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Research Article

Impact of Electromagnetic Field Radiation on Blood-Testis Barrier Regulatory Protein Levels in Rat Testes

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Abstract

The use of devices that generate electromagnetic fields (EMF), such as cell phones, is on the rise and has sparked interest in studying their effects of human health. We investigated of the proteins found in the entire testes of adult male Wistar rats that were exposed to EMF radiation at frequencies of 900 MHz and 2150 MHz. The exposure lasted for 2 hours per day over a period of 1 week and 10 weeks, which simulated various levels of cell phone use in humans. After the experiment, the subjects were sacrificed and testicular samples were collected Consider specifying the measurement technique used to assess the levels of N-cadherin and other proteins, such as immunoblotting or immunofluorescence, a protein that regulates the blood testis barrier (BTB). Immunohistochemistry was also used to identify other proteins involved in BTB regulation, namely Occluding, F-actin, Ezrin, and Fascin, and to confirm any protein misfolding in the BTB area. Our findings indicate that exposure to radio frequency modulated EMFs has significant effects on the BTB, specifically on the protein composition in the testes of rodents. The results suggest that 1 week and 10 weeks of EMF radiation exposure induce stress in the testicular tissue, potentially leading to a disruption in the physiological location of the BTB. This disorientation of the regulating proteins in the BTB interferes with the process of spermatogenesis. In conclusion, our study demonstrates that exposure to radio frequency modulated EMFs has significant effects on the blood testis barrier, particularly in the composition of proteins in the testes of rodents. The findings suggest that 1 week and 10 weeks of EMF radiation exposure induce disruption in the testicular tissue, leading to a disruption in the physiological location of the BTB. This disruption of the regulating proteins in the BTB hinders the spermatogenesis process.

Keywords: Electromagnetic Fields (EMFs); Cell Phones; Testes; Blood Testis Barrier (BTB); N-cadherin; Spermatogenesis

Introduction

In modern society, people are increasingly exposed to a wide range of EMFs that come from electricity generation and distribution, television sets, personal computers, radio communication, and mobile communication. The use of devices like radios, televisions, computers, and mobile phones in our daily lives results in continuous exposure to the effects of EMFs [1]. The popularity of mobile phones, with most individuals in many countries using them, has raised concerns about the potential impact of EMFs associated with mobile phone usage. Researchers are interested in studying the effects of using mobile phones during pregnancy on the development of the embryo and fetus. However, the exact nature and extent of the potential effects of EMFs on the embryo and fetus are still a subject of ongoing debate [2,3].

The effects of EMFs on living organisms have been a subject of ongoing debates and controversies. At the cellular level, EMFs have been linked to increased levels of free radicals and calcium concentration within cells, which can potentially hinder cell growth, disrupt protein structure, and cause DNA damage [4]. Additionally, EMFs can interfere with cellular signaling processes that rely on calcium. In vivo experiments have shown that a 900 MHz EMF can induce oxidative stress, resulting in histopathological impairments in the endometrium of rats. In the case of prostate cancer cells, exposure to a 60 Hz sinusoidal EMF has been found to generate reactive oxygen species (ROS), leading to inhibited cell growth through apoptosis and cell cycle arrest [5-7].

Exposure to a 2,450 MHz RF EMF has been found to cause rearrangement and breakage of DNA segments in testes [8]. Similarly, studies have shown that an 1,800 MHz EMF induced DNA breaks in human fibroblasts and rat granulosa cells, as observed in comet assays [8]. Chinese hamster lung cells also experienced DNA damage when exposed to an 1,800 MHz EMF [9]. Additionally, both a 900 MHz and 1.7 GHz RF EMF were found to induce DNA breakage in cauda epididymal spermatozoa and embryonic stem cells in mice. Taken together, these findings highlight the potential risk of protein misfolding associated with RF EMFs, particularly at a mobile phone frequency of 1.95 MHz [10]. The accumulating evidence suggests that EMFs can exert various effects at the cellular and molecular levels, impacting DNA integrity and cellular processes. However, further research is needed to fully understand the mechanisms and potential risks associated with EMF exposure [11-13].

Studies have reported that EMFs at a frequency of 900 MHz can cause moderate desquamation and vacuolization in the epithelium of testicular seminiferous tubules [14]. Furthermore, at a frequency of 1800 MHz, EMFs have been associated with severe vacuolization, necrosis, and desquamation in the seminiferous tubule epithelium [15]. In a study by Odacı., *et al*. [16], a decrease in granular cell numbers was observed in the dentate gyrus of rat pups exposed to a 900 MHz EMF during the prenatal period. Another study investigating the prenatal effects of EMFs reported a decrease in testicular seminiferous tubule diameter. These findings collectively demonstrate the adverse effects of EMFs on various organs [17].

Blood testis barrier (BTB)

BTB relies on the presence of proteins such as ezrin and fascin to maintain its integrity [18,19]. These proteins form adhesion complexes at the apical and basal ectoplasmic specializations (ES) and utilize F-actin for attachment. Interestingly, these adhesion complexes exhibit rapid deadhesion and readhesion capabilities [20,21]. In mammalian cells, ezrin, radixin, and moesin family proteins play a crucial role in tethering actin microfilaments to integral membrane proteins and peripheral proteins, such as adaptors [22-24]. This molecular interaction helps organize the apical membrane domain, which includes tight junctions (TJ) and adherents junctions (AJ) [25]. Consequently, this arrangement serves as a scaffold for signaling molecules involved in regulating cell migration, proliferation, adhesion, and polarity. Ezrin, specifically, has been found to associate with spermatozoa in humans and is known to be involved in sperm capacitation, a process crucial for fertilization [26]. Additionally, ezrin plays a structural role in the assembly of intercellular bridges known as tunneling nanotubes (TNT), which facilitate cell-cell communication in cancer cells [26,27].

BTB serves as a restrictive partition separating the blood vessels from the luminal compartment within the seminiferous tubules [18]. It is primarily composed of tight intracellular junctions established between adjacent Sertoli cells, effectively segregating the basal and adluminal compartments [28]. The tight junctions present in the BTB are responsible for regulating the permeability of the barrier, maintaining its selective nature [29]. the BTB plays crucial roles in the male reproductive system [29]. It creates a suitable microenvironment for the development

of germ cells, encompassing processes such as mitosis, meiosis, and differentiation. Additionally, the BTB acts as a shield, protecting the germ cells from harmful autoimmune responses and cytotoxic molecules [8]. Hence, beyond its physical role as a barrier, the BTB also possesses physiological components that govern the movement of substances into or out of the seminiferous tubules [28]. Furthermore, it serves as an immunological barrier, safeguarding the germ cells from potential threats posed by the immune system [30].

Human disease related to disruption of BTB

in the disruption of the BTB in Wistar rats, leading to a condition known as BTB dysfunction. BTB dysfunction refers to the impairment or breakdown of the protective barrier between the blood vessels and the seminiferous tubules in the testes [31]. The disruption of the BTB can have significant consequences on male reproductive health and may contribute to various diseases and conditions related to infertility, impaired spermatogenesis, and hormonal imbalances [32]. It is important to note that the EMF on the human body, particularly in relation to the BTB and reproductive health, are still a subject of scientific research and debate [33]. The studies mentioned in your phrase provide some insights into the effects of EMF exposure on male rats but may not directly translate to human health. further research is needed to fully understand the potential risks and mechanisms of EMF exposure on the BTB and human reproductive health. In the meantime, it is advisable to follow precautionary measures and guidelines regarding EMF exposure, as recommended by relevant health authorities and regulatory bodies [34,35].

This study aims to investigate the effects of EMF on the BTB utilizing both acute and chronic exposure models in rats. The primary objective is to determine whether EMF exposure disrupts the integrity of the BTB. To assess the integrity of the BTB, we focused on examining the expression and localization of specific proteins known to regulate BTB function. These proteins include N-Cadherin, Ezrin, Occludin, and Fascin, which play crucial roles in maintaining the structural and functional integrity of the BTB. By analyzing the expression levels and localization patterns of these proteins in testicular tissue samples from EMF-exposed rats, we aimed to identify any alterations or disruptions in BTB integrity. This provided valuable insights into the mechanisms through which EMF may affect the BTB and potentially impact male reproductive health. Our study design included both acute and chronic exposure models to assess the immediate and long-term effects of EMF on the BTB. We carefully controlled the exposure parameters, such as frequency, intensity, and duration, to mimic realistic EMF exposure scenarios. Overall, this study aimed to contribute to our understanding of the potential impact of EMF on the BTB and provide valuable insights into the mechanisms underlying BTB disruption. The findings may have implications for understanding the risks associated with EMF exposure and could guide the development of strategies to mitigate any adverse effects on male reproductive health.

Materials and Methods

Animal study

A total of one hundred twenty healthy male Wistar rats, aged 6-8 weeks and weighing 250-300 grams, were used in this study. All animal experiments were conducted in accordance with the requirements of the Animal Care Committee at the Institute. The animals were housed in a controlled environment with a 12/12-hour light/dark cycle, at a room temperature of 22 ± 2°C and a humidity level of 50% ± 10. They had ad libitum access to standard rat chow and tap water.

Sample collection

In this study, a total of 120 healthy male Wistar rats were divided into six groups, each consisting of 20 individuals. The rats in these groups were subjected to electromagnetic field (EMF) radiation at two different frequencies: 900 MHz and 2150 MHz. The exposure duration varied, with some groups exposed for 1 week and others for 10 weeks.

The groups were defined as follows: Group 1 represented the control group for acute exposure (C1/1 week), Group 2 served as the control group for chronic exposure (C10/10 weeks), Group 3 was exposed acutely to 900 MHz RF-EMF (91/1 week), Group 4 was exposed chronically to 900 MHz RF-EMF (910/10 weeks), Group 5 experienced acute exposure to 2150 MHz RF-EMF (21/1 week), and Group 6 underwent chronic exposure to 2150 MHz RF-EMF (210/10 weeks).

Throughout the designated exposure periods, the rats in each group were subjected to the specific EMF radiation conditions as outlined above. This experimental design allows for the investigation of the effects of EMF radiation on the rats' physiological and biological parameters.

EMF application

The animals in this study were individually placed inside Plexiglas tubes that were spacious enough to accommodate a single rat. These tubes were positioned in a radial arrangement, evenly spaced around the RF-EMF producing antenna. For Group 3, the rats were exposed to 900 MHz RF-EMF for 2 hours daily, consistently over a span of 1 week. Similarly, for Group 4, the rats received the same frequency of 900 MHz RF-EMF for 2 hours daily but for a duration of 10 weeks.

In the case of Group 5, the animals were subjected to 2150 MHz RF-EMF for 2 hours daily over a period of 1 week. For Group 6, the rats were exposed to the same frequency of 2150 MHz RF-EMF for 2 hours daily, but over a duration of 10 weeks. The weekly applications of RF-EMF were conducted for 5 days each week. As for the control groups, Group 1 served as the control for acute exposure and was kept within the same time as the other acute exposure groups. Group 2 acted as the control for chronic exposure, with the animals being placed in Plexiglas tubes for the same time period as the other chronic exposure groups, but without any RF-EMF administration (Figure 1). This experimental design allowed for the examination of the effects of different durations and frequencies of RF-EMF exposure on the animals, with the control groups serving as reference points for comparison.

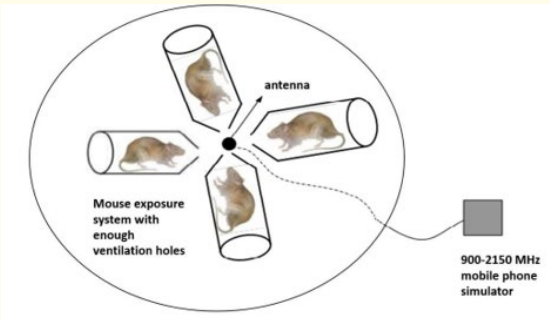

Figure 1: The experimental setup depicted in the schematic illustrates the dimensions of the Plexiglas tubes utilized for housing the rats during the study.

The diagram provides a visual representation of the arrangement of the tubes surrounding the RF-EMF producing antenna, emphasizing the equal spacing and radial positioning of the animals. This visual depiction serves as an overview of the experimental setup and serves as a reference to comprehend the physical configuration of the study environment.

Histological analysis

After the initiation of the experiment, all testes were surgically removed and examined after a 6-hour interval. The excised testicular tissue was then fixed in Bouin's solution, which consists of 7.5 ml saturated picric acid, 2.65 ml glacial acetic acid, and 2.5 ml 7% formaldehyde. Following fixation, the tissue samples were embedded in paraffin blocks. Thin sections measuring 5 µm in thickness were prepared from the paraffin-embedded blocks, and these sections were subsequently deparaffinized. To facilitate microscopic examination, the sections were stained with hematoxylin and eosin.

The evaluation of tubule degeneration in each experimental group was performed using a modified version of Johnsen's scoring system. This scoring system is primarily based on the progressive degeneration of the germinal epithelium and the subsequent loss of more mature cell types during testicular damage. Each tissue sample was assigned a score ranging from 1 to 10 based on the observed histological characteristics. A score of 10 indicates a normal tubule structure with complete spermatogenesis, while a score of 9 signifies a disorganized epithelium with some spermatozoa present. Scores of 8, 7, 6, and 5 indicate decreasing levels of spermatogenesis, with varying degrees of spermatozoa, spermatids, and spermatocytes. Scores of 4, 3, 2, and 1 represent further reductions in germ cell presence, with a score of 1 indicating the absence of germ cells and spermatogenesis.

The histological examinations were conducted in a random order under a standard light microscope, utilizing a magnification of 100x. The histologist performing the evaluations was blinded to the experimental group to ensure unbiased assessment of the tissue samples.

Immunohistochemistry

Immunohistochemistry staining was conducted on formalin-fixed, paraffin-embedded samples. The slides containing the tissue

sections were first deparaffinized using xylene and then rehydrated through a series of ethanol concentrations. To facilitate antigen retrieval, the samples were subjected to heat treatment in a citrate buffer solution with a pH of 6.0.

To block endogenous peroxidase activity and reduce nonspecific binding, the sections were incubated in methanol containing 3% H2O2 for 10 minutes and then treated with Ultra V Block (Labvision, Freemont, CA) for 7 minutes at room temperature.

Next, primary antibodies (refer to Table 1) were applied to the sections and allowed to incubate overnight at a temperature of +4°C within a humidified chamber. Following incubation, the sections were washed with Phosphate-buffered Saline (PBS) and subsequently subjected to consecutive incubations with a biotinylated secondary antibody and a streptavidin-peroxidase conjugate (Pierce, Rockford, IL).

Primary antibody	Source	Dilutions	Secondary Antibody	Visualization
N–Cadherin	Invitrogen	1:200	1:100/GAR	IHC/WB
Occludin	Invitrogen	1:200	1:150/GAR	IHC/WB
Cl-Caspase3	Cell Signaling	1:200	1:300/GAR	IHC/DAB

Table 1: Antibodies used for immunohistochemistry western blot and immunofluorescence.

Abbreviations: GAM, goat anti mouse; GAR, goat-anti rabbit; DAB, 3,3'-Diaminobenzidine tetrahydrochloride.

To visualize the antibody complexes, the sections were incubated with 3,3'-diaminobenzidine tetrahydrochloride (DAB, Bio-Gene, San Ramon, CA). Hematoxylin was used as a counterstain, and the sections were then dehydrated, cleared in histolene, and mounted under glass coverslips using Mounting Medium (abcam ab: 64230). Finally, the stained sections were evaluated under a light microscope to assess the localization and intensity of the immunohistochemical staining.

Immunohistochemical staining for cleaved caspase-3

To detect apoptotic cells, cleaved caspase-3 staining was performed on testicular tissue samples. The samples were fixed in 10% neutral buffered formalin and subsequently embedded in paraffin. Sections measuring 5 µm in thickness were obtained from the paraffin-embedded blocks. The sections were deparaffinized and then treated with methanol containing 3% H2O2 to block endogenous peroxidase activity. To minimize nonspecific binding, the sections were further blocked with Ultra V Block (Labvision, Freemont, CA) for 7 minutes at room temperature. Cleaved caspase-3 antibody (#9664, Cell Signaling, U.S.) was applied to the sections at a dilution of 1:200, and the sections were incubated overnight at +4°C in a humidified chamber. Following the incubation, the sections were washed with PBS.

Next, the sections were incubated with biotinylated horse anti-rabbit IgG (3 mg/mL; Vector, Burlingame, CA) at a dilution of 1:200 for 1 hour at room temperature. The antibodies were detected using Vectastain Avidin Biotin Complex (Vector PK 4000) for 30 minutes at room temperature. To visualize the antibody complexes, the sections were incubated with 3,3'-diaminobenzidine tetrahydrochloride (DAB, Bio-Genex, San Ramon, CA), resulting in the development of a brown precipitate. The stained sections were then mounted under glass coverslips using Mounting Medium and evaluated under a light microscope.

To analyze the cleaved caspase-3 positive cells, 100 seminiferous tubule cross-sections were counted per group. The numbers of apoptotic cells were assessed within each group of seminiferous tubules and compared between the groups by counting at least 100 seminiferous tubules per rat.

Western blot analysis

Testes lysates were prepared by homogenizing the tissue in lysis buffer for protein detection. Protein extraction was carried out using the BCA Assay Kit (Merck Millipore, #71285). Subsequently, the proteins were transferred onto membranes using standard blotting techniques and a Bio-Rad Germany electro transfer chamber. The blotting buffer used contained 39 mM glycine (ICN Biomedicals Ohio, USA), 48 mM Tris, and 20% methanol (J.T. Baker, The Netherlands). To obtain identical replicas from a single gel, the proteins were transferred to Immobilon P membranes (Millipore,

USA) using the double replica blotting technique described by Johannsson [14]. The transfer process took place for 8 hours at 250 mV.

For Western blotting, the membranes were blocked overnight with 3% BSA (Sigma, USA) in PBS saline buffer (pH 7.4). Subsequently, the membranes were incubated with patient sera at dilutions ranging from 1:100 to 1:500 for 1 hour at room temperature. After washing, the membranes were incubated with peroxidase-conjugated goat anti-human IgG, IgM, IgA, and IgE sera (1:250; Bio-Rad, Germany) in PBS saline buffer with 0.05% Tween-20. Finally, the appropriate substrate NBT/BCIP (50 mg/ml) (Bio-Rad, Germany) was added according to the manufacturer's recommendations. The antibodies used for Western blotting are listed in Table 1. Chemiluminescence was performed, and the immunoblots were analyzed as described previously [15].

Immunofluorescence staining

Testicular sections from both EMF-treated groups and control groups were stained with target proteins as phalloidin and fascin (Table 1.) with 49,6-diamidino-2-phenylindole (DAPI)-nuclear counterstain. A working solution (100 ng/mL DAPI in PBS prepared from DAPI stock solution (5 mg/mL DAPI in PBS) was used to perform DAPI staining. Sections were incubated in dark for 5 minutes at room temperature.

Statistical analysis

The experimental data were analyzed using one-way ANOVA, followed by the Tukey test, with the SigmaPlot 11.0 Program (Jandel Cooperation, San Rafael, CA). The results are presented as means ± SEM (standard error of the mean). Statistical significance was determined at a level of $P < 0.05$.

Results

The study found that exposure to EMF at a frequency of 2150 MHz had an impact on the organization of seminiferous tubules. Specifically, in the 21 group (exposed to 2150 MHz for 1 week) and the 210 group (exposed to 2150 MHz for 10 weeks), there was a significant decrease in Johnsen's scores compared to the control groups. The control groups, C1 (control group exposed for 1 week) and C10 (control group exposed for 10 weeks), had higher Johnsen's scores (9.35 ± 0.67 and 9.25 ± 0.71, respectively) compared to the exposed groups (7.85 ± 0.7 and 6.75 ± 0.7, respectively) ($p < 0.05$) (see Figure 2).

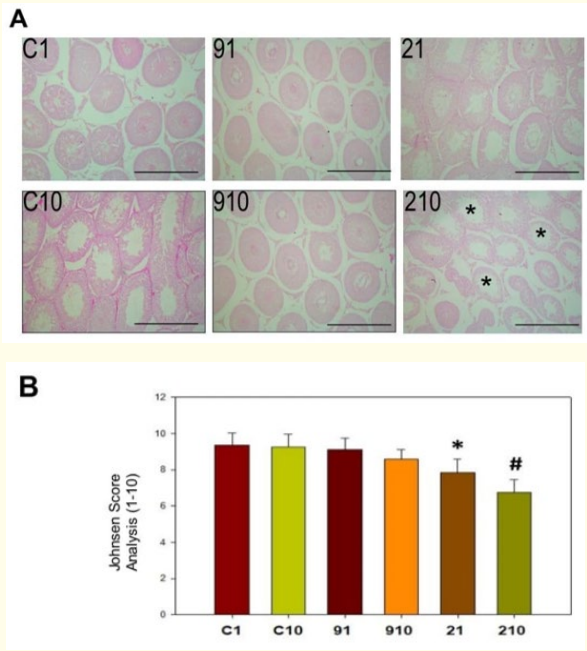

Figure 2: (A) Histological analysis of EMF effects on testis organization after 10 weeks under 900 and 2150 MHz EMF groups. Disorganized seminiferous tubules were detected in significantly in 2150 groups (B) Jhonsen score results of both groups.

*; 21 group (1 week under 2150 MHz) vs. control = $p < 0,001$. #; 210 group (10 week under 2150 MHz) vs. control = $p < 0,001$.

However, no disorganized seminiferous tubules were observed in the 91 group (exposed to 900 MHz for 1 week) and the 910 group (exposed to 900 MHz for 10 weeks). In these groups, the Johnsen's scores were 9.1 ± 0.64 and 8.6 ± 0.5, respectively, indicating that there was no significant impact on the organization of seminiferous tubules compared to the control groups.

Under exposure to EMF, the study observed a decrease in levels of N-cadherin, along with a disorientation of its distribution. The analysis involved chronic groups: C10 (Control/10 week), 910 (900 MHz/10 week), and 210 (2150 MHz/10 week). Among these groups, the most significant decrease in N-cadherin levels was observed in the BTB area after 10 weeks of exposure to 2150 MHz (210 group) (refer to Figure 3).

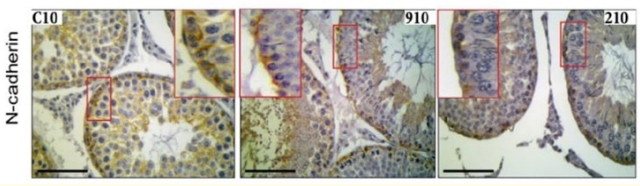

Figure 3: Effects of EMF exposure on N-cadherin levels and BTB integrity.

The study examined the impact of EMF exposure on N-cadherin levels in chronic groups: C10 (Control/10 week), 910 (900 MHz/10 week), and 210 (2150 MHz/10 week). The results revealed a significant decrease in N-cadherin levels in the BTB area after 10 weeks of exposure to 2150 MHz (210 group) (refer to Figure 3A).

BTB regulatory protein levels are decreased.

The study conducted immunohistochemical analysis of N-cadherin-Occludin expression under the exposure to EMF at frequencies of 900 MHz and 2150 MHz; the results showed clear disorientation of N-cadherin-Occludin expression in both the 900 MHz and 2150 MHz EMF-exposed groups. This disorientation is depicted in Figure 4 of the study. For further details and a visual representation of the disorientation of N-cadherin-Occludin expression, it is recommended to refer to Figure 4 in the original study.

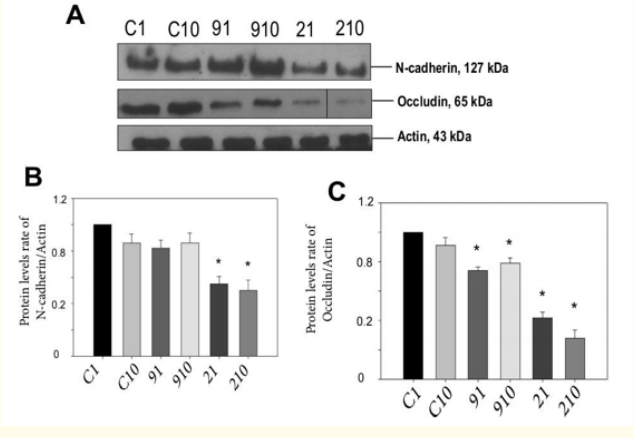

Figure 4: (A) Western Blot Analysis of N-cadherin after EMF radiation with control groups as C1: Control 1 week; C10: Control 10 week; 91: 900 MHz 1 week; 910: 900 MHz 10 week; 21: 2150 MHz 1 week; 210: 2150 MHz 1 week. (B) Histogram were demonstrated as the result of expression levels of N-cadherin and (C) Occludin. P < 0,05.

Cleaved Caspase-3 Expression as an apoptotic index

The study investigated the apoptotic index using cleaved caspase-3 expression as a marker. Among the groups studied, the highest apoptotic index was observed in the 21 group (2150 MHz/1 week) (7.3 ± 0.5) and the 210 group (2150 MHz/10 weeks), compared to the other groups. The control groups, C1 (Control/1 week) and C10 (Control/10 weeks), exhibited lower apoptotic indices (2 ± 1 and 3.4 ± 0.6, respectively). Similarly, the 91 group (900 MHz/1 week) and 910 group (900 MHz/10 weeks) showed apoptotic indices of 3.4 ± 0.6 and 3.6 ± 0.5, respectively. These differences were found to be statistically significant (p < 0.05), as illustrated in Figure 5.

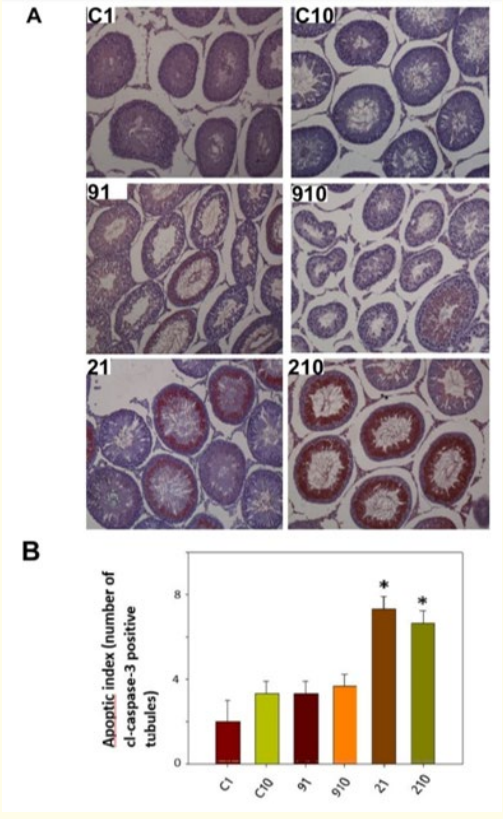

Figure 5: (A) Immunohistochemical analysis of cleaved caspase-3 (cl- caspase-3) EMF radiation with control groups as C10: Control 10 week; 910: 900 MHz 10 week; 210: 2150 MHz 10 week. Cl-Caspase-3 levels were increased after 1 week and 10 weeks both 2150 MHz EMF. (B) Histogram of immunohistochemical staining for cleaved caspase-3 (cl- caspase-3) positive cells as an apoptotic index, which were shown by counting 100 tubules per each group. *, P = <0,001.

Western blot analysis confirms immunohistochemistry results

To validate the findings obtained from immunohistochemistry, western blot analysis was performed. The results of the western blot analysis further supported the observed changes in protein expression. Specifically, the protein N-Cadherin, a crucial regulator of the blood-testis barrier (BTB) with a molecular weight of 127 kd, exhibited a significant decrease in both the 21 group (2150 MHz/1 week) and the 210 group (2150 MHz/10 week) (Figure 6). Moreover, the protein Occludin, with a molecular weight of 65 kd, demonstrated a marked decrease in expression in multiple groups, including the 91 group (900 MHz/1 week), the 910 group (900 MHz/10 week), the 21 group (2150 MHz/1 week), and the 210 group (2150 MHz/10 week).

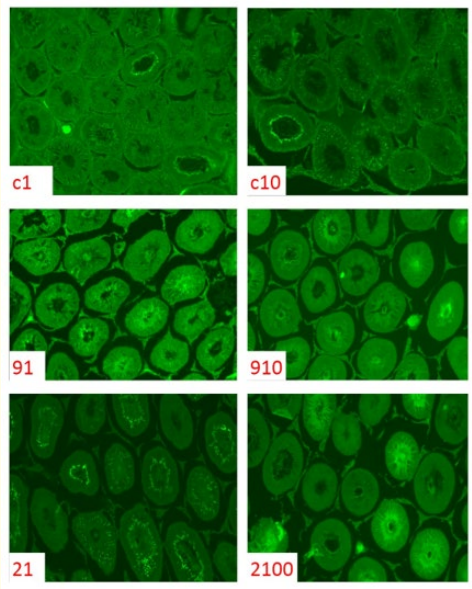

Figure 6: Immunofluorescence analysis of phalloidin as an actin detection.

To ensure accurate loading and comparison of protein levels, Actin (43 kd) was utilized as an integral control. The inclusion of Actin served as a reference for confirming equal loading of samples across the different experimental groups. These western blot findings provide additional evidence supporting the alterations in N-Cadherin and Occludin protein expression observed in the study. For a visual representation of the western blot results, please refer to Figure 6.

Discussion and Conclusion

Based on the study "Effects of exposure to electromagnetic field (1.8/0.9 GHz) on testicular function and structure in growing rats" by H. Ozlem Nisbet., *et al*. it was observed that exposure to electromagnetic waves at 1800 and 900 MHz frequencies resulted in increased testosterone levels, improved epididymal sperm motility, and enhanced normal sperm morphology in rats. These findings suggest that EMF exposure may have a positive effect on reproductive parameters in male rats [36]. In contrast, the study conducted by Guowei Zhang., *et al*. on the effects of cell phone use on semen parameters in a general population found that certain aspects of cell phone use, such as daily duration of talking on the cell phone and internet use via cellular networks, were associated with decreased semen parameters, including sperm concentration, total sperm count, and semen volume. These results imply that cell phone use may have negative effects on sperm quality and male fertility in humans [37].

Furthermore, the study by Teerapot Wessapan and Phadungsak Rattanadecho, investigating the temperature increases induced by EMF exposure at 900 MHz and 1800 MHz, revealed that the scrotum exhibited the highest specific absorption rate (SAR) values, indicating increased EMF absorption in this sensitive area. Although there were no significant differences in temperature increases between the two frequencies, the SAR value of the 900 MHz frequency was significantly higher in the testis, the most sensitive part of the male reproductive system. These findings suggest a potential for temperature-related effects on male reproductive organs due to EMF exposure [38].

Considering the study by Alper Kocyigit and Mesut Cevik on the effects of leukemia inhibitory factor (LIF) and insulin-like growth factor I (IGF-I) on bovine embryo development and cryotolerance, it was observed that LIF, either alone or in combination with IGF-I, significantly influenced in vitro bovine embryo development, particularly the blastocyst formation ratio. The addition of IGF-I along with LIF to the culture medium was found to be beneficial for bovine embryonic development and cellular cryotolerance after vitrification [39].

Taking all these findings into account, it is clear that the effects of EMF exposure on reproductive health are complex and can vary depending on the frequency, duration, and specific parameters

being studied. The results from the rat study suggest that EMF exposure at 1800 and 900 MHz frequencies may have positive effects on testosterone levels, sperm motility, and sperm morphology in rats. However, the study on cell phone use in humans indicates potential negative effects on semen parameters, such as sperm concentration and count. The temperature-related effects observed in the EMF exposure study further highlight the complexity of the issue. It is essential to note that the studies discussed here were conducted on animals (rats) and in vitro bovine embryos, and further research is needed to determine the direct implications for human reproductive health. Future studies should consider the specific mechanisms underlying these effects, including the role of temperature changes and the potential involvement of growth factors. Additionally, large-scale studies involving human subjects are necessary to establish more conclusive evidence regarding the impact of EMF exposure on male fertility and reproductive health.

In conclusion, the studies reviewed in this discussion provide valuable insights into the potential effects of EMF exposure and cell phone use on reproductive parameters in both animals and humans. While the rat study suggests positive effects on testosterone levels and sperm quality, the study on cell phone use indicates potential negative effects on semen parameters. The temperature-related effects observed in the EMF exposure study further highlight the complexity of the issue. Further research is needed to fully understand the underlying mechanisms and to establish the implications for human reproductive health. These findings underscore the importance of continued investigation into the potential risks and benefits of EMF exposure in relation to male fertility and reproductive health.

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Conflict of Interest

The authors declare no conflicts of interest.

Ethical Approval

All the experimental animal protocols for this study were approved by the Akdeniz University Local Ethics Committee for Animal Experiments (protocol number: 758/2018.10.07).

Author Contribution

NEGO designed the experiment and interpreted the data; ESA, HE, MEO, MT, SE, and NEGO performed the experiments, collected the data, and analyzed the results; ESA and NEGO wrote the manuscript. All authors edited the manuscript and have given approval for the publica-tion of the present version of this manuscript.

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