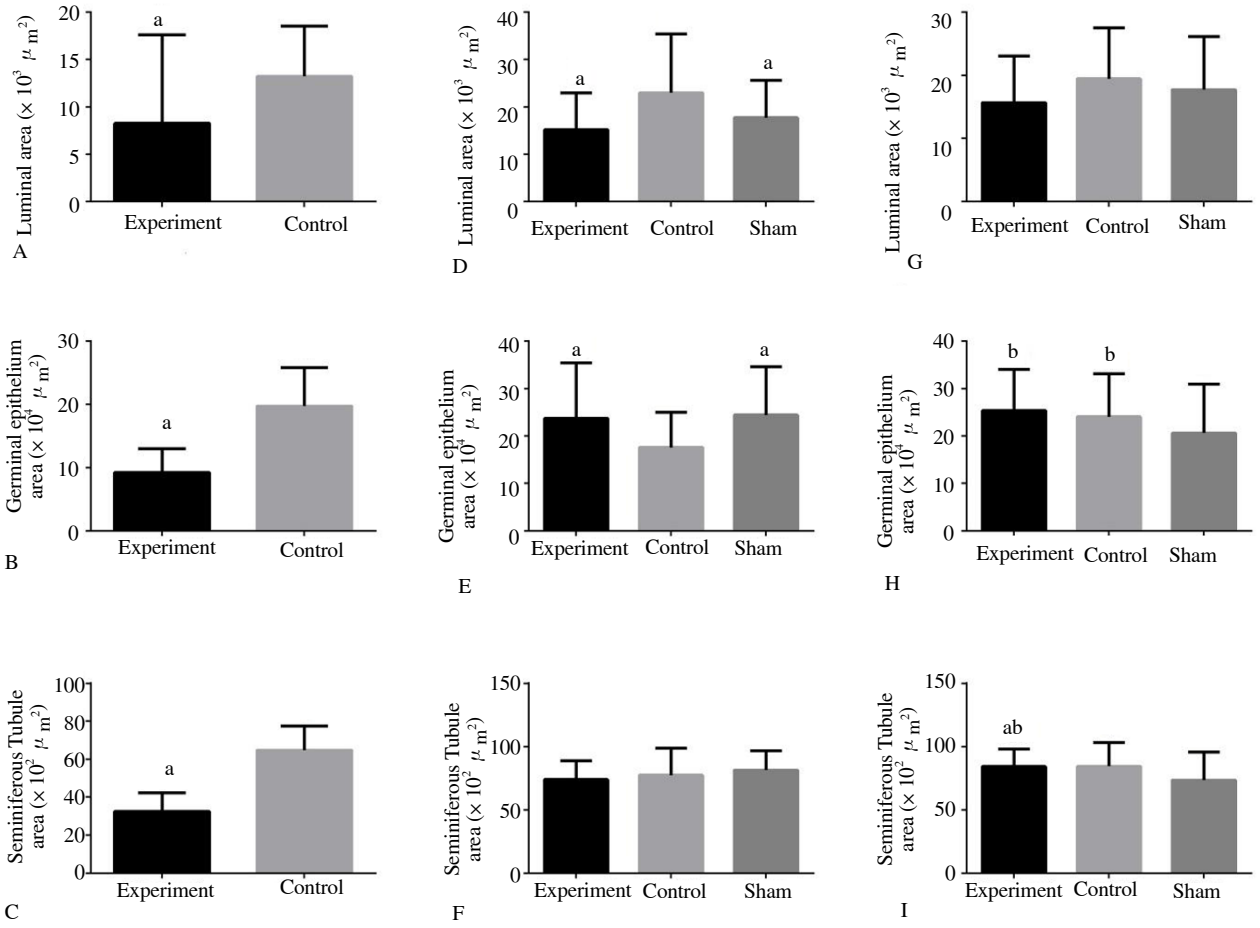

Figure 5. Mean±SE for some stereological indices of seminiferous tubules amongst rat groups.
Luminal area (μm²) in A: neonatal, D: immature, G: mature rats. Cellular area (μm²) in B: neonatal, E: immature, H: mature rats and cross sectional area of the tubule (μm²) in C: neonatal, F: immature, and I: mature rats. a represents significant differences with control group (P<0.05); b represents significant differences with sham group (P<0.05); ab showed significant differences with control and sham groups (P<0.05).

be various based on the frequency, exposure period, and the strength of EMF at cellular and organism levels and life stage. For instance, Salama *et al.*[13] showed that exposure to 800 or 900 MHz GSM RF radiation (8 h/d for 12 wk) in standby mode impeded a significant decrease in the diameter of seminiferous tubules in adult rabbits. Furthermore, Bahaodini *et al.*[7] reported that long-term exposure to low frequency EMF significantly decreased seminiferous tubules diameter and increased the number of seminiferous tubules per unit area of testes[10]. And to 10 Hz and 1 mT EMF showed a decrease in the tubular diameter, seminiferous tubules area, seminiferous epithelium height, total volume of seminiferous tubule, tubular lumen, seminiferous epithelium in Wistar rats[14]. Being exposed to 10 GHz microwave radiation for 2 h per day for 45 d revealed shrinkage in the seminiferous lumen[4]. Exposure to 900-MHz EMF between 13-21 d after pregnancy, showed a decreased diameter in seminiferous tubules and thickness of epithelium in the newborn rats[15]. In addition, Ozguner *et al.*[16] stated that seminiferous tubules diameter was significantly reduced in adult male rats exposed to EMF. In contrast, Al-Dameghstated that electromagnetic radiation caused a significant enhancement in the diameter of the seminiferous tubules with a disorganized seminiferous tubule sperm cycle interruption of rat[17]. Whereas, RF radiation emitted from cellular phones has no significant effect on testicular function or structure[18]. Additionally, exposure to a mobile phone radiation during pubertal development for 1 h a day for 45 d did not have harmful effects on testicular histology in rats[19]. Moreover, Trosic *et al.*[20] reported no significant effect of the applied RF radiation on testicular function or structure. Saygin *et al.*[21] indicated no differences in the diameter of the seminiferous tubules in the Wistar rats exposed to 2.45 GHz EMF, for 60 min/d for 28 d. Rats exposed to mobile phone radiation for 1 h/d for 28 d showed reduced percentage of motile sperm[10]. Sperm motility in rats exposed to 50 Hz EMF for 24 h/d for 85 d decreased. On the contrary, EMF did not affect the total sperm concentration and viability. Cell phone waves decreased sperm parameters in human semen samples[22]. Sperm count and motility in Wistar rats decreased as the magnetic field strength increased[7]. Sperm count in rats exposed to EMF in various manner by placing a mobile phone over the cage decreased[23].

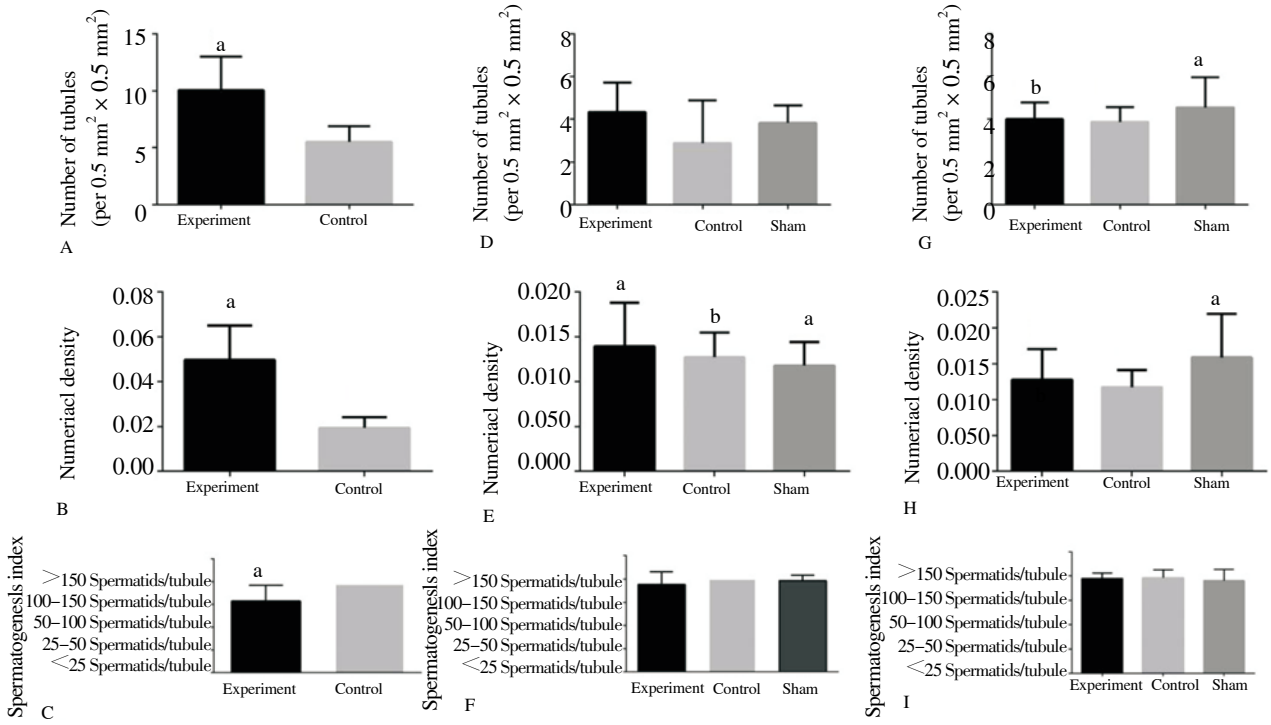

Figure 6. Mean±SE for some stereological indices of seminiferous tubules amongst rat groups.
Number of seminiferous tubules (per 0.5 mm^2 × 0.5 mm^2) of testis in A neonatal, D immature, G mature rats. Numerical density of the seminiferous tubules in B neonatal, E immature, H mature rats, and the spermatids number of seminiferous tubules in C neonatal, F immature, and I mature rats. a represents significant differences with control group (P<0.05); b represents significant differences with sham group (P<0.05).

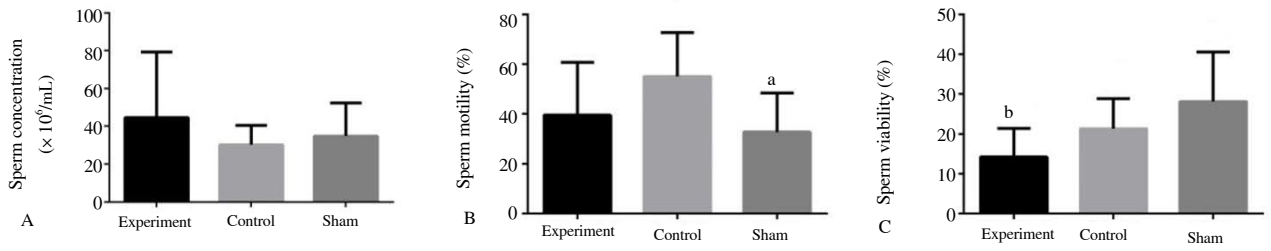

Figure 7. Mean±SE for sperm quality evaluation between subgroups in mature rats.
A sperm concentration (× 10^6/mL), B percentage of sperm motility, and C Percentage of sperm viability. a represents significant differences with control group (P<0.05); b represents significant differences with sham group (P<0.05).

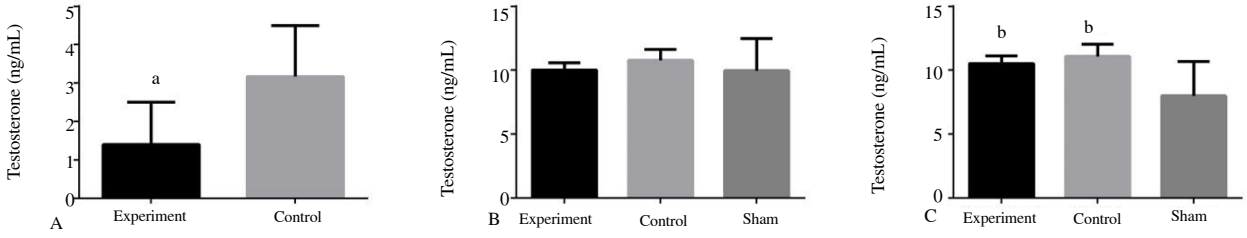

Figure 8. Comparison of Testosterone levels (ng/mL) between subgroups.
A: neonatal, B: immature, and C: mature rats. a represents significant differences with control group (P<0.05); b represents significant differences with sham group (P<0.05).

Furthermore, magnetic fields created by laptop computers may reduce sperm count and sperm motility in men. Also, sperm count and motility reduced as the magnetic field strength enhanced[1]. Cell phones usage reduced semen quality in men by lowering the sperm count, motility, viability, and normal morphology[5]. Sperm suspension was exposed to an internet-connected laptop by Wi-Fi for 4 h, showed a significant decrease in progressive sperm motility[9]. Odaci *et al.*[24] reported exposed pregnant rats to 900 MHz EMF for 1 h each day during day 13-21 of pregnancy had a higher apoptotic index, greater DNA oxidation levels and lower sperm motility and

vitality compared to the control group[24]. RF radiation exposure from cell phones adversely affects male fertilizing potential of spermatozoa[4]. Microwave exposure may have a significant effect on reproductive system of male rats, which may be a symptom of male infertility[4]. Exposed to an internet-connected laptop by Wi-Fi for 4 h decreased significantly sperm motility[9]. The prolonged use of cell phones may have negative effects on the human sperm motility and morphology[25]. Exposure to electromagnetic field through cell phones reduced in the human semen quality; including sperm motility and morphology but, does not affect the total sperm count[6]. The spermatozoa in both experimental animals and humans exposed to RF electromagnetic radiation for the longest time periods decreased motility, concentration, and viability[26]. In contrast to our results, exposure to EMF did not induce any adverse effects on the reproductive capacity including sperm quantity, quality, and morphology[20,27].

About 50 Hz sinusoidal magnetic field decreased testosterone levels of adult male rats significantly only after 6 and 12 wk of the exposure period[27]. Exposure to 900 MHz radiofrequency electromagnetic field decreased testosterone level of male Sprague–Dawley rat[28]. Long-term exposure to mobile phone radiation leads to reduce in serum testosterone levels. Exposed to 30 min per day, 5 d/wk for 4 wk to 900 MHz EMF causes significant decrease in serum total testosterone level[16]. Exposure to static magnetic field (128 mT; 1 h/d for 30 d) decreased rat testosterone levels[29]. Exposure to mobile phone radiation 60 min/d for 3 mo significantly decrease the serum testosterone level of Wistar rats[28]. Exposed to 10 GHz microwave radiation for 2 h/day for 45 d significantly decreases the testosterone level of seventy day-old rats[30]. In spite of, exposure to electromagnetic field 1 800 and 900 MHz for 2 h continuously per day for 90 d[31] and exposure to 1 800 MHz GSM-like[32] caused an enhance in testosterone level. However, exposure to circularly polarized with 50 Hz magnetic fields continuously for 6 wk in rats[33]; exposure to static magnetic fields 50 Hz for 40 min daily for 17 d[34]; and exposure to 50 Hz, 5 mT magnetic field for periods of 1, 2 and 4 wk[35] have no significant effects on testosterone level of male rats.

Some researches with similar protocols have evaluated the effects of RF radiation exposure on the male reproductive system in the rat, mice, rabbit and human. The results of such studies have shown a decrease in sperm quality (including motility, count, *etc.*) and testis morphometric parameters. Jammer exposure to rats also induced the same effects that were against or along with the aforementioned studies[1,2,26]. These data depend on the duration of using and device distance with the body. The strong documents explain RF radiation at the limitation of specific absorption rate (0.4-1.6&2.0W/Kg) have no thermal actions, but have non-thermal effects such as production of oxidative stress, shortage of antioxidant enzymes in cell membrane, changes in phosphorylation status and protein express level. Enhances the generation of ROS lead to reduction of motility and cell viability[36,37].

Findings of this study indicate that long-term exposure to RF radiations emitted from common mobile jammers could adversely affect neonatal rat fertility. However, it had no significant effect on male mature and immature rat reproduction parameters.Even though restraint stress induced by immobilizing the rats for long-period could adversely affect male mature rat's reproductive parameters, but it had no significant effect on male immature rat reproduction parameters.

Conflict of interest statement

The authors declare that thay have no conflict of interest.

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