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Study of Histological Changes of Testis in Rats of Different Ages when Exposed to Frequency 10GH$_Z$ within the X-Band Microwave

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ABSTRACT

This study investigates the impact of 10 GHz microwave exposure, within the X-band range, on rat testicular tissue and sperm development across different age groups. Laboratory rats were divided into a control group of six, which was not exposed to microwaves, and a treatment group of 18, further split into three age-based subgroups (8–12 weeks, 12–16 weeks, and 16–20 weeks), each containing six rats exposed to 10 GHz microwaves for 2.5 hours daily over five weeks. The findings revealed no histological changes in the control group. However, in the treated groups, significant alterations were observed: the youngest group showed a lack of sperm formation and loss of spermatocytes; the middle group displayed malformed sperm and reduced numbers; and the oldest group experienced a decrease in sperm count and minor shape alterations. Additionally, after five weeks, all treated groups exhibited damage to the testicular interstitial tissue, including ruptures and space formation between seminiferous tubules. This study suggests potential risks of microwave exposure on reproductive health and lays the groundwork for future research in this area.

1. Introduction

The heavy reliance on electromagnetic wave-operated devices that has accompanied recent global advancements has exposed its users to additional doses as a result of scientific advancements in the fields of communications, data transmission, personal wireless devices, and medicine. Modern industrial applications, which can raise the natural background radiation. These developments in the field of technology have added factor for pollutants to the environment in which we live as a result of the increase in density and frequency emitted by electromagnetic wave technologies without considering the health consequences resulting from them [1]. Which leads to an increase in researchers' concern about the effects of these waves on biological systems in general [2], and there are many scientific indications related to the effect of microwaves and radio frequencies that are part

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of the electromagnetic spectrum on the cells of the organism when they interact with it and the resulting effects biological tissues [3,4].

Histological and physiological studies have shown that exposing animals to electromagnetic fields has a negative effect on the development of sperm and Sertoli cells (Leydig). However, the evidence that comes out every day about the effects of these waves is conflicting and unclear [5].

Microwaves are located within the non-ionizing rays of the electromagnetic spectrum and can travel in space and in material and immaterial mediums [6]. Accordingly, the transmission medium must be known in order to understand the mechanism of its transmission in the medium and then its interaction with it [7], where the frequency range of these waves ranges (300 GHz–300 MHz), which corresponds to a wavelength 1mm to 1m [8], where this wavelength is widely used in transmitting radar as well as microwave ovens and in medical applications through diathermy, as well as other applications. Although the energy of these waves is weak enough to break down atomic bonds, they cause biological effects. It is known that biological effects occur by raising the temperature, changing the course of chemical reactions, or creating electric currents in cells and tissues [9]. Living organisms are frequently exposed to these waves as a result of using applications that operate with these waves, which leads to their interaction with living tissues. The total effects resulting from this interaction share three main phenomena: the penetration of the energy of these waves into the living system and its spread in it, and the basic interaction of waves with tissues. Biological and possible side effects caused by the interaction [10]. Understanding the mechanism of microwave interaction with living tissue is not easy because of the complexity of living tissue and the multiple layers of biological systems in living organisms [11].

In fact, the x-beam frequencies penetrate living tissue to a depth of a little less than (5mm) to a little more than (7) mm, and a significant proportion of their energy is absorbed [3,4]. Observing the biological effect in itself does not indicate the existence of biological risks or harmful health effects [12], while the effects are harmful and negative only when the body cannot cancel the high temperature with the normal activity of the body such as blood flow and sweating [13].

Although the human body can compensate and manipulate the additional energy load through thermoregulatory mechanisms without a clear increase in temperature, the biological effects of microwaves are divided into thermal and non-thermal effects [14]. One of the characteristics of these waves that makes them dangerous to the system biologically it works inside the material (i.e., penetrates the material and interacts with every particle of the material), and accordingly heat is generated, although it is cold waves. The amount of heat generated depends on the electrical properties of the material in the first place, where there is a wide range of electrical properties possessed by biological tissues [15].

Based on the above survey, it is worth noting that the research on the effects of the x-beam with wide applications is limited and not enough to prove the biological effects resulting from it. Therefore, this study focused on the effect of the x-band with a frequency range of 12–8 GHz corresponding to a wavelength of 3.7–2.5 cm, which is part of the microwave spectrum, on the testis in rats. This band is known for its wide applications in the modern communication system, as well as the fact that most medical devices use this band technology, as well as other civil and military applications, in addition to being located in the middle region of a common dangerous frequency.

2. Physical properties of tissues and their role in histological changes

In recent decades, microwave waves have become in increasing demand in many fields, especially the communications and medical fields, because these waves are characterized by the fact that they work inside matter, that is, they penetrate living matter (tissue) and interact with it, which leads to the generation of internal heat without feeling it, and the amount of heat produced depends primarily on the physical (electrical) properties of the target tissue at these frequencies [12].

Although the human body can compensate and manipulate the additional energy load through thermoregulatory mechanisms without a clear increase in temperature, the biological effects of microwaves are divided into thermal and non-thermal effects [15]. One of the characteristics of these waves that makes them dangerous to the system biologically, it works inside the material (i.e., penetrates the material and interacts with every particle of the material), and accordingly heat is generated, although it is cold waves. The amount of heat generated depends on the electrical properties of the material in the first place, where there is a wide range of electrical properties possessed by biological tissues [16]. One of the important physical characteristics is the specific absorption rate (SAR). It is a term used to describe the absorption of electromagnetic radiation of microwave frequencies in the body. It is the rate of energy absorbed by tissues, expressed in a unit (W/kg). It has been specified in most organizations as the amount (1.6 W/kg) accordingly, the biological effects depend on the amount. The energy absorbed in the body of a living organism and not just what is present in space. In addition, there are other factors that affect the interaction between waves and biological systems, including radiation specifications (such as continuous or non-continuous radiation, frequency (f), exposure period and energy flux density) and Specifications of the exposure environment (such as temperature, pressure, humidity, and components of the surrounding environment, as the metal reflects these waves), in addition to the biological specifications of the exposed body, including age, sex, type of organ, geometry and size of the tissue, the rate of blood reaching it, and above all these factors. The dielectric properties of tissues, such as (complex permittivity, conductivity, and constant) are considered the main factor for the reaction mechanism [17].

3. Materials and methods

In general, the depth of penetration of the wave energy to the body is determined by the following relationships [16]:

$$\alpha = \sqrt{\pi\mu\sigma f} \quad (1)$$

$$\delta = \frac{1}{\alpha} \quad (2)$$

where α represents the attenuation constant of the wave, δ the penetration depth of the biological material f the frequency used, μ the magnetic permeability (since biological tissues are non-magnetic materials Eq. (3), σ represents the AC electrical conductivity $\mu_0 = \mu = 4\pi * 10^{-7}$

It is clear from the relations that the penetration depth is inversely proportional to the frequency. Therefore, as we will notice through the histological results of the testis, the effect of waves of lower frequency will be large compared with the higher frequencies within the same band. Relationship (2) shows that microwaves can be transmitted over long distances at the lowest frequency. Among these facts, companies and related industries are encouraged to design their equipment at a high frequency and try to avoid operating the equipment or devices at a frequency of 2.36 GHz or less than that, which can reduce the biological effect of these waves on internal organs [16].

The study was conducted on 24 laboratory rats of the type Sprague-dawley rats, all males, with weights ranging between 250 and 300 grams, and weighed using a dahongying scale. These animals were obtained from the animal house at the College of Science and University of Baghdad. At the beginning of the experiment, male and female rats were brought and then bred in the animal house, and the males were isolated for study and to control the required ages and exposed to radiation according to the time period of the required age. These animals were placed in plastic cages with metal lids furnished with fine sawdust and supplied with water from plastic bottles. They were also prepared according to the rules of the standard diet in the animal house at the College of Veterinary Medicine/University of Basra at a temperature ranging from (30-25)°C and a regular life cycle of 12 hours of light and 12 hours of darkness. It was left for three weeks for the purpose of adaptation, and then the experimental animals were divided into two groups as follows:

i. The first group represents the control group and includes six animals.
ii. The second group represents the treatment group and includes 18 animals, which were divided into three groups (first, second, and third) so that each group includes 6 animals, where all animals were exposed to the same frequency (10 GHz), where the animals of the first group were aged 8–12 weeks, and the animals of the second group at the age of 12–16 weeks, and the animals of the third group at the age of 16–20 weeks. The animals were exposed to the treatment for two and a half hours daily for five weeks.

The microwave system was installed in the Department of Physics, College of Science, University of Basra, as shown in Figure 1, where the microwave antenna was placed directly on the plastic cage with the plastic cover as well, to avoid the reflection processes caused by the metal cover, and another antenna received the waves. Through the received wave, whose value appears on the power sensor, it was found that the plastic cage used during exposure does not affect the process of receiving waves.

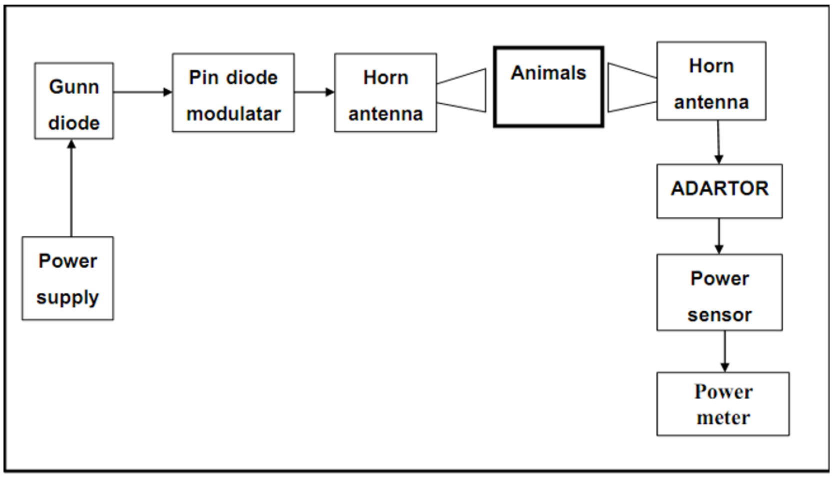

Fig. 1. Diagram of the microwave system used in the process of exposing animals

Animals were dissected for all groups (control, treatment) and for days (17, 35) of exposure to microwaves after being anaesthetized with ketamine or xylazine at a dose of BC 50 mg/kg 5 mg/kg) [17], then the testicles were raised and cut. It was cut into small pieces and fixed well in formalin 10% for 48 hours. The current study relied on what was mentioned by [18] in the technique of preparation for histological examination and staining of histological slides. Microscopic examination of the sections was carried out using a light microscope, and then the tissue sections were photographed using a microscope equipped with a camera. The behavior of the animals was followed up, and the clinical signs that appeared during the experiment were recorded.

4. Results and discussion

The results of the current study showed that when examining the histological sections of the rat testis (for the control group), they were normal and did not suffer any kind of histological changes due to not being exposed to microwaves. It was noted that the testicular tissue consisted of a group of seminiferous tubules in which the sperm swims in the middle of a cavity. The spermatic tube, as well as the presence of the primary spermatozoa, the secondary spermatocyte, the progenitor sperm finally the spermatids as shown in Figure 2. These results are consistent with the study of Meo *et al.,* [19] when studying a species of Wister albino rat.

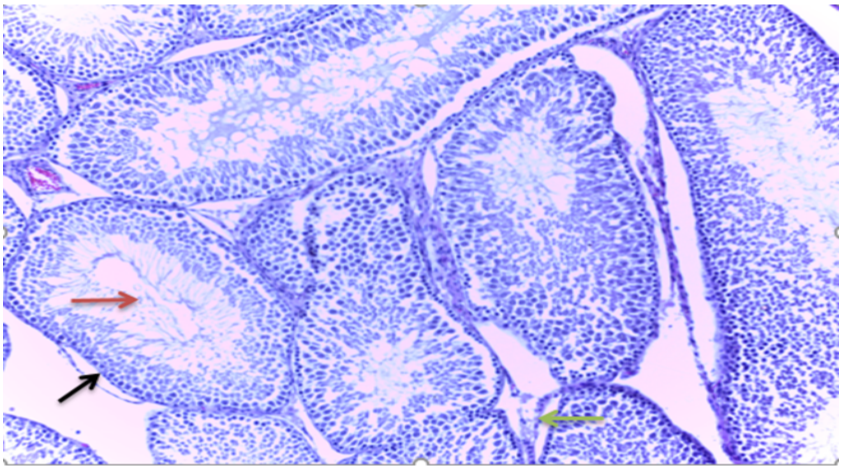

Fig. 2. A cross section of the rat testes of the control group showing the seminiferous tubules, sperm and Laydig cell (H&E) (100X)

The results of the (first) group exposed to a frequency of 10 GH_Z after 17 days of exposure showed that there are histological changes that show the lack of sperm formation and the loss of the stages of sperm formation, the loss of the primary and secondary spermatocytes, as well as the loss of some spermatozoa, as in the two Figures 3 and 4. This result is in line with that of researchers Kesari *et al.,* [20], concerning the study of the rat. They demonstrated that the radiation from a microwave oven, to which the rats were subjected for five days, could decrease sperm cell production. This is also confirmed by Agarwal *et al.,* [21], whose analysis of semen, exposed to waves from a mobile phone, decreases the stages of sperm cell formation.

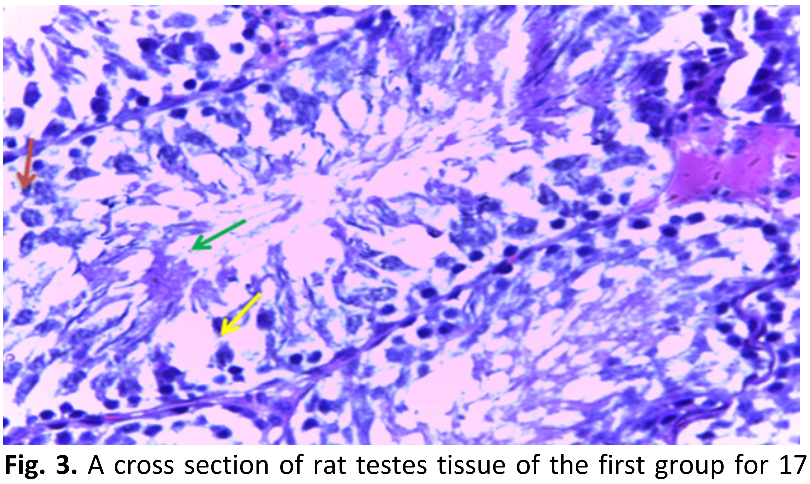

Fig. 3. A cross section of rat testes tissue of the first group for 17 days showing the histological changes in the seminiferous tubule, sperm (PSP) and primary sperm cell (Spar) (H&E) (400X)

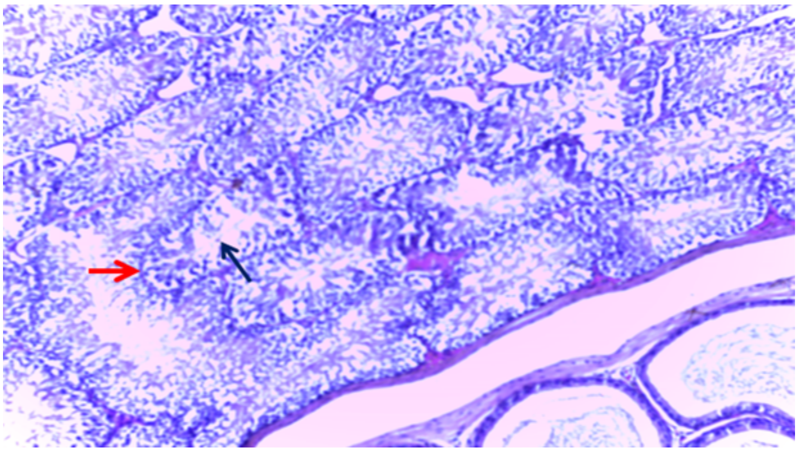

Fig. 4. A cross section of rat testes tissue for the first group for 17 days showing the histological changes in sperm count and the loss of some stages of their formation, seminiferous tubule (H&E) (100X)

The results of the second group exposed to the frequency of the user in this experiment after 17 days showed that the microwaves that were exposed to the rats affected the shape of the sperm, their low number, and the stages of their formation, as shown in Figure 5. The results of the study agree in part with the study by Khayyat [22], which showed that when rabbits are exposed to mobile waves for three days, it leads to changes in the shape of the seminiferous tubules as well as the loss of sperm formation from the normal level. It also partially agrees with the study of Ahmed *et al.,* [23].

Group (the third) and after 17 days of exposure, a loss of numbers of sperms and the stages of their formation was observed, as well as simple changes in the shape of the sperm, but these numbers are simple compared to the groups (first and second) and as shown in Figure 6, and this was confirmed by the study of Duan *et al.,* [24] confirm that when exposing the rat to electromagnetic fields, EMF causes a change in the shape of the sperm as well as the germ cells that make up them, as well as what was confirmed by Yahyazadeh *et al.,* [25] who confirmed when exposing the rat to the field of magnetic rays that there are significant changes in the number of Sperm progenitors. These are the findings of the current study.

As for the results of exposure to the waves, after 35 days of exposure, it showed that there are changes in the testicular tissue (interstitial tissue), as well as the presence of rupture between one tubule and another and the emergence of spaces with the loss of tissue connecting to the (first) group, as shown in Figures 7 and 8. This was confirmed by Khayyat [22]. Also, the researchers confirmed that Khaki *et al.,* [26] studied the rat and found that when exposed to electromagnetic fields, EMF caused histological changes in the seminiferous tubules as well as in their constituent tissues.

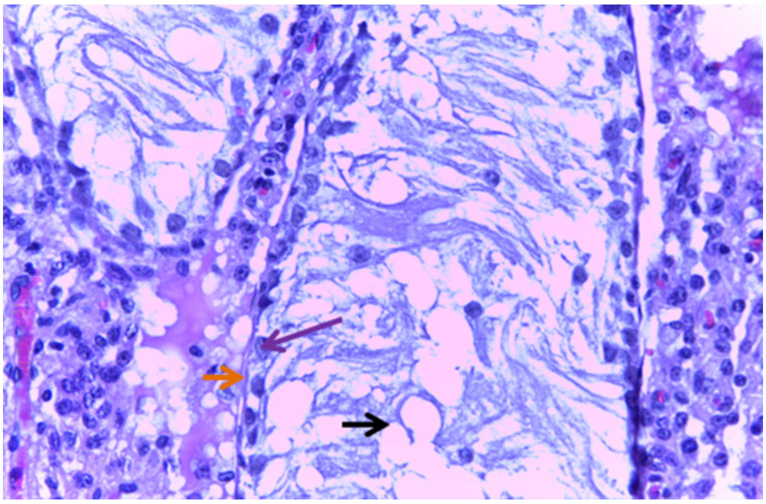

Fig. 5. A cross section of the testes of a rat of the second group for a period of 17 days showing the morphological changes in sperms and their decrease in their number and the loss of some stages of their formation, as well as the separation of the basement membrane at the walls of the spermatic tubule and the primary sperm cell (H&E) (400X)

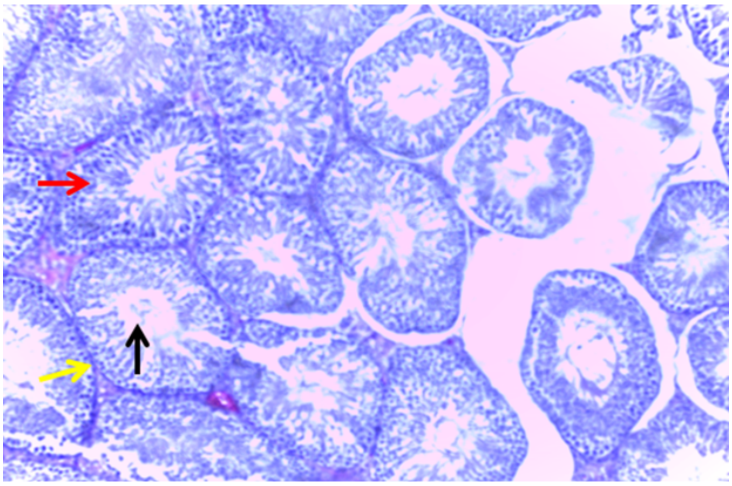

Fig. 6. A cross-section of the testicular tissue of a rat for the third group for a period of 17 days showing a slight change in the number of sperms and the loss of some stages of their formation and the primary sperm cell (H&E) (100X)

Also, the results of the current study are similar to those of researchers Odacı and Özyılmaz [27], who confirmed that exposure to radiofrequency for a period of two weeks led to the appearance of abnormal symptoms in the histological structure of sperm and seminiferous tubules. Also, the results of the current study agree with those of researchers Ozguner *et al.,* [28], who showed the presence of changes in the shapes of sperm in the testes of rats exposed to microwave radiation for a period of less than two weeks.

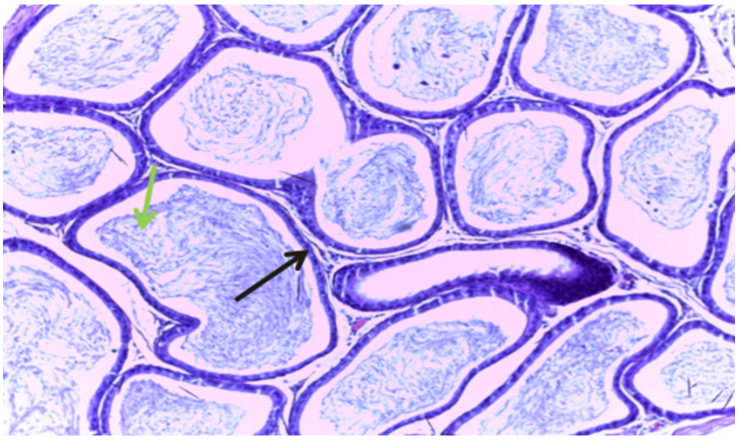

Fig. 7. A cross section of the rat testes tissue of the first group for 35 days showing the loss of sperm formation stages in addition to the low number of sperms (H&E) (100X)

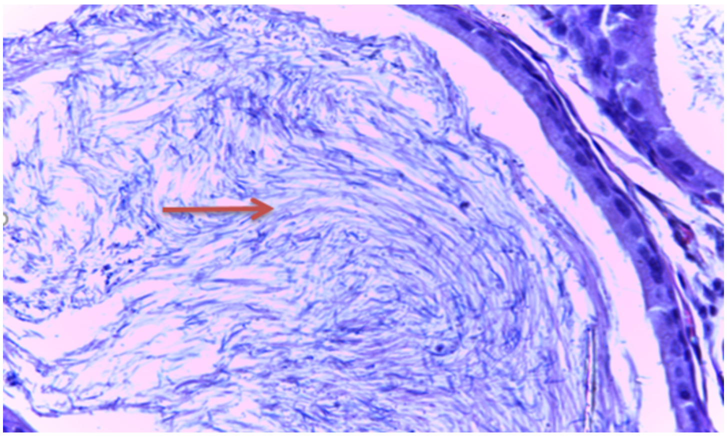

Fig. 8. A cross section of rat testes tissue of the first group for 35 days showing sperms, seminiferous tubules, tubule walls and the basement membrane of the tubule (H&E) (400X)

Likewise, the results of the second and third groups, are shown in Figures 9 and 10, respectively, where the first figure indicates the presence of blood bleeding in the seminal tubules and a decrease in the sperm count, while the second figure, within the time period of one 35 day, indicates a decrease in the sperm count. Changing shapes and the presence of space between the tubules, the stages of spermatogenesis, and the basement membrane.

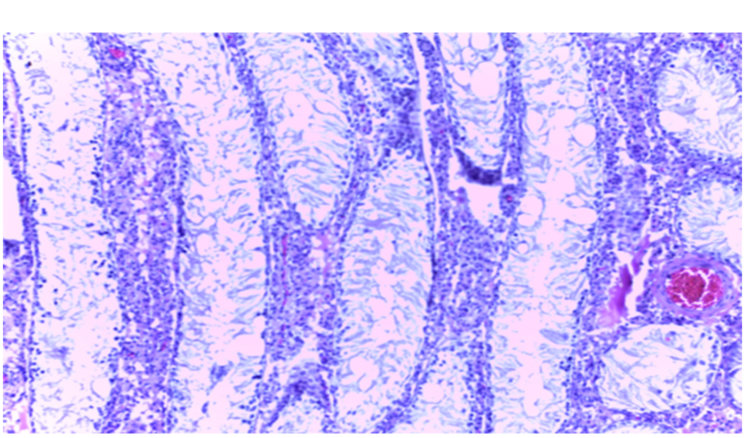

Fig. 9. A cross section of rat testes tissue for the second group for 35 days (H&E) (400X)

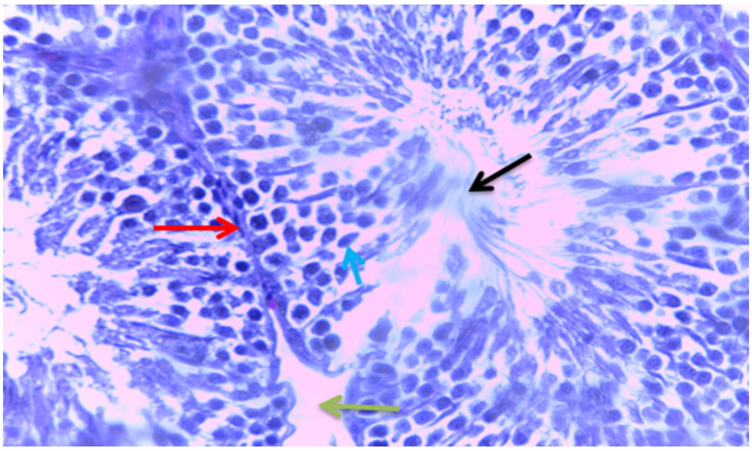

Fig. 10. A cross section of the rat testes tissue of the third group, showing low sperm count. Changing shapes and the presence of space between the tubules, the stages of spermatogenesis and the basement membrane (H&E) (400x).

The increasing rate of growth in the world today and the tendency of companies to improve the strength and quality of their equipment have raised questions about the health risks to factory workers and the general public. Therefore, this event provides an impetus for carrying out large research projects and collecting a large amount of data and clinical experimental observations. Before beginning any interpretation with respect to the results obtained, it is necessary to reconsider the basic phenomena involved in the fundamental interaction of electromagnetic radiation with biological systems. Therefore, the first step is to understand bio-electromagnetic bio-analysis so that users of microwave applications (the idea of research) are exposed to different frequencies in different countries of the world. The frequencies of these waves that are generated by these applications can affect the sperm, their shape and motility, and cause histological changes in the testicle through three mechanisms: MW-specific effect, thermal molecular effect, or a combination of both (of the two influences) [29].

Studies on animals indicate that electromagnetic field radiation has a wide range of harmful effects on the male reproductive system and sperm parameters, and this was confirmed by the current study, where it found clear histological changes to different degrees according to the different ages of the animals, and this difference can be explained depending on the value of penetration depth. Penetration depth waves of the target tissue, as its value is inversely proportional to the frequency used, and this is confirmed by relationship (2), as well as the electrical properties of the fabric, and since the experimental animals are of the same type and sex, they possess similar electrical properties, so the penetration depth depends on the frequency only, and this is the reason that led to the emergence of different histological changes in the exposed groups, where it was found that the histological changes are inversely proportional to the frequency (that is, the higher the frequency, the less effects on the testicular tissue). This confirms that ages play a role in reducing the dangerous effects of microwave radiation, where mutations are more harmful than adults. As a result of exposure to radiation, it is noted from the tissue sections that the third group is exposed to a frequency (i.e. the presence of a change). Simple histological results in addition to the presence of clear changes in the number of sperms, while in the group (the first) and the second, the changes were clear.

This result is consistent with the properties of high-energy (frequency) rays that cause the phenomenon of electronic build-up that occurs in the first millimeters of the skin, or subcutaneous tissue, and this means that high frequencies accumulate within the layers of the tissue, and this is the specific effect of waves [30].

As the study mentioned earlier, microwaves are fundamentally different from other energies, as they have the advantage that they work inside matter (that is, they penetrate matter and interact with every atom of matter), and therefore heat is generated. This feature in general, while the interaction of these waves with biological tissues is the interaction of the wave energy (frequency) with the entire tissue and not with the components of the tissue (cells), is because the wavelength of these waves is much greater than the dimensions of the cells (absorption cross-section) [31].

The increase in temperature during exposure to microwaves is a result of the increased movement of tissue particles when absorbing MW energy, as the tissue contains a high percentage of water, meaning the presence of dipoles. These are dipoles. In addition to other factors on which the increase in the temperature generated in the tissue depends, such as the duration of exposure, the efficiency of the body to eliminate the temperature, the thickness of the tissue, and the specific body area for exposure [32], any increase in the temperature of the testicle that has limited blood circulation and accordingly The efficiency of the testis to eliminate heat is limited, which leads to clear biological effects, and as a result, the cells most affected by these waves are sting cells, and the injury of these cells may affect the sperm because these cells are the ones that produce sperm, and ultimately sperm production decreases in the testicle, and this is what was found in this study.

It is worth noting that the biological effects and histological changes that were diagnosed on the tissue sections are due to the duplication of the two effects (limited and partial thermal).

4. Conclusions

The present investigation centers on examining the impact of microwave frequency in the gigahertz range (specifically 10 GHz). Furthermore, it seeks to analyze the alterations that develop in the different stages of sperm formation in laboratory rats of varying age groups. Due to the above-listed background and introduction, the recent discovery has identified that there were no histological changes in the control group because they have not encountered microwave waves. The histological examination results of the initial treatment group showed histological changes such as sperm voidance and depletion with different sections of sperm formation. It is clear that primary and short predictory cells suffered the most evident changes along with quantitative decrease of spermatozoa. Similar to the first treatment group, the second one shows histological changes affects not only the shape of sperm but also the stages of their development. There was a defined fall in the quantity of sperm and their decline. The same occurs with the third group The third group demonstrated histological changes showed a similar decrease in sperm quantity and stages of spermatogenesis, while the shape of sperm changed even less. However, it is important to note that the changes depicted a less severe alteration than in the first and second groups. With respect to the effect of microwave waves after the reception by the testicles of the presented for 5 weeks, cod is the following. It results in newly changes in interstitial tissue of the testis. Simultaneously the seminiferous tubules received a severe break into separately tubules and voids visualized after connective tissue has been spent. The present work has a valuable contribution demonstrating novel and potential directions for future researchers and functional and pathologic studies.

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