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ORIGINAL ARTICLE

Effect of long-term exposure of 2.4 GHz radiofrequency radiation emitted from Wi-Fi equipment on testes functions

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Abstract

The aim of this study was to investigate long-term effects of radiofrequency radiation (RFR) emitted from a Wireless Fidelity (Wi-Fi) system on testes. The study was carried out on 16 Wistar Albino adult male rats by dividing them into two groups such as sham (*n*: 8) and exposure (*n*: 8). Rats in the exposure group were exposed to 2.4 GHz RFR radiation for 24 h/d during 12 months (1 year). The same procedure was applied to the rats in the sham control group except the Wi-Fi system was turned off. Immediately after the last exposure, rats were sacrificed and reproductive organs were removed. Motility (%), concentration ($\times 10^6$/mL), tail defects (%), head defects (%) and total morphologic defects (%) of sperms and weight of testes (g), left epididymis (g), prostate (g), seminal vesicles (g) were determined. Seminiferous tubules diameter (μm) and tunica albuginea thickness (μm) were also measured. However, the results were evaluated by using Johnsen's score. Head defects increased in the exposure group ($p < 0.05$) while weight of the epididymis and seminal vesicles, seminiferous tubules diameter and tunica albuginea thickness were decreased in the exposure group ($p < 0.01$, $p < 0.001$, $p < 0.0001$). However, other alterations of other parameters were not found significant ($p > 0.05$). In conclusion, we observed that long-term exposure of 2.4 GHz RF emitted from Wi-Fi (2420 μW/kg, 1 g average) affects some of the reproductive parameters of male rats. We suggest Wi-Fi users to avoid long-term exposure of RF emissions from Wi-Fi equipment.

Keywords

2.4 GHz Wi-Fi, electromagnetic fields, long-term exposure, radiofrequency, reproduction, testes

History

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Introduction

Widespread use of wireless technologies such as Wireless Fidelity (Wi-Fi) communication devices has rapidly increased over the past several years. Nowadays, it is very easy to reach access points or Wireless Local Area Networks (WLAN) in houses, workplaces, public areas, schools, etc. Rapid development of wireless technologies has increased the environmental electromagnetic field (EMF) levels while providing big comfort in our daily life. Even relatively new EMF sources like wireless headphones, wireless printer and hard discs, etc. took place in the workplaces and houses. Therefore, public and scientific awareness that was previously focused on the adverse health effects of EMF emitted from mobile phones has been steadily focusing on biological hazards of wireless equipment such as Wi-Fi. Because the health hazards of these equipment are still unclear, The Council of Europe is recommending restrictions to be put in place on the use of mobile phones and access to the internet in all schools across the continent to protect young children from potentially harmful radiation (Watson, 2011).

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In Bioinitiative report (2012), which is published recent years, the authors stated that radiofrequency radiation (RFR) exposures can alter and damage genes, trigger epigenetic changes to gene expression and cause *de novo* mutations that prevent genetic recovery and healing mechanisms. They explained that these exposures may interfere with normal cardiac and brain function; alter circadian rhythms that regulate sleep, healing, and hormone balance; impair short-term memory, concentration, learning and behavior; provoke aberrant immune, allergic and inflammatory responses in tissues; alter brain metabolism; increase risks for reproductive failure (damage sperm and increase miscarriage risk); and cause cells to produce stress proteins (Bioinitiative report, 2012). Brain was believed to be the most affected organ simply because head is exposed to radiofrequencies (RF) due to mobile phone usage. Therefore, at the beginning, studies mainly focused on the relation between RF emitted from mobile phones and its adverse health effects especially brain tumors (Dasdag et al., 2004, 2009, 2012; Hardel et al., 1999). Afterwards, studies on mobile phone exposure focused on other organs especially on reproductive organs (Çelik et al., 2012; Nisbet et al., 2012). The results of these studies in this field are still contradictive (Agarwal et al., 2011; Akdag et al., 1999; Dasdag et al., 1999a, 2003, 2008; Lee et al., 2012).

Parallel to mobile phone studies, recently studies have been undertaken to focus on health hazards of other wireless technologies such as Wi-Fi, which also emits RF (Avendano et al., 2012; Atasoy et al., 2013; Saygin et al., 2011). It is obvious that RF emitted from Wi-Fi equipment affect entire body. Therefore, it is difficult to estimate sensitive organs during Wi-Fi exposure because of the whole body exposure. Thus, large scale and long duration studies are necessary to answer this question. As it is evident from other radiation types, one of the radiosensitive organs is the reproductive organs. Thus, studies should focus on the effects of RF emitted from Wi-Fi equipment on reproduction as well.

Dasdag et al. (1999b) reported that certain type of occupational RF exposure increases hormones such as testosterone and progesterone. Aissa et al. (2012) exposed pregnant rats to 2.45 GHz RF emitted from Wi-Fi generator at whole-body specific absorption rates (SAR) of 0, 0.08, 0.4, and 4 W/kg for 2 h/d and 5 d/week from days 6 to 21 of gestation. After the birth, they exposed newborns to the same RF exposure from birth to day 35 postnatal. They found no change in humoral response of young pups and suggested a lack of adverse effects of Wi-Fi exposure on delivery and general condition of the animals. Avendano et al. (2012) exposed sperms *in vitro* to RF emitted from internet-connected laptop (Wi-Fi) for 4 h. They reported that *ex-vivo* exposure of human spermatozoa to a wireless internet-connected laptop decreased motility and induced DNA fragmentation by non-thermal effects. They speculated that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility. Atasoy et al. (2013) investigated the effects on rat testes of RF emitted from indoor Wi-Fi Internet access devices. In their study, the experimental group was exposed to RF for 24 h/d for 20 weeks. They observed significant increases in serum 8-hydroxy-2′ deoxyguanosine levels and 8-hydroxyguanosine staining in the testes of the experimental group indicating DNA damage due to exposure ($p < 0.05$). They suggested that these findings raise questions about the safety of radiofrequency exposure from Wi-Fi Internet access devices for growing organisms of reproductive age, with a potential effect on both fertility and the integrity of germ cells. De Gannes et al. (2012) assessed the effects of *in-utero* exposure to 2.45 GHz Wi-Fi signal (2 h/d, 6 d/week for 18 d) on pregnant rats and their pups. They showed no teratogenic effect of repeated exposures to the Wi-Fi wireless communication signal even at 4 W/kg, which is the highest level in their study. Saygin et al. (2011) investigated the effects of 2.45 GHz RF on rat testes. They showed that 2.4 GHz RF reduced number of Leydig cells of testis tissue. They consequently stated that 2.4 GHz RF affects spermatogenesis and causes apoptosis. Based on these studies, the subject is still perceived as controversial. Therefore, the purpose of this study is to investigate the effects of 2.4 GHz Wi-Fi exposure on rat testes.

Materials and methods

Subjects and animal care

Sixteen Wistar Albino adult male rats with initial average weight of 313 ± 25 g were acquired from the Medical Science Application and Research Center of Dicle University. The rats fed with standard pelleted food (TAVAS Inc., Adana, Turkey) in a standard Plexiglas cage. Final average weight of the animals was 348 ± 28.8 g. They were separated equally into two groups such as sham exposed ($n = 8$), and exposure ($n = 8$), and kept on a 14/10 h light/dark schedule. During the study, the ambient temperature (22 °C) and the relative humidity (45%) were maintained in the normal range for these animals. All animal procedures were in agreement with the Principles of Laboratory Animal Care and the rules of Scientific and Ethics Committee of Dicle University Health Research Center.

Exposure and field measurements

A generator, which emits 2.4 GHz RF radiation, was used to represent exposure of Wi-Fi systems. Rats in the sham and exposure groups were confined in a Plexiglas cage ($55 \times 32 \times 20$ cm). Rats were kept freely in the cage and no movement restriction was applied to them during the study. The rats in the sham and exposure groups were survived in the cage under normal daily circumstances. Rats in the exposed group were exposed to 2.4 GHz RF radiation 24 h/d during 12 months. The rats in both groups were kept 50 cm far from the antenna of the generators. Same experimental circumstances were applied to the rats in the sham group, except the generator was turned off. Power density and electrical field inside plexiglas cage were measured by field probe EMR 300 (NARDA, Pfullingen, Germany). The study was performed in Faraday cages to eliminate outdoor electromagnetic fields during 12 months.

Specific absorption rate (SAR) measurement

In our experimental setup, the electromagnetic field values were measured with an Electric-field probe while the transmitter was on, then, these measured values were used in an electromagnetic field solver to find the electric field distribution in the cage and inside the rat. We used CST Microwave Studio, an electromagnetic field solver based on finite integration technique (FIT) for numerical simulations. FIT is in general very similar to finite-difference time domain (FDTD) technique, but it performs discretization on non-orthogonal grids using integral form of Maxwell's equations. Charge and energy conservation inherit to Maxwell's equations are all preserved within FIT which leads to stable numerical results in time-domain. Voxel (volumetric pixel) Rat model which was formed using computerized tomography scans of a rat was obtained from CST and this model was used in the field simulations. Simulated field values were compared to those of measured electric field values which were obtained with the field probe, and when close corroboration was achieved, simulations were rerun for SAR calculations. Wi-Fi equipment emits 50 mW rms RF signal. At the far field of the Wi-Fi antenna, the electromagnetic wave can be regarded as a plane wave. SAR calculations were based on plane wave incidence originating from Wi-Fi antenna which was modeled with equipment's transmit power.

Evaluation of sperm parameters

At the end of the 12 months, the rats were intraperitoneally administered a combination of 6 mg/kg of 2% xylazine

hydrochloride (Rompun) and 75 mg/kg ketamine hydrochloride (Ketalar) for anesthesia. Afterward, the testes of each rat were located and the testes, epididymis, seminal vesicles, and ventral prostate were removed, cleared of adhering connective tissue. The testis, epididymis, seminal vesicles, and ventral prostate weight were evaluated along with epididymal sperm concentration, sperm motility, and sperm morphology. One of the testes was fixed in 10% Bouin fixative for histopathologic examinations.

Epididymal sperm count

The epididymis was finely minced with anatomical scissors in 10 mL of physiologic saline, placed in a rocker for 10 min, and allowed to sit at room temperature for 2 min. After incubation, supernatant fluid was diluted 1:10 with a solution containing 5 g sodium bicarbonate, 1 mL formalin (35%), and 25 mg eosin per 100 mL of water. Total sperm number was determined using counting chambers. The cells were counted with the help of a light microscope (magnification, 200×).

Epididymal sperm motility evaluation

The fluid obtained from the cauda epididymis with a pipette was diluted to 2 mL with Tris-buffer solution. A slide was placed on phase-contrast microscope, and an aliquot of this solution was placed on the slide and percent motility was evaluated visually at a magnification of 400 times. Motility estimations were performed from three different fields in each sample. The mean of the three estimations was used as the final motility score. Samples for motility evaluation were kept at 37 °C.

Epididymal sperm morphology evaluation

To determine the percentage of morphologically abnormal spermatozoa in the cauda epididymis, the slides stained with eosin–nigrosin (1.67% eosin, 10% nigrosin and 0.1 M sodium citrate) were prepared. The slides were then viewed under a light microscope at 400× magnification. Two-hundred spermatozoa were examined on each slide, and the head and tail and total abnormality rates of spermatozoa were expressed as percent.

Histological analysis

The right testis from the rats were placed in 10% Bouin solution for 24 h for fixation and further pathologic examination. After fixation, the sections were subjected to boutine histologic tissue preparation and dehydrated and embedded in paraffin. Paraffin blocks were sliced to 5-m thickness with a microtome and the slices were subjected to routine hematoxylin and eosin (H&E) staining and were then examined under a light microscope (Nikon ECLIPSE 80i, Nikon, Tokyo, Japan) by a histologist blinded to the groups. For each sample, 100 randomly selected seminiferous tubule diameters were measured. In addition, for each section, 100 randomly selected seminiferous tubules were evaluated using the Johnsen classification (Johnsen, 1970).

Statistical analysis

Mean values and standard deviations were calculated, and statistical significance of the differences between exposed samples and controls was evaluated. A computer program (SPSS 11.5, SPSS Inc., Chicago, IL) was used for statistical analysis. Data were analyzed by Mann–Whitney *U*-tests. In all hypothesis tests, a criterion level of $p < 0.05$ was used.

Table 1. Spermatological, histological evaluation and organ weights of both group.

	Groups		
Traits	Control	Wi-Fi	*p*
Motility (%)	63.0 ± 3.31	71.8 ± 3.61	>0.05
Concentration ($\times 10^6$/mL)	33.8 ± 1.04	31.7 ± 2.35	>0.05
Tail defects (%)	9.9 ± 0.31	12.0 ± 1.07	>0.05
Head defects (%)	2.3 ± 0.37	6.5 ± 1.69	<0.05
Total morphologic defects (%)	12.2 ± 0.28	18.5 ± 2.59	>0.05
Testes (g)	1.46 ± 0.037	1.35 ± 0.028	>0.05
Epididymis (g)	0.58 ± 0.021	0.48 ± 0.021	<0.01
Prostate (g)	0.73 ± 0.036	0.59 ± 0.096	>0.05
Vesiculaseminalis (g)	1.37 ± 0.084	0.83 ± 0.050	<0.001
Seminiferous tubules diameter (μm)	394.7 ± 5.55	376.0 ± 4.71	<0.01
Tunica albuginea thickness (μm)	21.0 ± 0.39	18.8 ± 0.24	<0.0001
Johnsen's Biopsy score	9.64 ± 0.052	9.63 ± 0.058	>0.05

Results

Cauda epididymal sperm characteristics

The effects of 2.4 GHz RF on epididymal sperm concentration, sperm motility and abnormal sperm rate are presented in Table 1. The percentage of head defects in the exposed rats was found higher than the unexposed rats ($p < 0.05$) while any statistically significant difference was not observed in the sperm concentration ($\times 10^6$/mL), the percentage of epididymal sperm motility, tail and total morphological defects among the groups ($p > 0.05$).

Prostat, seminal vesicle, testis and epidydimis weights

The effects of 2.4 GHz RF on organ weights are presented in Table 1. Weights of epidydimis (g) and seminal vesicles (g) were found lower in the exposed rats ($p < 0.01$, $p < 0.001$) while any difference was not observed in weight of testes and prostate in both group.

Histological evaluation of the testes

Results of histological analysis showed that 2.4 GHz RF alter general morphology of rat testes (Figure 1, Table 1). For instance, seminiferous tubules diameter (μm) and tunica albuginea thickness (μm) were decreased in the testes of exposed rats ($p < 0.01$, $p < 0.0001$).

Specific absorption rate (SAR)

Point, 1 g and 10 g average SAR level of testes and prostate were found as 4880 μW/kg, 2420 μW/kg and 1020 μW/kg, respectively (Figure 2).

Discussion

Use of wireless internet has been growing rapidly due to abundant existence of internet providers in the form of WLAN and Wi-Fi communication services. Nowadays, access points

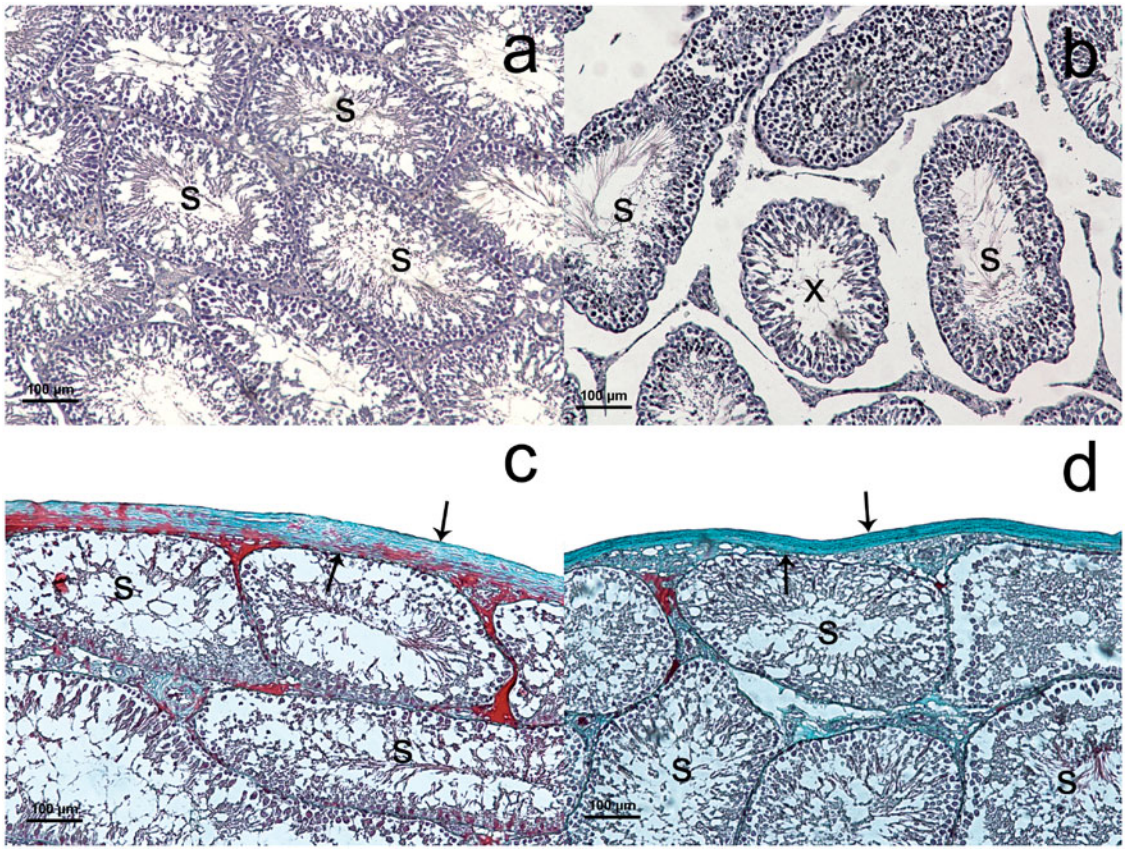

Figure 1. Testes sections of control group (a, c) and exposed group (b, d). Seminiferous tubules diameter (S) and Tunica albuginea thickness (arrows) decreased in exposed group (a, b). Any difference was not observed in both groups in terms of Johnsen Score. However, a tubule with disorganized view due to loss of germinal epithelium (b) seen in the figure (arrowhead) H&E (a, b), Masson Trichrome (c, d).

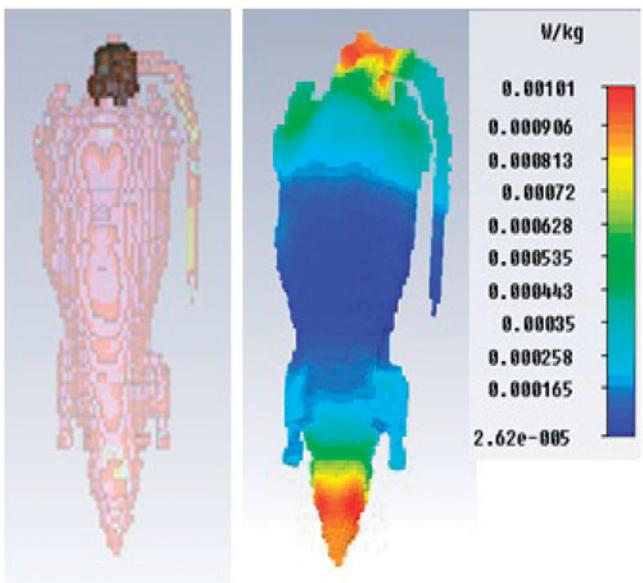

Figure 2. Rat simulation model and SAR distribution (10 g avg).

can be found in workplaces, public places, houses, and schools. These relatively new sources of RF radiation are raising public concern regarding the health effects of wireless internet providers. Presumably, most popular users of these systems are children and young population. However, evidence is not available on whether these systems are harmful or not. Therefore, people must be careful around these equipment until sufficient data are available.

Most of the studies on the effects of RF focused on mobile phone exposure. However, studies on health concerns of Wi-Fi are scarce. This study aims to bridge this gap and it is the first to investigate the long-term effects of Wi-Fi exposure on rat testes functions (24 h/d, 7 d/week for 1 year). Therefore, there are no data in open literature to compare the long-term effects of RF to the results of this study. Nevertheless, two studies have been performed on the effects of Wi-Fi exposure and testes functions (Avendano et al., 2012; Saygin et al., 2011). Avendano et al. (2012) performed an *in vitro* study and evaluated the effects of laptop computers connected to local area networks wirelessly on human spermatozoa. They collected semen samples from 29 healthy donors by swim up. They exposed *ex vivo* one sperm aliquot from each patient to a Wi-Fi internet-connected laptop for 4 h. They found a significant decrease in progressive sperm motility and an increase in sperm DNA fragmentation. They speculated that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility. However, we did not found a decrease in sperm motility in our study. Therefore, the results of Avendano et al. (2012) on sperm motility do not support our results. Conversely, sperm motility increased in rat exposed to 2.4 GHz RF emitted from Wi-Fi in our study. But the increase was not found statistically significant ($p > 0.05$). Although there is a contradiction between these studies on motility, we also found that head defects increased in exposure group ($p < 0.05$) while weight of epididymis and seminal vesicles, seminiferous tubules diameter and tunica albuginea thickness were decreased in

exposure group ($p<0.01$, $p<0.001$, $p<0.0001$). Therefore, we can state that long-term exposure of 2.4 GHz RF emitted from Wi-Fi affects some of reproductive parameters of male rats. It is clear that further, more conclusive studies are necessary with ongoing research to bring clarity to this public health issue.

In another study, Saygin et al. (2011) investigated the effects of 2.45 GHz RF on apoptosis and histopathological changes in rat testes. The study was on the short-term effects of RF, which was 1 h/d for 28 d. However, SAR level measured in their study (3.21 W/kg) was much higher than the SAR levels in our study and the study of Khalid et al. (2011). Therefore, there is a big contradiction between the studies mentioned here in terms of SAR. Saygin et al. (2011) stated that 2.45 GHz RF did not affect seminiferous tubule diameter although RF exposure reduced the number of Leydig cells of testes. The difference between the results of our study and Saygin et al. (2011) originates from the design of the study. As it is stated before, we aim to investigate extraordinary long-term effects of 2.4 GHz RF instead of short-term effects, which was performed by Saygin et al. (2011).

It is not known exactly molecular mechanisms of non-ionizing electromagnetic radiation on sperm and testes tissue. However, Ayrapetyan et al. suggested that the hydration of water ionization products (H_3O^+ and OH^-) reduces their chemical activity, which may be a factor in their increased reactivity when subjected to electromagnetic effects that are disruptive to hydrogen bonds (Ayrapetyan et al., 2009). The total or partial dipolar vibration-induced removal of the hydration shell of these ions could reactivate them and result in interaction with soluble gas components of water, causing the formation of free radicals (Ayrapetyan et al., 2009). Some studies have shown that the metabolic control of cell volume (membrane surface) is extremely sensitive to weak chemical and physical signals, including nonionizing radiation-induced structural changes in the cell's bathing medium (Ayrapetyan, et al., 1984; Deghoyan et al., 2012; Haussinger, 1996; Hoffmann et al., 2009). It has been suggested that some non-conductive membrane mechanisms are responsible from cell volume regulation such as Na+/ K+ pump, Na+/Ca++, Na+/H+ exchangers, changes of cytoskeleton contractility and membrane fluidity and others (Ayrapetyan et al., 2013; Hoffmann et al., 2009). Therefore, it can be suggested that some histomorphometric changes such as head defects on cauda epididymal sperm cells, the decreasing in weights of epidydimis and seminal vesicles and the decreasing of seminiferous tubules diameter and tunica albuginea thickness after long-term WiFi exposure, which determined in the present study, may be due to changes of cell volume regulation mechanisms such as Na+/K+ pump, Na+/Ca++, Na+/H+ exchangers, changes of cytoskeleton contractility and membrane fluidity. In order to explore underlying molecular mechanisms in terms of reproductive effects of long-term WiFi exposure, it should be carried out molecular study.

Because the studies on bio-effects of 2.4 GHz Wi-Fi exposure are very limited, we do not have the privilege to discuss our results with other studies. 24 h/d for 1 year is a very important exposure duration to have enough information about the effects of 2.4 GHz RF on testicular functions.

Conclusion

The results of our study revealed that 2.4 GHz RF emitted from Wi-Fi equipment affects testicular function and histology. Therefore, we suggest Wi-Fi users to avoid long-term exposure of RF emitted from Wi-Fi equipment. Especially, more attention should be paid to children and adolescents that are known to be frequent Wi-Fi users.

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Declaration of interest

The authors report no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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