

Low-power Density Radiations Emitted from Common Wi-Fi Routers Influence Sperm Concentration and Sperm Histomorphometric Parameters: A New Horizon on Male Infertility Treatment

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ABSTRACT

Background: Male infertility is defined as an inability to impregnate a fertile female; it is a widespread problem which is usually caused by some male factors such as low quantity and quality of sperm, specifically oligospermia and azoospermia.

Objective: This study aimed to evaluate the bio-positive effects of low power density Wi-Fi radiation on the reproductive system of infertile and healthy mice.

Materials and Methods: In this experimental study, thirty adult male Balb/c mice were randomly divided into 5 groups. Groups oligospermic-sham (OS), oligospermic-exposure 1 (OE1) and oligospermic-exposure 2 (OE2) received Busulfan, 10 mg/kg, intraperitoneally, but the control-sham (CS) and control-exposure (CE) groups left without Busulfan therapy. Groups CE, OE1 and OE2 were exposed to 2.4 GHz Wi-Fi radiation while, the CS and OS were sham exposed to Wi-Fi radiation without energizing the Wi-Fi router. The right and left testes and right epididymis were dissected out and histopathological, histomorphologic changes and the quality of the sperms were analyzed.

Results: Low power density Wi-Fi radiation significantly increased sperm concentration in the CE group compared to that in CS, while enhancement of spermatid cells was not significant. Sperm concentration in OE2 was more than that in OE1 as the spermatid cells enhanced.

Conclusion: Findings revealed that radiation hormesis induced by low power density Wi-Fi radiation have biological beneficial effects on mouse sperm concentration and sperm histomorphometric parameters.

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Keywords

Electromagnetic Field; Microwave; Wi-Fi Router; Busulfan; Oligospermia

Introduction

Living in the modern era has led to widespread human exposure to various types of electromagnetic fields (EMFs). Tremendous use of wireless communications and information technology in various sectors such as industry, commerce, agriculture, medicine, radar and communication systems has transformed the electromagnetic waves as an undeniable part of the biosphere [1]. Due to the enhance-

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ment of human exposure to Wi-Fi radiation, global concerns regarding the detrimental effects of microwaves are increasing [2]. In the last decade, numerous investigators evaluated the bio-effects of microwaves at different levels to shed light on the precise mechanisms of microwave radiation interacting with the living organisms [3]. In this regard, several researchers reported that EMF radiation induced serious health outcomes in people who inhabit around Wi-Fi and cell phone sources [4-6]. Some studies have revealed that EMF radiation emitted from cell phone, Wi-Fi router and other sources induce infertility in male reproductive system by decreasing the quality of semen [7-9]. *Mortazavi et al.* [10] for the first time, demonstrated that laptop computers can affect male reproductivity which means reduction in the sperm count and motility. *Avendano et al.* [7] showed that radiation emitted from Wi-Fi internet-connected laptop significantly decreased the motility of the human sperm, and sperm DNA fragmentation enhanced. Therefore, recent studies have demonstrated the significant role of Wi-Fi radiation on morphology, viability and motility of sperm and caused growing concerns about increasing infertility [11]. On the other hand, other researchers have claimed that low dose EMF radiation, with low frequency or low power density, not only eliminates the detrimental effects but also induces beneficial outcomes [12]. Power density can be used to characterize an RF field and has been defined as power per unit area using the following formula:

$$S = \frac{PG}{4\pi r^2}$$

Where, S is the power flux density in W/m², P is the maximum power output in watts, G is the gain from the directional antenna in dB, 4πr² is the surface area of a sphere and r is the direct distance between the antenna and the exposure point in meters. We hypothesize that short-term exposure to microwave radia-

tion with low power density can induce bio-positive effects on the reproductive systems and stimulate the infertility treatment. Infertility is a widespread problem which affects 15-20% of couples during their reproductive ages [13]. That is, they will be unable to conceive a child which is usually caused by some male factors such as low quantity and quality of sperm, specifically oligospermia (less than 20 million spermatozoa/mL) [14] and azoospermia (complete lack of spermatozoa in semen). Various factors such as exposure to heat, lifestyle, smoking, injuries, diseases and use of some drugs like Cisplatin and Busulfan results in oligospermia [15]. Busulfan which has two common brands, including Myleran (in tablet form) and Busilvex (injection form), is an alkylating agent that can decline spermatogenesis leading to infertility through two functional groups that inhibit cell division by binding to nucleic acid strands [16] which causes delay in meiosis. This drug is used for producing oligospermia model in rats and hamsters [17]. There are few reports on Wi-Fi radiation effects and its relation to infertility [18-19]. In the present study, Busulfan was applied to produce oligospermic model in mice and the bio-effects of short term exposure of 2.4 GHz microwave radiation emitted from Wi-Fi router on the quality of sperm, and histomorphometric changes on both infertile and healthy mice were evaluated.

Material and Methods

Animals

In this experimental study, thirty adult (7-8 weeks) male Balb/c mice, weighing 30-35 g, were kept under a 12 h-12 h light/dark cycle (light 7.00 a.m. to 7.00 p.m.) at a constant temperature (22±1°C). The mice were kept in standard Plexiglas cages with free access to water and food *ad libitum*. The body weight of the mice was measured weekly. All experimental procedures were performed based on the approval of the Ethics Committee of Ani-

mal Care, using the guidelines of Shiraz University of Medical Sciences, Shiraz, Iran.

Animal Treatment

Mice were randomly divided into 5 equal groups (n=6, Table 1). Those in the OE1 and OE2 groups served as the oligospermic exposure 1 and oligospermic exposure 2, respectively. They received Busulfan (10 mg/kg, intraperitoneally, Busilvex, Pierre Fabre Medicament, Boulogne, France); hence, they were exposed to 2.4 GHz microwave radiation. The mice in the OS group served as the oligospermic sham; they received Busulfan but were sham exposed to Wi-Fi router without energizing. The mice in CE group served as the control exposure. They did not undergo Busulfan therapy but they were exposed to 2.4 GHz microwave radiation. The mice in the CS group served as the control sham; they did not undergo Busulfan therapy and sham exposed to Wi-Fi radiation without energizing the Wi-Fi router. Those in all, except OE2 group, were placed at a distance of 100 cm from the router and in the OE2 group, they were placed at 150 cm from the router. Since the spermatogenesis cycle in the mice is 35 days [20], the mice in the CE, OE1 and OE2 groups were exposed to Wi-Fi radiation 35 days after Busulfan therapy, according to the exposure protocol (2 h/day for 4 consecutive days). In this phase of the study, a laptop which was placed in the adjacent room exchanged data via Wi-Fi router. However, for the mice in the CS and OS groups, the laptop did not exchange data

via Wi-Fi router (sham exposure).

Wi-Fi Router

A Wi-Fi router (D-link, Wireless N 150 Home Router, D-link Corporation, Taipei, Taiwan) was used in this study to generate 2.4 GHz Wi-Fi radiation. The electromagnetic quantities were measured using spectrum analyzer (Tektronix, 2754p, Oregon, United States). The results obtained were as follows: the power density was 3125 $\mu\text{W}/\text{m}^2$ and 1401 $\mu\text{W}/\text{m}^2$, electric field $E=1.06$ V/m and 0.613 V/m, magnetic field 2.8 mA/m and 1.6 mA/m at 100 cm and 150 cm, respectively. Whole body average SAR, specific absorption rate, for the mice weighing 30-35 g were determined to be 30mW/kg and 92mW/kg, respectively.

Sperm Evaluation

The mice in all groups were sacrificed 24 h after the last exposure, and semen samples were analyzed. The volume of the right testes was measured using a digital scale (Radwag, WPS 1100/C/10, Radom, Poland). The testes were suspended in a container filled with water to record the testis volume. Their right epididymis was separated, chopped and placed in isotonic solution (5 ml phosphate buffered saline) for 20 min at 37 °C. Then, sperm concentration, motility and viability were investigated [21]. Semen sample slides were evaluated with light microscopy to investigate the percentage of the sperm motility. In order to estimate the sperm count, semen samples were

Table 1: Experimental groups for evaluation of the effects of short-term exposure to microwave radiation emitted from Wi-Fi router on oligospermic mice.

Groups	Radiation	Oligospermia	Distance
Control sham (CS)	-	-	100 cm
Control exposure (CE)	+	-	100 cm
Oligospermic sham (OS)	-	+	100 cm
Oligospermic exposure 1 (OE1)	+	+	100 cm
Oligospermic exposure 2 (OE2)	+	+	150 cm

transferred in Neubauer slides; then, the number of sperms was calculated via microscopic examination, hence; eosin-nigrosin staining was used and viability of sperms was analyzed by light microscope.

Histomorphometric Evaluation

Left testes of the mice were dissected and fixed in 10% formalin buffered (contains 4% (w/v) formaldehyde and 0.075 M sodium phosphate buffer). To assess histological changes of the mice testes seminiferous tubules, each testis was sampled for ten vertical sections from the equatorial regions. Once ethanol and xylene were used for dehydration step, each sample was placed in paraffin. They were sectioned at thicknesses of 5 μ m and were stained with hematoxylin and eosin. Eventually, our indices were evaluated by light microscopy (Nikon, E-200, Japan). Spermatids were observed and assessed in five circular-transverse sections of testicular tubules. Lumen, cellular and total diameters (μ m), lumen, cellular and cross-sectional area ($\times 10^3 \mu\text{m}^2$), number of tubules (per 5 \times 5 mm²) and numerical density were calculated in 10 circular transverse sections of different regions of the testis [22]. The average of two diameters, D1 and D2 at the right angles were measured, and the mean seminiferous tubule diameter (D) was evaluated. Cross-sectional area (A_c) of the seminiferous tubules was analyzed by the equation $A_c = \pi (D/2)^2$ where π is equivalent to 3.14 and D, is the mean diameter of seminiferous tubules. The number of profiles of seminiferous tubules per unit area (NA) was determined with the unbiased counting frame [20]. Numerical density (Nv) of the seminiferous tubules and the number of profiles per unit volume were calculated using the modified Floderus equation: $Nv = NA / (D+T)$ [20] where NA is the number of profiles per unit area, D is the mean diameter of the seminiferous tubule, and T is the average thickness of the section (μ m). Ten tubules per testis of mice were evaluated and the number of spermatids

was estimated.

Statistical Analysis

To evaluate the normal distribution of data, Kolmogorov-Smirnov test was used. One-way ANOVA parametric test with LSD *post hoc* was used to detect significant differences among groups and sperm assessment parameters. All statistical analyses were performed using SPSS version 11.5 (SPSS Inc, Chicago, Illinois) and P-value <0.05 was regarded as statistically significant. Spermatogenesis index of the seminiferous tubules in different groups was evaluated by Mann-Whitney U test. Group means and their standard errors (SE) were reported in the text and graphs (GraphPad Prism version 5.01 for Windows, GraphPad Software Inc, San Diego, CA, USA).

Results

Sperm Evaluation Findings

Total volume of the right testes in oligospermic groups including OS, OE1 and OE2 significantly decreased compared to the control groups including CE and CS (P<0.001, for all three groups). An increase was observed in CS group in comparison with the CE group (P=0.02; Figure 1A). Sperm concentration in oligospermic groups (OS, OE1 and OE2) showed a statistically significant reduction in comparison with CS and CE groups. The highest sperm concentration was revealed in the CE group in comparison with other groups. Sperm concentration between the CE and CS groups proved a statistically significant difference (P=0.04). Moreover, sperm concentration of the right epididymis in OE2 was more than that of OE1 based on different distances of 150 and 100 cm, respectively, but the difference was not significant (P=0.56; Figure 1B). The sperm motility percentage in OS, OE1 and OE2 groups had a statistically significant reduction compared to CE and CS groups (P<0.001 for all three groups). In contrast, the

percentage of sperm motility between CS and CE groups was not significant ($P=0.51$; Figure 1C). Sperm viability percentage in OS, OE1 and OE2 groups significantly diminished in comparison with CE and CS groups ($P<0.001$ for all three groups). In addition, the percentage of sperm viability in CS and CE groups showed no statistically significant difference ($P=0.81$; Figure 1D).

Histopathologic and Histomorphometric Changes

Lumen diameter of seminiferous tubules in oligospermic groups including OS, OE1 and OE2 significantly decreased compared to the control groups including CE and CS ($P<0.001$, for all three groups). There were no significant differences between CS and CE groups ($P=0.11$). In contrast, lumen diameter in OE2 showed a statistically significant reduction in comparison to OE1 group ($P=0.002$; Fig-

ure 2A). Cellular and total diameters of the seminiferous tubules in OS, OE1 and OE2 groups significantly declined compared to those in CS and CE groups ($P<0.001$ for all three groups; Figure 2B and 2C) but the difference between CS and CE groups was not significant. The number of tubules per unit area in the OS, OE1 and OE2 groups significantly increased in comparison with CS and CE groups ($P<0.001$ for all the three groups; Figure 2D). Lumen area of the tubules in the CS group had the highest amount compared to other groups. Lumen area in CE group significantly decreased in comparison with the CS group ($P=0.04$). The amount of lumen area in OE2 group had a statistically significant decline compared to OE1 group ($P=0.05$; Figure 2E). Cellular area of the seminiferous tubules in OS, OE1 and OE2 groups significantly decreased in comparison with CS and CE groups ($P<0.001$ for all three groups). Cellular area

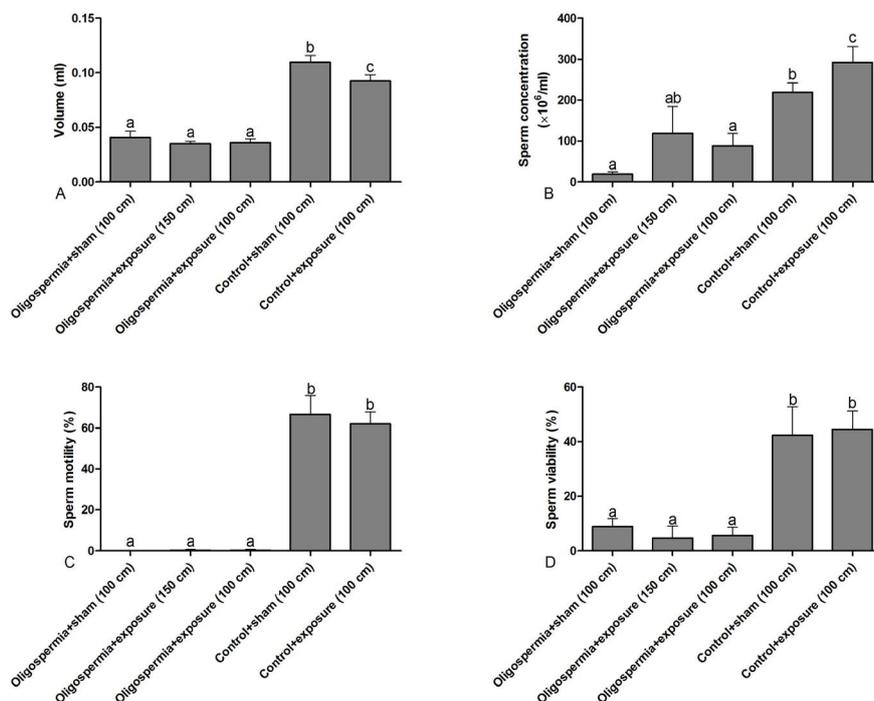


Figure 1: Mean and standard error of A) testis volume (ml), B) sperm concentration ($\times 10^6$ /ml), C) sperm motility (%), and D) sperm viability (%) after short-term exposure to microwave radiation emitted from Wi-Fi routers in different groups of busulfan-induced oligospermic mice ^{a,b,c}; different letters indicate significant differences between groups ($P<0.05$).

in the CS and CE groups was not significantly different ($P=0.20$; Figure 2F). The cross-sectional area of the seminiferous tubules in CE group significantly decreased compared to the CS group ($P=0.01$). Moreover, the amount

of cross-sectional area in OE2 group had a non-significant reduction in comparison with OE1 group ($P=0.12$; Figure 2G). Numerical density of the seminiferous tubules in the OS, OE1 and OE2 groups significantly increased

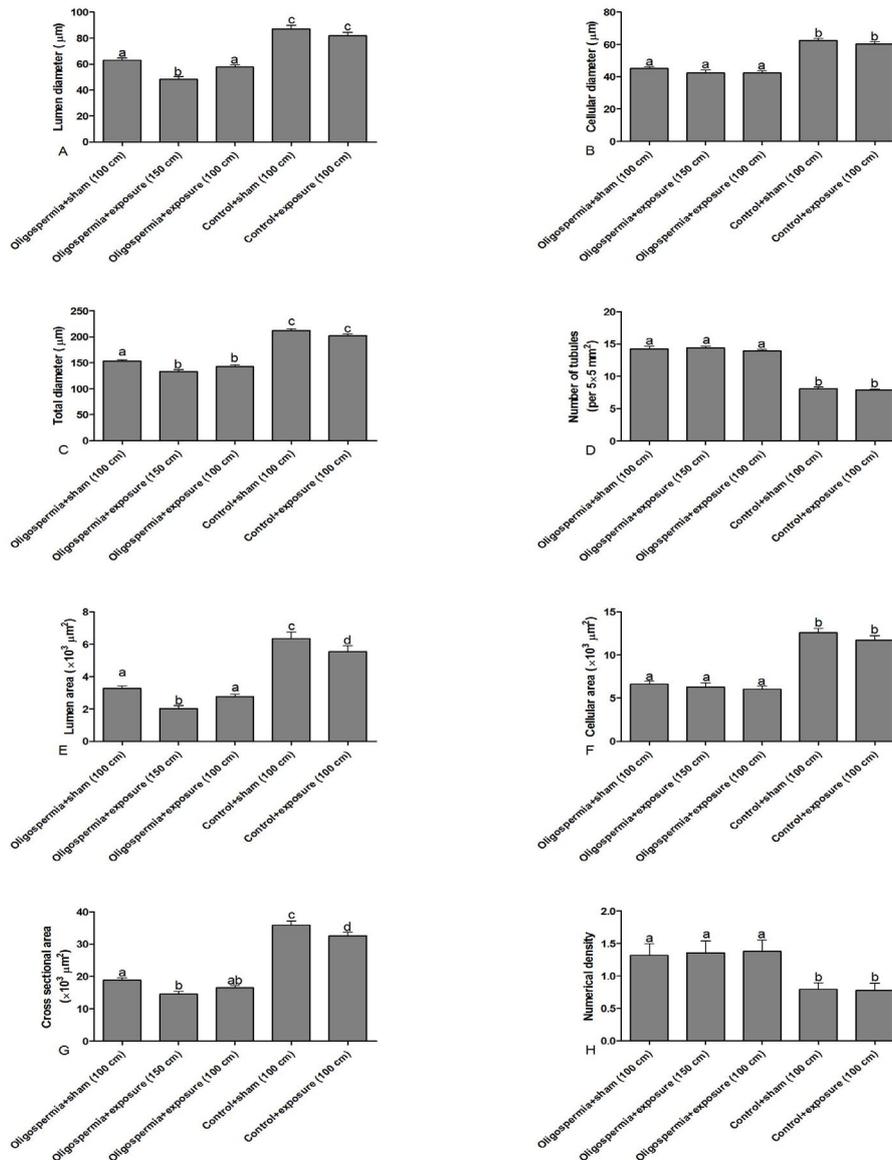


Figure 2: Mean and standard error of stereological indices of seminiferous tubules of A) Lumen diameter (μm), B) cellular diameter (μm), C) total diameter (μm), D) number of seminiferous tubules per unit area of testis, E) luminal area ($\times 10^3 \mu\text{m}^2$), F) cellular area ($\times 10^3 \mu\text{m}^2$), G) cross-sectional area of the tubule ($\times 10^3 \mu\text{m}^2$) and H) numerical density of the seminiferous tubules after short-term exposure to microwave radiation emitted from Wi-Fi routers in different groups of busulfan-induced oligospermic mice. ^{a,b,c} Different letters indicate significant differences between groups ($P < 0.05$).

in comparison with the CS and CE groups ($P < 0.01$; Figure 2H). The spermatogenesis index of seminiferous tubules in the OE2 group was more than that in the OE1 group ($P = 0.71$; Figure 3 and Figure 4A, B). Furthermore, the amount of spermatogenesis index in the CS and CE groups had no statistically significant difference ($P = 0.75$; Figure 3 and Figure 4D, E).

Discussion

Our findings revealed that treatment with short term exposure to microwave radiation at a frequency of 2.4 GHz with $3152 \mu\text{W}/\text{m}^2$ power density in the CE group significantly increased sperm concentration in epididymis; however, increase in the spermatid cells was not significant. We used Busulfan to induce oligospermic mice in the OS, OE1 and OE2 groups and observed that 2.4 GHz microwave

radiation in OE2 group with lower power density ($1401 \mu\text{W}/\text{m}^2$) than the OS and OE1 groups increased the sperm concentration in the epididymis and the spermatid cells in the testis. However, this increase was not significant.

These results are in line with the radiation hormesis theory defined as a biological dose response inducing stimulatory or inhibitory effects by low or high doses of irradiation respectively and not only eliminate the detrimental effects but also induce beneficial outcomes [23]. So far, beneficial effects of Wi-Fi common routers on the living organisms have not been reported and it is the first study to investigate the short-term and low power density effects of Wi-Fi exposure on the male mice testis functions. Therefore, no data are available in the literature to compare the results of this study. *Maioli et al.* [24] evaluated the ef-

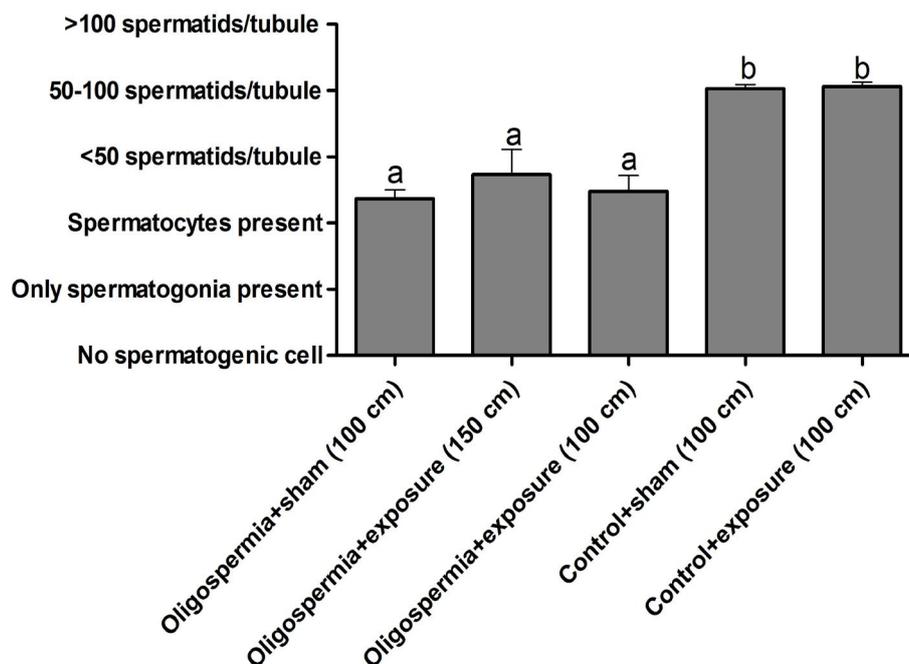


Figure 3: Mean and standard error of spermatogenesis index of seminiferous tubules in different groups after short-term exposure to microwave radiation emitted from Wi-Fi routers in different groups of busulfan-induced oligospermic mice. ^{a,b,c} Different letters indicate significant differences between groups ($P < 0.005$).

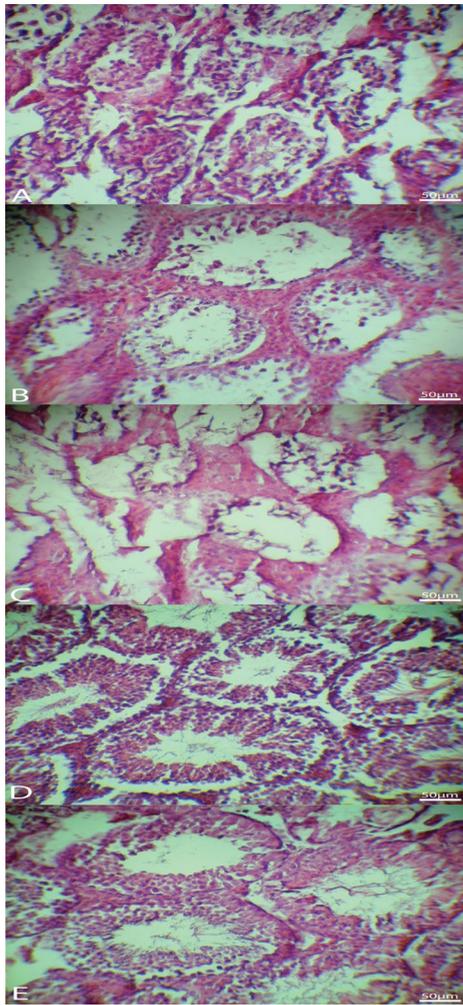


Figure 4: Seminiferous tubules in different groups. A) oligospermic exposure 2, B) oligospermic exposure 1, C) oligospermic sham, D) control exposure, E) control sham. Scale bar is 50 μm (hematoxylin and eosin staining).

fects of 2.4 GHz microwave radiation emitted from Radio Electric Asymmetric Conveyor (REAC) with a power density of $400 \mu\text{W}/\text{m}^2$ and $\text{SAR}=0.128 \mu\text{W}/\text{g}$ on the embryonic stem cells in mice. Results of this investigation revealed that expressions of the skeletal, cardiac and neuronal marker proteins were enhanced. These findings are in line with the results obtained by *Lee et al.* [12] who demonstrated that Wi-Fi signals with 2.4 GHz frequency, $26 \mu\text{W}/\text{cm}^2$ power flux density and $\text{SAR}=240 \text{mW}/\text{kg}$ emitted from smartphone significantly

increased the adipose stem cell proliferation. On the other hand, in our study, motility and viability in the CE and CS groups were not statistically significant; this is in contrast with the results reported by *Aweda et al.* [18]. He indicated that 2.4 GHz microwave radiation with $6 \text{mW}/\text{cm}^2$ power density reduced the sperm motility and concentration, and increased the abnormal sperm cells of the male rats. In this study, the groups were exposed to various values of SAR including 0.48, 0.95, 1.43, 1.91 and $2.39 \text{W}/\text{kg}$, respectively. The effects were enhanced by increasing SAR. Moreover, *Moon et al.* [25] evaluated rat reproductive function after 8 weeks of exposure at a 2.4 GHz frequency for 1-2 hours/day. The results revealed that the number of spermatocytes significantly decreased. In our study, the lumen and cross-sectional areas in the CE group significantly reduced in comparison with those in the CS group. Also, the lumen diameter and area in the OE2 group significantly reduced in comparison with those in OE1 group. These findings are in line with the results obtained by *Dasdag et al.* [19]. They stated that long-term exposure of radiofrequency radiation emitted from Wi-Fi equipment at 2.4 GHz frequency, for 24 h/day for one year, reduced some of the male rats reproductive functions such as the weight of epididymis, tunica albuginea thickness and seminiferous tubules diameter while a non-significant enhancement was observed in the sperm motility. The exact molecular mechanisms of microwave radiation on the sperm and testis tissues are obscure. However, researchers have claimed that increase in ROS generation affects ERK (extracellular signal regulated kinase) signaling pathway which can be activated by different mediators such as ROS, cytokines and growth factors [26]. Furthermore, ATP enhancement can stimulate ERK1/ERK2 and PI3K (phosphatidylinositol 3-kinase) activity pathways [27]. These factors are the major regulators in the proliferation and cell survival [28]. Also, *Ristow and Zarse* [29] demonstrated that mitochondrial

respiratory chain activation increases the supply of adenosine triphosphate (ATP), leading to reactive oxygen species (ROS) enhancement within the mitochondria; this reduces the oxidative stress and eventually induces bio-positive effects. Moreover, ATP binding to multiple P2 nucleotide receptors increases intracellular calcium concentration (Ca^{2+}) [30-32]. By enhancing the intracellular Ca^{2+} , the activities of various enzymes such as protein kinase C (PKC) and ERK1/ERK2 will increase and can trigger cell differentiation and proliferation [33, 34]. It can be suggested that microwave radiation emitted from Wi-Fi router at a frequency of 2.4 GHz with low power density such as $3152 \mu\text{W}/\text{m}^2$ and $1401 \mu\text{W}/\text{m}^2$ could stimulate the spermatid cells and may lead to increase in spermatids proliferation. In addition, it could possibly accelerate the spermatids differentiation to the sperm in the testis or may cause the enhancement of the sperm concentration in the epididymis, also, it could possibly lead to infertility treatment while the cellular diameter, number of tubules, numerical density and cellular area approximately remained constant. We do not have the privilege to discuss our results with other researches due to complete lack of information on bio-positive effects of 2.4 GHz Wi-Fi exposure. Further studies may shed light on the exact mechanisms of low dose and low power density Wi-Fi exposure on reproductive system.

Conclusion

Overall, it can be concluded that microwave radiation with low power density emitted from common Wi-Fi router has biological beneficial effects on mouse sperm concentration and sperm histomorphometric parameters.

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Conflict of Interest

None

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