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INVESTIGATION OF SOME EFFECTS OF RADIO WAVES ON THE MALE REPRODUCTIVE SYSTEM

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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INVESTIGATION OF SOME EFFECTS OF RADIO WAVES ON THE MALE REPRODUCTIVE SYSTEM

By Sarah Abdulameer Gshayyish GSHAYYISH

February 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

INVESTIGATION OF SOME EFFECTS OF RADIO WAVES ON THE MALE REPRODUCTIVE SYSTEM

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Master of Science in Physics

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This study aims to investigate the increase in enzyme activity due to the increase in body temperature caused by exposure to radio waves and to reduce electromagnetic pollution by the optimal and correct use of these devices by finding the effect of radio frequency emitted by wireless devices on the male hormones. Communication technologies based on radio frequency (RF) emissions provide great benefits to our daily lives. However, the rapid development of these technologies raises concerns about their potential impact on public health. In the thesis study, we investigated the effects of non-thermal RF on the body temperature of mice and related mechanisms. Changes in estrogen and testosterone levels were observed in groups exposed to radio waves. It was determined that radiation application caused degeneration in testicular tissue and structural disorder in testicular morphology, melatonin alone had a positive effect on sperm production in healthy rats without radiation application, and melatonin given together with radiation application had a partial protective effect. It was found that the average body temperature in the light phase was higher in the exposed group compared to the control group. TRPM8 gene expression was not affected by RF in trigeminal ganglia.

2023, 65 pages

Keywords: Radio waves, Enzyme, Testosterone, Estrogen

ÖZET

RADYO DALGALARININ ERKEK ÜREME SİSTEMİ ÜZERİNDEKİ BAZI ETKİLERİNİN İNCELENMESİ

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Bu çalışma, radyo dalgalarına maruz kalındığında meydana gelen vücut ısısındaki yükselme nedeniyle enzim aktivitesindeki artışları araştırmayı ve kablosuz cihazların yaydığı radyo frekansının erkeklik hormonları üzerindeki etkisini bularak, bu cihazların optimal ve doğru kullanımı ile elektromanyetik kirliliği azaltmayı amaçlamaktadır. Radyo frekans (RF) emisyonlarına dayalı iletişim teknolojileri günlük hayatımıza büyük faydalar sağlamaktadır. Ancak, bu teknolojilerin hızla gelişmesi, halk sağlığı üzerindeki potansiyel etkileri konusunda endişelere yol açmaktadır. Bu tez çalışmasında, termal olmayan RF'nin farelerin vücut ısısı üzerindeki etkileri ve ilgili mekanizmaları araştırılmıştır. Radyo dalgalarına maruz kalan gruplarda östrojen ve testesteron seviyelerinde değişiklik gözlenmiştir. Radyasyon uygulamasının testis dokusunda dejenerasyona ve testis morfolojisinde yapısal bozukluğa neden olduğu, radyasyon uygulaması yapılmayan sağlıklı ratlarda tek başına melatonin uygulamasının sperm üretimi üzerinde olumlu etkisi olduğu, radyasyon uygulaması ile birlikte verilen melatoninin ise kısmi koruyucu etkisi olduğu tespit edilmiştir. Maruz kalan grupta ışık fazındaki ortalama vücut sıcaklığının, kontrol grubuna göre daha yüksek olduğu tespit edilmiştir. TRPM8 geninin ifadesi, trigeminal ganglionlarda RF'den etkilenmemiştir.

2023, 65 sayfa

Anahtar Kelimeler: Radyo dalgaları, Enzim, Testosteron, Östrojen

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Sarah Abdulameer Gshayyish GSHAYYISH
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CONTENTS

LIST OF SYMBOLS

%	Percentage
A/m	Ampere per meter
°C	Degree Celsius
g	Gram
kg	Kilogram
m^2	Square meter
mg	Milligram
mL	Milliliter
ng	Nanogram
nm	Nanometer
nmol	Nanomole
rpm	Revolutions per minute
µL	Microliter
µmol	Micromole

LIST OF ABBREVIATIONS

17β-HSD	17β-hydroxysteroid dehydrogenase
3β-HSD	3β-hydroxysteroid dehydrogenase
ABP	Androgen-binding protein
DNA	Deoxyribonucleic acid
EMF	Electromagnetic field
EMR	Electromagnetic radiation
FDTD	Finite difference time domain
FEM	Finite element method
FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone
HSF1	Heat shock transcription factor 1
LH	Luteinizing hormone
MRI	Magnetic resonance imaging
RF-EMR	Radiofrequency electromagnetic radiations
RFR	Radiofrequency radiation
ROS	Reactive oxygen species
SAR	Specific absorption rate
TSPO	translocator protein
UHF	Ultra high frequency
UNEP	United nations environment program
WHO	World health organization
Wi-Fi	Wireless fidelity

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1. INTRODUCTION

Human reproduction is complex and inefficient compared to other mammals, in addition to the active participation of the limbic system, the important role of environmental factors is also described. In the world, infertility is estimated between 15 to 20% in couples of childbearing age with free sexual demand; In Iraq, infertility affects approximately 15% of these couples (Al-Jebouri 2015). Similar figures are reported in the United States (10-15%) (Chandra *et al.* 2013) and England (10%) (Spencer *et al.* 2016). The etiology of infertility is very varied, with more than one cause found in a considerable number of couples (10% to 30%) and encompassing a wide range of physical and emotional factors (Direkvand-Moghadam *et al.* 2014).

1.1 Male Genital System

The male genital system of mammals is responsible for the continuous production, nutrition and temporary storage of sperm, in addition to their delivery to the female genital tract. This system consists of the testes, sperm pathways, attached sex glands and the copulatory organ, the penis (Mohanty and Singh 2017).

The testes are responsible for both the production of sperm and the production of androgens, which are essential for the maintenance of male fertility. In addition, the testes produce considerable amounts of estrogen, being one of the main sources of this steroid in males (Mesbah and Salem 2016).

The spermatic pathways comprise the straight tubules, testicular network, efferent ductules, epididymis, vas deferens and urethra, where sperm produced in the testes are transported to the female genital pathways during copulation. The epididymis is a transitional organ where spermatozoa develop and acquire the motility and fertilisation ability necessary to reach and fertilise the oocyte at its end (Ramírez-González and Sansone 2022).

Attached sex glands include the prostate, seminal vesicles, and bulbourethral glands, androgen-responsive organs whose function is to produce the constituents of seminal plasma, which are important for nourishing and maintaining sperm. Seminal plasma also functions as a means by which male gametes will be transported to the female genital tract (Ramírez-González and Sansone 2022).

1.2 Testicles

The testis corresponds to the male gonad, with exocrine and endocrine function, producing both sperm and hormones, including androgens that serve to maintain reproductive function and secondary sexual characteristics. The testis also produces estrogens from the aromatization of androgens (Titi-Lartey and Khan 2022).

The testis is surrounded by a thick capsule of dense connective tissue, called tunica albuginea, from which fibrous septa depart into the testicular parenchyma, dividing the organ into lobules. Morphologically, the testicular parenchyma consists of the seminiferous tubule compartment and the intertubular or interstitial compartment (Fietz and Bergmann 2017).

The seminiferous tubule compartment is neither innervated nor vascularized and constitutes the bulk of the organ in most species. Each seminiferous tubule is constituted by the tunica propria and seminiferous epithelium, delimiting the tubular lumen. The tunica propria has one or more layers of contractile myoid cells, depending on the species, and intercellular material, including collagen and elastic fibers and amorphous ground substance. The seminiferous epithelium consists of concentric layers of germ cells associated with a single type of somatic cell, the Sertoli cell. Germ cells develop in close interaction with Sertoli cells that play an important role in regulating spermatogenesis (Griswold 1998).

Sertoli cells perform specialized functions, most of which are related to the maintenance, proliferation and differentiation of germ cells. Among these functions,

there is the support and nutrition of cells, release of mature spermatids to the lumen of the seminiferous tubule, secretion of testicular fluid and proteins, such as androgen binding protein (ABP) and inhibin, among others. In addition to phagocytosis of degenerating germ cells and residual bodies resulting from spermiogenesis. Also noteworthy is the compartmentalization of the seminiferous tubule established by tight junctions that unite Sertoli cells together, forming the Sertoli cell barrier, which provides a protected and specialized environment for the germ cells. The Sertoli cell is still a key cell that mediates the actions of follicle stimulating hormone (FSH) and testosterone. In the intertubular compartment are found Leydig cells, associated with blood and lymph vessels, which are essential for the transport of hormones and nutrients in the testis, in addition to fibers and connective tissue cells, especially macrophages and occasionally mast cells. The Leydig cell has abundant smooth endoplasmic reticulum and mitochondria with tubular cristae, which contain enzymes associated with steroid synthesis (Russel *et al.* 1990), such as 3β-hydroxysteroid dehydrogenase (3β-HSD), responsible for several steps in the steroidogenic pathway, including the synthesis of androstenedione, a precursor of testosterone (Zirkin and Papadopoulos 2018).

Leydig cell-derived testosterone is essential for male sexual differentiation during embryonic development and for the onset and maintenance of spermatogenesis and the expression of secondary male sexual characteristics after birth (Smith and Walker 2014).

1.3 Physiology of the Sperm Process

Each testicle within the scrotum produces millions of sperm that are expelled in the semen. Spermatogenesis depends on the interaction of gonadotropins, testosterone, Y chromosome genes, intratesticular autocrine and paracrine factors, which gives rise to sperm in normal quantity and quality, which must find a permeable excretory pathway and an adequate seminal medium produced by the attached glands (Esteves and Miyaoska 2015).

Sperm is a liquid that contains sperm in suspension, which are produced in the testicle in two stages that last approximately 60 to 90 days in total; and according to different authors, it can be divided into two stages: spermatogenesis, which is the production of spermatids from stem cells, and spermiogenesis, which consists of a morphological modification of the spermatid into spermatozoa, with formation particularly of the acrosome and flagellum. In the testes, spermatozoa are immotile or motile in situ and nonfertilizing. These are evacuated towards the epididymis by the intratesticular spermatic ducts (straight tubules, rete testis) and epididymal reabsorption and secretion occur at the same time in the epididymis. Only here the spermatozoa acquire much of their progressive mobility and their fertilizing capacity. As the secretion of spermatozoa between two ejaculations is continuous, they are stored in the tail of the epididymis. At the time of ejaculation, the epididymal fluid that contains the sperm is expelled through the vas deferens that ends in the ampulla deferens, then passes through the ejaculatory ducts to the prostatic urethra. At this point the prostatic secretion is added. Then, in a second time, when the seminal vesicles contract, their secretion is discharged into the ejaculatory ducts. In total, the volume of sperm represents 5% of the epididymal secretion, 30% is prostatic secretion and 65% is secretion from the seminal vesicles (Marchiani *et al.* 2017). In order to make possible the comparison of the different series in the world, the International Andrology Association and the Group of Experts of the World Health Organization have recommended using a descriptive classification to carry out the measurements in a spermiogram with the aim of creating uniformity and avoid confusion in the final reports.

1.4 Spermatogenesis

Spermatogenesis is the process by which haploid cells, or male gametes, are generated from diploid germ cells, the spermatogonial stem cells (Chen *et al.* 2017). The production of haploid spermatids is the result of a highly organised and cyclical process in which immature germ cells divide, undergo meiosis, and then differentiate. Together with Sertoli cells, this process takes place in the seminiferous tubules. After spermatogenesis has occurred, spermatozoa are released into the tubule lumen and

travel the testicular network to the epididymis via efferent ductules. Fertilization-ready spermatozoa mature as they move through the epididymis.

Mammalian spermatogenesis can be broken down into three stages, each with its own set of morphological and functional hallmarks: (a) the proliferative or spermatogonial phase, in which spermatogonia undergo a series of mitotic divisions; (b) the meiotic or spermatocyte phase, in which spermatogonia already committed to differentiation form primary spermatocytes, which initiate the first meiotic division, forming secondary (Sharma *et al.* 2013).

1.5 Hormonal Control of Spermatogenesis

LH (Luteinizing Hormone) and FSH (Follicle Stimulating Hormone) are two gonadotropins secreted by the pituitary gland in response to GnRH (Gonadotropin Releasing Hormone), which is produced by hypothalamic neurons and essential for spermatogenesis (Figure 1.1). Kisspeptins are natural ligands for the orphan G protein-coupled receptor (Kitahashi *et al.* 2013). They regulate GnRH release. Kisspeptins positively and negatively feedback on the hypothalamic-pituitary-gonadal axis. They mediate testosterone's negative feedback on GnRH and LH's positive feedback (Marques *et al.* 2022). Testosterone, produced by LH-stimulated Leydig cells, regulates spermatogenesis induction and maintenance. It inhibits LH and GnRH release. FSH and T both have receptors on Sertoli cells and regulate spermatogenesis. When stimulated by FSH, these cells secrete glycoproteins crucial to healthy spermatogenesis, such as androgen-binding protein (ABP), which protects androgens from degradation while transporting them to the testes for concentration and release. male genital organs. Inhibin and activin are also secreted, with activin stimulating FSH production and inhibin regulating FSH release (Ramaswamy and Weinbauer 2014). Aromatization of androgens to estrogens mediates indirect negative feedback on gonadotropin secretion. Estrogen receptor alpha and beta are nuclear membrane proteins that mediate estrogen's physiological effects. Peritubular myoid cells, interstitial Leydig cells, Sertoli cells, and germ cells (except elongated spermatids) express ER, but spermatocytes do it best in the testis. ER is limited to Leydig and peritubular myoid cells. Estrogens regulate germ cell

survival, inhibit apoptosis, and stimulate germ cell proliferation, differentiation, and maturation (which become spermatids). Final sperm maturation is estrogen-dependent and delicate (Carreau *et al.* 2003).

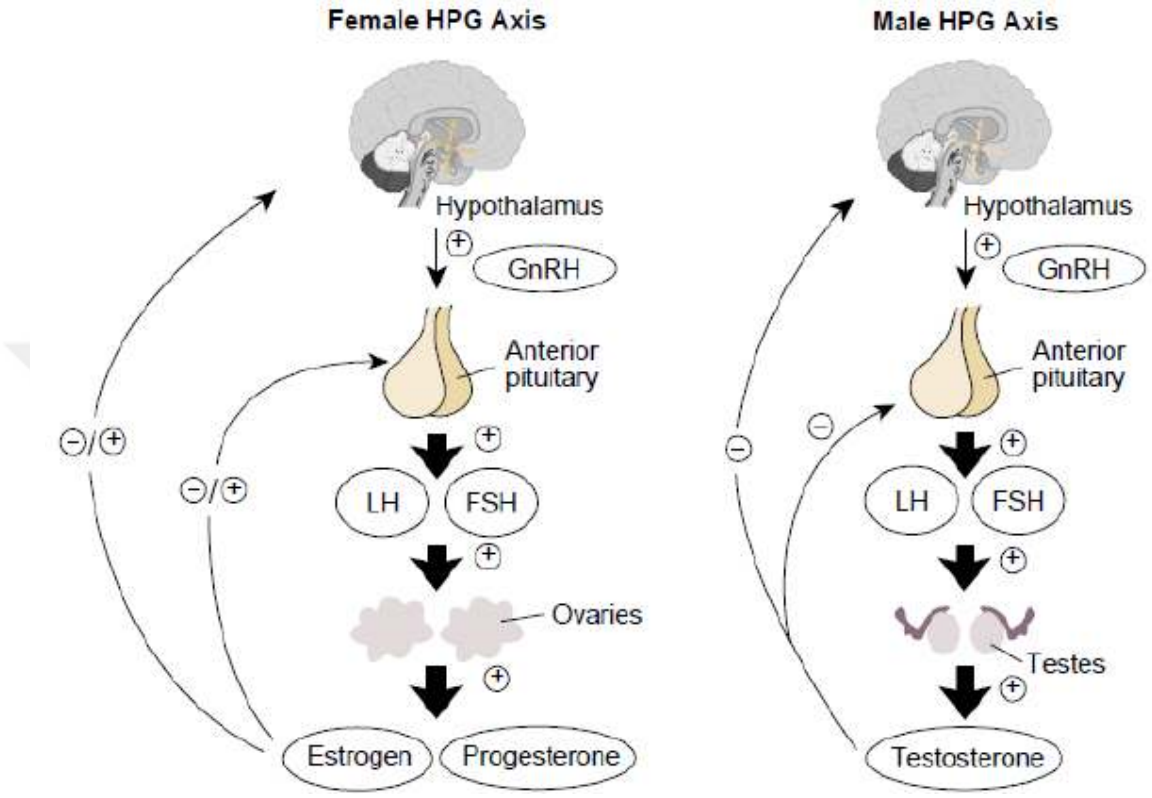


Figure 1.1 Schematic representation of the Hypothalamus-Pituitary-Testis axis

1.6 Steroidogenesis

Steroid hormones are compounds derived from cholesterol, which have 17 carbon atoms in their basic structure, forming the so-called cyclopentanoperihydronanthrene nucleus, with 4 non-planar rings A, B, C and D, joined together (Figure 1.2). Other carbon atoms may be added at positions 10 and 13 or to a side chain attached at carbon 17 (Nes 2011).

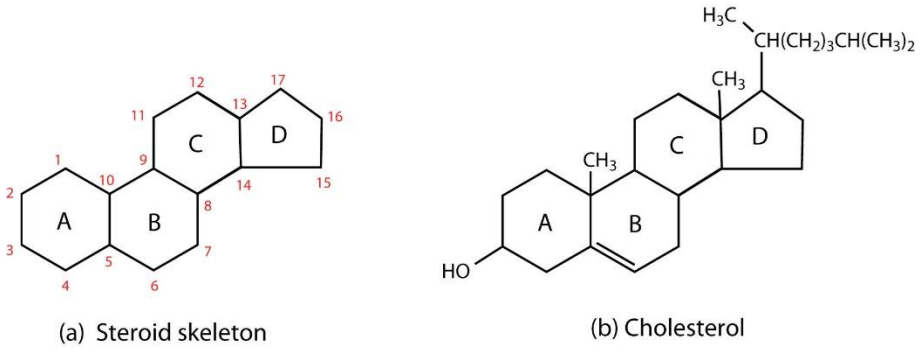


Figure 1.2 Basic structure of the cholesterol molecule, precursor of steroids (cyclopentanoperhydrophenanthrene nucleus with A-D nuclei and side chain)

Steroid hormones are synthesized in the adrenal glands, gonads, adipose tissue and the central nervous system. Different steroid hormones have different physiological effects, and the type of hormone produced depends on the arrangement of steroidogenic enzymes present in the tissue in question (Schiffer *et al.* 2019).

Steroid biosynthesis occurs in two steps: (1) transport of cholesterol to mitochondria and formation of pregnenolone; (2) metabolism of pregnenolone by steroidogenic enzymes in specific tissues. In the first step, the transport of cholesterol from the cytoplasm to the interior of the mitochondria is carried out by the protein StAR (steroidogenic acute regulatory protein) together with the translocator protein TSPO (translocator protein), a protein with high affinity for cholesterol that is located in the membrane. external mitochondrial membrane (Figure 1.3). Once in the mitochondrial matrix, cholesterol is cleaved into pregnenolone by the enzyme CYP11A or cholesterol side chain cleavage (P450scc) enzyme, present in the inner mitochondrial membrane. Free pregnenolone diffuses out of the mitochondria and from then on will be used for the formation of steroid hormones (glucocorticoids, mineralocorticoids, androgens, estrogens, progestins) (Miller and Bose 2011).

This next step of steroidogenesis depends on the action of the enzyme 3β-hydroxysteroid dehydrogenase, which converts pregnenolone into progesterone. Considering the testes, pregnenolone and progesterone are the precursors in the

biosynthesis of testosterone and, subsequently, estradiol (Schiffer *et al.* 2019) (Figure 1.3).

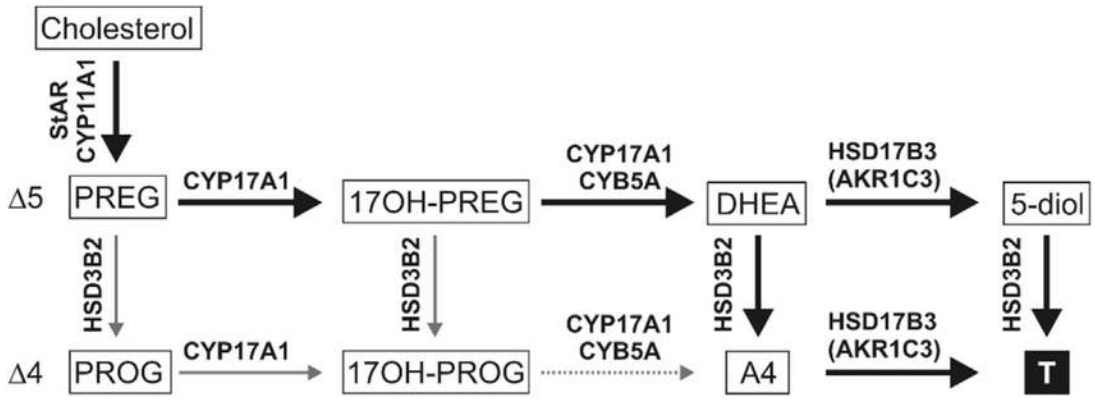


Figure 1.3 Steroid biosynthetic pathway in the testes

In the testes, steroidogenesis is basically composed of androgen production by Leydig cells. The most important androgen synthesized in the testes is testosterone, which is required for sperm production and maintenance of male sexual function (Schiffer *et al.* 2018). For its production, there is the participation of a cascade of enzymes, such as CYP17 (P450c17 or 17α-hydroxylase/C17-20 lyase), an enzyme that continues the biosynthesis of testosterone, inducing the conversion of progesterone to 17α-hydroxyprogesterone, and then to androstenedione. Finally, androstenedione is converted, through the action of the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD), into testosterone. Testosterone produced in the testes can be metabolized by the enzyme 5α-reductase to 5α-dihydrotestosterone (DHT), a metabolically more active androgen than testosterone (Russell *et al.* 1990), or it can be aromatized to 17β-estradiol by the action of the enzyme. P450-aromatase (Schiffer *et al.* 2018).

1.7 Electromagnetic Waves

Electromagnetic waves are made up of an electric field and a magnetic field.

Electric field: The electric field is a field that gives rise to forces that act on electric charges and that in turn is produced in the presence of electric charges. The intensity of the electric field is measured in volts per meter (V/m).

Magnetic field: The magnetic field is also a field of forces that are applied to electric charges, originating from the movement of these charges. The strength of magnetic fields is measured in amperes per meter (A/m), although a related quantity, flux density (μT), is more frequently used. Unlike the electric field that appears with the sole presence of electric charges, a magnetic field to originate requires the movement of at least some electric charge, that is, they only appear when an electrical device is started and current flows.

Electromagnetic fields: Energized and current-carrying electrical conductors, as well as antennas, are sources of both electric fields as they support electric charges and magnetic fields, as said charges are in motion. For this reason, it is referred to as an electromagnetic field (EMF), as it is in the simultaneous presence of both fields.

Absorption of electromagnetic waves by living things is dependent on the electric field's orientation in space. Radiation in the form of electromagnetic waves is produced when two or more electrically charged particles collide. The electromagnetic field is the area defined by this wave as it travels through space. When an electromagnetic field generates waves, such waves propagate at the speed of light (3×10^8 metres per second). The wavelength of a propagating periodic wave is the shortest distance between two consecutive points along the wave's path where the oscillation is in phase with itself. Water makes the wavelength shorter in biological media than air does. Electromagnetic fields permeate our world, yet they are too weak for the human eye to detect. Storms cause electric charges to build up in specific parts of the atmosphere, creating electric fields. However, developments in man-made radiation, such as electricity and radio frequencies, began to spread to all corners of the world at the turn of the twentieth century. Transformers, transmission lines, motors, freezers, electrical heating systems, communication systems, such as TV, radio, computers, and mobile phones, are all examples of devices that use electricity in various forms (Sgrignani *et al.* 2016).

People are naturally subjected to this type of fields, but also artificially due to all electrical devices. That is why the magnitude and frequency of the EMF to which an individual or group of individuals (population) is exposed in their environment must be determined (Czajka-Oraniec and Simpson 2010). Electromagnetic fields and their possible effects on human health are an issue of growing concern in society, which directly involves electric power and telecommunications companies, universities, regulatory bodies, municipalities, etc. It is a complex issue, but where there is already a significant amount of information prepared by international scientific organizations, which has resulted in a large number of publications where criteria, recommendations and guidelines are established and based. The position of the World Health Organization is that the different countries adopt the ICNIRP recommendations, since they are based on the best available information, as well as being issued by an independent body (Harada *et al.* 1999).

Directly health-based limits on exposure to time-varying electric, magnetic, and electromagnetic fields are referred to as "fundamental restrictions," and the current density, specific energy absorption rate, and power density are the physical parameters used to establish these limits (Selek *et al.* 2015). Below is presented in Table 1.1, the description of the electromagnetic spectrum, indicating the services that belong to each frequency band, highlighting the band that was evaluated in this project and that corresponds to the UHF (Ultra High Frequency).

Table 1.1 Description of the electromagnetic spectrum of different bands

Frequency Range	**Band**	**Description**	**Type of services**
30-300KHz	LF	Low frequency	Long wave radio and LF transmitters
300-3000KHz	MF	Medium frequency	AM radio, radio navigation
3-30MHz	HF	High frequency	CB radio, amateurs, HF radio communications
30-300MHz	VHF	Very high frequency	FM radio, VHF TV, emergency services, amateurs
300-3000MHz	UHF	Ultra high frequency	UHF TV, cell phones, amateurs
3-30GHz	SHF	Super high frequency	Microwave, satellite communications, radar, point-to-point microwave
30-300GHz	EHF	Extremely high frequency	Radar, radio astronomy, short microwave links

The UHF is a band of the electromagnetic spectrum that occupies the frequency range of 300–3000 MHz. The main systems that work in UHF are: television, radio links for non-professional use and mobile telephony.

The effects on human health that will result from the constant rise in electromagnetic radiation (EMR) exposure caused by the widespread use of wireless connection (WiFi: wireless fidelity) network technologies have started to draw attention by being more thoroughly studied in both social and scientific environments today (Akdag *et al.* 2016). Due to the fact that using the Internet has become a necessity in modern life, there is increased risk of electromagnetic fields (EMF) exposure from a variety of radiation sources, including WiFi, laptop and desktop computers, connected office equipment, and devices used at home, at work, in public places, and in schools (Hardell 2017). In the part of the electromagnetic spectrum known as non-ionizing radiations, which lack the energy to produce electromagnetic ionisation, are radiofrequency (RF: radio frequency) waves and microwaves (MW: microwaves). Mobile phones, WiFi-enabled telecommunications networks, military radar systems, and satellite communications all emit radiations known as radiofrequency electromagnetic radiations (RF-EMR), which cover a large portion of the electromagnetic spectrum in the frequency range of 3 kHz to 300 GHz. Although RF-EMR interacts with several biological creatures and affects physiological systems in both thermal and non-thermal ways, its mode of action is still not completely understood (Verma *et al.* 2019). A subset of RF-EMRs called microwave electromagnetic radiations (MW-EMR) have a high frequency range (300 MHz to 300 GHz) and can have an impact on our health depending on their frequency and power. Microwave ovens, short-range wireless connections, Bluetooth in mobile phones, cable-displacer devices such wireless printers, keyboards, and mouse for PCs, and microwave ovens all use these frequencies. So, in daily life, high frequency (2.45 GHz) microwave radiation is used by current cell phones, laptop computers, WiFi technology, and microwave ovens. Lipids, proteins, and nucleic acids, which are biological macromolecules of living cells that transport information in non-thermal (non-thermal) ways, interact with MW-EMRs (Megha *et al.* 2015).

The thermal effect, one of the health impacts of RF/MW-EMR, causes cellular functioning to deteriorate based on the local increase in tissue temperature. The power density and specific absorption rate (SAR: specific absorption rate) of electromagnetic waves released by radiation-emitting apparatus determine the extent of local warming. The interaction between radiation and body cells causes stress, which is the principal cause of the non-thermal (non-thermal) effect, in cells and tissues (Varghese *et al.* 2018). Watts per square metre (W/m^2) is a unit of measurement for power density, which is power per unit area. The rate at which energy is absorbed by the human body when exposed to RF-EMR is measured by the SAR parameter. The watts per kilogramme (W/kg) unit of measurement for this characteristic is the amount of power absorbed per kilogramme of tissue. In most cases, the SAR parameter displays the average value over either the entire body or a small portion of the body (typically 1 g or 10 g of tissue). The greatest level recorded in the bodily part under study for the given volume or mass is represented by this value. SAR ratings for mobile phones always refer to the highest transmit power achievable. In accordance with the safety standards established by radiation limitations regulating authorities in various countries, SAR is a trustworthy scale used to measure the RF-EMR exposure characteristics of mobile phones. Although the "FCC: Federal Communications Commission" has set a 1.6 watts per kilogramme (1.6 W/kg) SAR limit for mobile phones for public exposure, this figure exceeds the radiation restrictions of every nation 'regulating agency' requirements could be very different (Narayanan *et al.* 2019).

One of the most common mental health issues in the globe is anxiety and depression. Anxiety, fear, anxiety/anxiety, avoidance, withdrawal, and other anxiety-related symptoms, including medical and psychological problems that induce general symptoms and depression, which is an emotional (emotional) behavioural disorder characterised by extreme sadness and dysfunction (Narayanan *et al.* 2019). Due to similarities between how humans and animals exhibit their emotions and the impossibility of studying the effects of stress on humans due to ethical and related considerations, several experimental animal models and tests are employed to investigate behavioural problems (Koç *et al.* 2013).

In the case of Wi-Fi radiation, the effect at the cellular level is probably due to irregular blockage of ion channels in cell membranes, possibly caused by magnetic microfields, leading to disruption of cell electrochemistry and its functions. This refers to the fact that what is important is not the power of the Wi-Fi signal itself, since it is of low intensity, but rather the modulation of the signal, which is the way in which its intensity rises and falls when receive and send data from its internal antenna and it is this that potentially disturbs cell membranes and causes some of the effects named.

In the case of sperm cells, the problem is even more critical, since they are haploid, which means that they only have one set of genes and, if the cell is injured, it would not be able to repair the double strand of DNA if it were damaged break (Obajuluwa *et al.* 2017).

Exposure limits: One of the most controversial issues regarding the effect of electromagnetic fields is the maximum levels of exposure recommended to avoid health problems. Currently, the most accepted recommended limits are those published in 1998 by the ICNIRP, a non-governmental organization recognized by the World Health Organization (WHO) (Ibrahim *et al.* 2017). For the general public, 2 mA/m^2 is recommended. This value is called the basic restriction, that is, the level of current induced by an electromagnetic field that must not be exceeded in any case (Grandolfo 2009).

1.8 Association of Hyperthermia with Male Infertility

In the last 50 years and especially in Western countries, male infertility has been increasing. There is no doubt about this, studies carried out by international health organizations indicate that in 1940 men were capable of producing, on average, 113 million sperm per millimeter, a figure that has fallen precipitously to 66 million by 1990 (Kumar and Singh 2015).

Among the different causal factors of male infertility, there are some in which there is full agreement, such as: Klinefelter syndrome, Noonan syndrome and cystic fibrosis, but in others there is great discussion and uncertainty about their causes. Currently studies have shown a clear decline in the quality of human semen and an increased risk of male subfertility (Othman *et al.* 2017). The potential harmful action that some environmental factors seem to have on reproductive organs such as: tobacco, alcohol, the use of pesticides, ionizing radiation and high temperature has been suggested as a possible cause of this phenomenon. Studies on the effects of heat on mammals have shown that this is also the case with humans, whose testicles are temperature-dependent. In order to operate normally, the testicles need to be kept at a temperature that is 2 to 4 degrees Celsius lower than the rest of the body. In 1945, Badenoch was the first to record such a temperature disparity, and later authors have corroborated his findings (Thonneau *et al.* 1998). The scrotum is responsible for the initial level of regulation of testicular temperature; it lacks subcutaneous fatty tissue, and the total surface area of this skin varies with temperature, allowing it to dissipate heat to the outside if necessary. The second system of temperature control is found in the spermatic cords, where a countercurrent between the incoming arterial blood and the departing venous blood causes a temperature shift due to the loss of heat through the scrotal skin. As a result, the testicles receive pre-cooled blood. Several lines of evidence from studies of patients with cryptorchidism and varicoceles imply that heating the scrotal sac may be deleterious to sperm generation, both from the standpoint of the stem cell population and the later stages of the process the later phases of sperm development. Continuous exposure to high temperatures for an average of 8 to 10 years, generated sperm production disorders such as teratospermia and azospermia. According to a study conducted in Egypt comparing 40 fertile men (with an average of 2.5 children) with 90 men from the same factory who were exposed to heat (Kesari *et al.* 2018). Therefore, it can be stated that male infertility, which impairs morphology and ultimately leads to an inability to obtain pregnancies, is a risk factor for occupational exposure to high temperatures (Kesari *et al.* 2018).

Experimental investigations show that certain types of heat exposure decrease semen quality, and a high scrotal temperature is a common observation in infertile patients.

The average scrotal temperature rises as the number of men in sedentary jobs grows. The scrotal skin temperature of 99 healthy males was monitored continuously for 24 hours and correlated with samples of their sperm and blood. High scrotal temperatures were found to have a deleterious effect on sperm count. For every 1 °C increase in mean scrotal temperature throughout the day, sperm concentration reduced by 40%.

A healthy man's scrotal temperature shouldn't go above 33 degrees, says the World Health Organization. Spermatogenesis can be inhibited if the core body temperature rises above 38.5°C. The temperature difference between the body and the scrotum can be affected by many external factors (such as posture, clothes, lifestyle, and season). For quite some time now, experimental investigations in animals and humans have shown the effect that exposure to external heat has on male fertility. Indeed, epidemiological research has pointed to potential consequences of numerous occupational exposures on male fertility (Mieusset *et al.* 2007).

High temperatures have been shown to reduce sperm count and motility in the testicles when they are subjected to them on a frequent basis. Although scientists are still trying to determine the full extent of heat's impact on sperm production, some of the known impacts at the cellular level, based on studies primarily conducted in mice, are:

- Heat exposure slows DNA synthesis in the developing sperm nucleus, and it halts mitosis in the cells.
- Experiments on male rats show that heat exposure reduces inhibin production by 60%. Low levels of inhibin cause elevated FSH levels, which in turn triggers testicular cells to self-protect by eliminating FSH receptors from the cell surface, completing the negative feedback loop.
- Pachytenic spermatocytes and juvenile spermatids are more vulnerable to heat damage than mature sperm. Some of the processes of self-elimination in these sperm growth pathways are comparable to those utilised by other cells in the body, while others seem to be unique to sperm. The heat shock transcription factor 1 (HSF1) is activated in response to heat stress, however this does not result in the induction of heat shock proteins in male germ cells. Using HSF1 null animals, researchers were

able to demonstrate that HSF1 promotes apoptotic cell death of pachytenic spermatocytes exposed to stress, as pachytenic spermatocyte apoptosis was significantly decreased in testes with a single heat exposure and also in cases of cryptorchidism. thermal. However, in testes exposed to high temperatures, HSF1 also functions as a cell survival factor in more immature germ cells, perhaps including spermatogonia. The findings here show that HSF1 has two distinct functions in male germ cells, one of which is unrelated to whether or not heat shock genes are activated. How germ cells regulate their heat stress levels to be lower than those of other cells in the body is still a mystery.

- Furthermore, just 6 hours after being exposed to heat, these cells undergo programmed cell death. Studies in mice lacking a gene required for a specific activation pathway have shown (Khan and Brown 2002).

However, it has been found that heat-induced germ cell apoptosis is not completely prevented when heat-dependent apoptotic cell (FasL) is activated, demonstrating that heat-induced apoptosis in the testes can utilise other unique signalling pathways. Collectively, these findings support the hypothesis that mitochondria, and perhaps the endoplasmic reticulum, play a role in heat-induced apoptotic pathways during testicular germ cell death.

The effects of fever on spermatogenesis were studied in a group of 27 healthy males in a Danish study (mean age 24.4 years). Over the course of 16 months, they were monitored by collecting monthly samples of sperm and keeping daily records of any episodes of fever. The analysis included the total volume of sperm, the concentration of sperm, the proportion of immotile sperm, and the proportion of morphologically normal sperm. After a temperature increase during meiosis, sperm concentration dropped by 32.6% (p 0.01) and dropped by 35.0% (p 0.01) after a temperature increase during the postmeiotic phase of spermatogenesis (spermatids). Having a fever during spermiogenesis can decrease the percentage of sperm with normal morphology by 7.4 percent and increase the percentage of immotile sperm by 20.4%. The men's semen parameters changed dramatically with the length of time they were feverish. As a result, sperm concentration drops by 7.1% and 8.5% each day of fever during meiosis and

spermiogenesis. Per day of fever during spermiogenesis, the percentage of morphologically normal sperm drops 1.6% and the percentage of immotile sperm rises 4.5%. However, there was a wide range of reactions to fever (Carlsen *et al.* 2003).

Therefore, a fever during the postmeiotic phase of spermatogenesis has a detrimental effect on the concentration, morphology, and motility of a semen sample (spermatids). Fever has a detrimental effect on sperm concentration during the meiotic phase; however, fever does not appear to significantly affect these sperm parameters throughout other phases of spermatogenesis and a fever.

Similarly, several scientists (Garolla *et al.* 2013) have demonstrated such a decrease in spermatozoa motility following sauna exposure, despite no significant changes in seminal volume, cell count, or morphology. Infertility-inducing factors that are reversible within a week and do not alter the reproductive capacity. The same study also discovered that testosterone and gonadotropin concentrations in men did not alter, suggesting that sauna heat does not have a negative effect on fertility (Othman *et al.* 2017).

Research on healthy males has also revealed that changes in their sperm count were brought about by things such as their body position, the clothes they wore, and their surroundings. Human experiments have shown a decrease in spermatozoa concentration in the ejaculate and spermatogenesis when the testes were intermittently immersed in heated water, when athletic supports were applied, and when the testes were pushed into the inguinal canal, all of which raised intratesticular temperature. After the scrotal temperature normalised, reversibility was shown in these trials (Jóźków and Rossato 2017). This study joins an in vitro study on Wi-Fi radiation in sperm cells presented at the last world congress on Reproductive Health in 2010. This research intends to investigate these usage settings and determine if there is any interference with the regular functioning of the man's fertilising capacity under these circumstances (Krzastek *et al.* 2020).

1.9 Biological Effects

The scientific interest in carrying out studies that lead to establish the possible biological effects that electric fields generated by current could have on human beings is relatively recent (Kavya *et al.* 2014). It is based on the compilation of the most important information and it aims to to be the base that in the near future can count on norms that regulate the exhibition. Biological effects are measurable responses to a stimulus. It is important to note that not all biological effects are generators of a detrimental effect on health, that is, harmful to health (Cabal *et al.* 2005).

A biological effect occurs when exposure to an electromagnetic field causes some detectable physiological effect in a living system. Health effects are frequently the result of biological effects that accumulate over a certain period of time and also depend on the dose received. Therefore, knowledge of these is important to understand the risks generated for health. Electromagnetic fields, especially electromagnetic radiation in the radiofrequency and microwave range, have been related to different biological effects. It has been documented that they cause effects on biomolecules, cell proliferation, interference with immune processes, effects on reproductive capacity, genotoxic effects, effects on the nervous system, on the circulatory system and a decrease in the number of births (Balmori 2021). Table 1.2 below generalizes some biological effects produced by radiation and the possible processes involved.

Table 1.2 Biological effects and processes involved in them when radiation strikes

Processes and compounds of the human body	**Function**	**Conclusions**
Calcium Flow	Basic in the functions of neurons and other cells.	Its relationship to RF radiation exposure is unclear and no health risks have been clarified.
Melatonin	This hormone is produced by the pineal gland and is responsible for controlling the daily circadian rhythm.	Not many studies have been done on this subject and they do not suggest that there is any alteration in the function of the pineal or in the production of melatonin.
Movement of substances across the cell membrane	The membranes that protect the cell are extremely important for its proper functioning, having various functions such as receptors, detection, change activation or ion transport.	There is evidence that RF radiation can have effects on proteins in membranes and can alter the movement of ions. However, these effects are detected only at radiation levels that produce excessive heating. Effects on human health are uncertain.
Cancer and genes	Disease characterized by abnormal regulation of cell growth and proliferation. The possibility of generating cancer is always latent, without the need to have implicit genotoxic damage.	In relation to the genotoxic potential, there are reports on research works in most of which no significant genotoxicity has been found. There are no studies that demonstrate this epigenetic oncogenic potential of radiofrequency radiation.

2. LITERATURE REVIEW

2.1 Exposure to Electromagnetic Fields

Electronic items, in addition to their effect on scrotal temperature, emit radiation that is harmful to male reproduction. There is a growing demand for electronic items that operate at various frequencies and at the same time increase environmental exposure to electromagnetic fields (EMFs). Testicular function is particularly susceptible to EMF-emitted radiation due to the constant proliferation or mitosis of testicular stem cells and the meiosis that occurs when these testicular cells initiate differentiation during spermatogenesis.

Currently, EMFs have been associated as one of the environmental pollutants that affect the fertile capacity of exposed individuals. EMFs remain under study by various research groups with the aim of evaluating their effects on health, still obtaining controversial results, however, significant advances have been made in the characterization of their possible interactions with living organisms. Likewise, it has been studied whether or not the results obtained in vitro are potentially harmful in vivo, and whether these effects are transient or permanent. However, due to the methodology of these studies, it has not been possible to directly extrapolate its effects to human health. Adverse effects of low-frequency and radiofrequency EMF have been documented on testosterone, FSH, LH, and prolactin levels, in addition to affecting sperm parameters such as motility, morphology, concentration, vitality, acrosomal reaction, and DNA fragmentation. In individuals exposed to CEM, apoptotic events in testicular cells and ROS 44 production are increased.

During the last two decades, there has been a significant increase in the use of mobile telephony around the world. The effect of radiation from mobile devices on sperm parameters and male fertility is a topic of recent interest and is the subject of numerous investigations. There are several studies that demonstrate the direct in vitro and in vivo influence of non-ionizing radiation, such as that emitted by mobile phones or wireless

connections (Wi-Fi), which increase sperm DNA fragmentation and alter gamete mobility male affecting seminal quality (Gorpinchenko *et al.* 2014).

In an analysis carried out by (Avendano *et al.* 2012), working with seminal samples incubated under a laptop connected to the internet via Wi-Fi, and as control samples under similar conditions but without a computer; did not find differences in the percentage of dead spermatozoa ($p>0.05$) but found a progressive decrease in motility ($p<0.05$). On the other hand, (Yildirim *et al.* 2015) did not find alterations in the sperm parameters with the different times of use and portability of the mobile; but they found a lower concentration of progressively motile spermatozoa in the group of patients who used Wi-Fi internet and this decrease in mobility worsened the longer the time of exposure to this radiation.

Interaction of RF fields with tissues occurs depending on some physical, biological and environmental factors. Variables such as the physical dimensions of the biological structure, the dielectric and conductivity properties of the exposed tissue, the tissue geometry, and the position of the organism according to the polarization of radiofrequency radiation can change the interaction.

As radiofrequency radiation passes from one tissue to another tissue, some of the wave is reflected, while some of it passes to the other tissue (Perry *et al.* 2011). This RF energy transfer depends on the impedances (electrical -ε- and magnetic permeability -μ-, conductivity -σ-) of the tissues. RF radiation proceeds by expending its energy in the tissue and the wave energy decreases with the e-2 factor in the tissue (e=2.718). The distance in which the energy of the RF wave falls to $1/e^2$ (one in 7.38) within the tissue is defined as "impactness" (Vicenta Paparella *et al.* 2015). As the frequency increases, the propagation of the RF wave in the tissue, in other words, the penetration decreases and the RF wave at the same frequency has different intensities in different tissues. In general, the health and biological effects of RF radiation are classified in two ways as thermal and non-thermal (non-thermal). If an increase in temperature is observed in biological structures after RF exposure, biological changes due to temperature increase are observed. These changes are called thermal effects (Cook *et al.* 2011). As a result of

the temperature increase, viable attached cells may die or mutagenesis may occur. The temperature causing the death of cells was determined as 43 °C (Bungum *et al.* 2011). The thermal effects are caused by an increase in heat in the system due to increased molecular motion and friction. Dielectric properties are an important parameter for this effect. When RF radiation hits the body surface, some is reflected, some is absorbed by entering the body. As RF radiation passes through the tissue, its velocity changes depending on the electrical properties of the medium. When the dipole comes under the influence of an electric field, its direction is determined, it creates an order. If the field is variable with time, the dipole oscillates under the field effect and the heat increase due to oscillation occurs. With the increase in frequency; As the oscillation/vibration increases and the losses due to friction increase, the same results occur due to the increase in frequency. tissue warming; increases with frequency and field strength. When electric fields act on charged particles, they force them to move, and this causes changes in the system. If the applied electric field disappears, after a certain time, it is observed that the system returns to its original state. This time is called the relaxation time. The relaxation time depends on the particle charge, the properties of the medium and the temperature of the medium. As the temperature increases, the relaxation time decreases. The relaxation period for water molecules at room temperature is 10-11 seconds. Again, energy absorption for water molecules reaches a maximum in the range of 1–100 GHz. Although the applied radiofrequency radiation does not have a heat-increasing effect, if the RF field causes biological changes, these effects are defined as non-thermal (non-thermal) effects. The skin and other superficial tissues generally absorb the athermal radiation emitted by mobile phones, which causes an insignificant increase in temperature in the brain and other organs in the body (Hossain *et al.* 2010).

The 0–300 GHz frequency range standards, which have been created up to now and are widely used all over the world, are standards prepared by considering thermal effects. Since the power to raise the temperature of 1 kg of tissue by 1 °C is 4 W, the SAR value of 4 W/kg is based on the safety limit for humans and 1/10 (0.4 W/kg) of this value is the general occupational exposure standard. Radio Frequency Radiation limit values have been established based on 1 in 50 (0.08 W/kg) as a public standard.

2.1.1 Specific absorption rate (SAR, W/kg)

Radiofrequency Radiation (RFR) absorbed by the tissues causes a temperature increase in the tissue; This absorption, expressed in Watts per kg, is called the Specific Absorption Rate (SAR). In other words, the Specific Absorption Rate (SAR) is a measure of the amount of energy stored in the biological system exposed to RF. The commonly used unit is W/kg, but mW/gr can also be used. In addition, SAR can be defined as the electromagnetic energy absorbed by the living thing or as the energy absorption rate of the living thing. The amount of RF energy that raises the temperature of 1 kg of tissue that absorbs RF energy by 1 °C is defined as 4 W. As a result, the SAR value of 4 W/kg was taken as the dose at which biological effects could be observed in the establishment of the standards. SAR is generally used to discuss the biological effects of RF radiation in the kHz-10 GHz range. In the 100 kHz-10 MHz frequency range, SAR is used with current density. At actual live exposure, SAR cannot be measured. It can be calculated experimentally, by measuring in the phantom, or by computer modeling. The SAR calculation can be calculated with programs using the mathematical FEM (Finite Element Method) and FDTD (Finite Difference Time Domain) methods. In SAR studies, live models with at least three-dimensional MRI (Magnetic Resonance Imaging) images are used (Hoque *et al.* 2013).

2.1.2 Different models of mobile phones and their respective SAR values

In our country, mobile phones operate at frequencies of 900 MHz, 1800 MHz, 2100 MHz and 2600 MHz. The technical features of mobile phones are not the same, and the RF radiation they emit is not the same. It is possible to estimate the radiation emitted by mobile phones by knowing their SAR values. The SAR information of mobile phones of different models and brands can be accessed from the websites of the relevant companies and from the websites related to the health and biological effects of mobile phones (Varshney *et al.* 2018).

2.1.3 Literature studies on 900 MHz/1800 MHz or 800 MHz /1900 MHz EMR exposures

Five rats were randomly assigned to each of the following groups: group I (control), exposed to a mobile phone in the off position; group II (EMR), exposed to a mobile phone in the silent position; group III, exposed to a mobile phone in the vibration position; group IV, exposed to a mobile phone in the ringing position; and group V, exposed to a mobile phone in the silent, vibrate, and ringtone positions. An EMR source for the study was a mobile phone operating in the 900/1800 MHz GSM band. 30 missed calls (20 seconds each) totaling 10 minutes per day for four weeks were used to expose rats in Groups II and V to EMR. Additionally, biochemical analyses were used to detect the levels of lipid peroxidation (MDA levels), antioxidant enzymes (SOD, CAT activities), and glutathione peroxidase (GPx) activity in the rat brain tissues. Additionally, when compared to the control group, rats in groups IV (EMR+R) and V (EMR+R+V) showed significantly lower CAT activity (p 0.05). However, there was no statistically significant change in MDA concentration, SOD activity, or GPx activity between the control groups and other experimental groups (groups II, III, IV, and V). The study's findings showed that four weeks of EMR, vibration, ringing, or mixed exposures had a substantial impact on young Wistar Albino rats' oxidative stress (Shehu *et al.* 2016).

2.1.4 Effects on reproduction, growth and development

In many experimental studies done so far, it has been determined that lethal effects can only be seen with a biologically harmful temperature increase. High doses of RF radiation in rats have been reported to cause a decrease in fetal weight and an increase in miscarriage. In the epidemiological examination conducted on women working with short wave diathermy devices operating with 27 MHz Radio Frequency radiation and plastic welding machines operating at the same frequency, low and sick baby birth rates were observed compared to control groups (Heynick and Merritt 2003).

In studies conducted in the field of RAD Fadiation, only negative effects were not observed. For example, in one study, no abnormality was found in pregnant women exposed to RF fields below the standard values. In a study conducted on rats, it was found that when 1300 MHz RF radiation was applied at a dose of 7.7 W/kg, it did not harm the reproductive cells of rats (Forgács *et al.* 2005). In another study, when 100 MHz RF radiation exposure was applied at a SAR value of 0.018–0.023 W/kg, no statistical difference was found in the sperm quality and number of rats (Berman *et al.* 1980).

According to a study conducted close to the working range of our current research, it was determined that 2450 MHz RF radiation did not affect the testicles when applied to rats at a power density of 50 W/m^2 and a SAR dose of 0.9–4.5 W/kg. It was stated that even though the power density was increased to 100 W/m^2, the SAR value was increased to 2 W/kg, and 360 minutes of exposure was applied for 5 days, it still did not cause any change in the testicles (Berman *et al.* 1980). In the study conducted with 289 married radar workers and 148 married control group, a questionnaire was applied and RF exposure was measured. In the study, sexual dysfunction (reduction in giving birth) was observed in 43.6% of the radar workers, while this value was found to be 24.4% in the control group. As a result, long-term exposure to low-intensity RF radiation was found to be effective on reproduction (Yan *et al.* 2007).

2.2 Effect of Heat on Male Reproduction

Testicular function is temperature dependent, being regulated between 2 °C and 4 °C below body temperature by the scrotum, which structurally lacks adipose tissue with an appropriate surface to dissipate heat and also contributes to the spermatic cord, which has a heat exchange, which results in the pre-cooling of arterial blood upon arrival at the testis (Kierszenbaum and Tres 2015).

Heat has a direct inhibitory effect on spermatogenesis. High temperatures are deleterious for sperm production. In humans, heating the scrotum to 43 °C for 30 minutes causes a significant increase in germ cell death and an 80% decrease in the

number of sperm in the ejaculate. Gabrielli *et al.* (2011) demonstrated the deleterious effects of heat in retrospective studies of bakers and welders, concluding that occupational exposure to heat is a risk factor for male fertility.

Intermittent exposure to sources that radiate heat or prolonged contact of the testicles with body temperature by sitting for many hours a day, as occurs with drivers, alters male reproductive health (Vicenta Paparella *et al.* 2015) compared the concentration of immature germ cells in seminal samples of infertile men exposed to heat and observed an increase in the concentration of germ cells either by exposure to sources that radiate heat or by sitting for many hours, due to the rise in temperature scrotal due to prolonged body contact ($p<0.005$); found no differences between the means of exposed groups ($p= 0.2122$). The increase in testicular temperature produces disorders in the development of spermatogenesis and triggers apoptotic events in the cells of the germinal epithelium and in the spermatozoa; destabilizes and alters sperm DNA to the detriment of male reproductive capacity (Bouvet *et al.* 2003).

As a result of many studies examining the biological and health effects of Radio Frequency radiation, it is thought that the formation of free radicals lies behind the damage it causes. Oxidative substances, defined as free radicals, can damage proteins, enzymes, cell membranes, and DNA molecules, leading to disruption of many functions in the body. It has been shown that free radicals formed as a result of RF radiation exposure have harmful effects on the calcium balance in the body. This effect is more pronounced especially in the central nervous system, brain and heart. With the decrease in intracellular calcium level, disruptions occur in cell growth, reproduction and intercellular communication. The effect of RF radiation increases in direct proportion to the exposure time (Hauser 2006).

Another metabolic disorder caused by oxidative damage caused by free radicals is peroxidation of membrane lipids. Lipid peroxidation, which occurs as a result of complex chain reactions involving free radicals, means the disruption of the stable arrangement of polyunsaturated fatty acids, which are the basic components of the cell membrane, which is vital in cellular functions, and the formation of damage to the cell

by facilitating the entry of harmful molecules and ions that cannot enter the cell (Scaglia *et al.* 2009).

Recent studies show that RF fields are one of the important effects that cause oxidative stress by inducing the formation of free radicals.

2.3 Justification

Because the information available on the effect of exposure to electromagnetic fields is contradictory, for example in cancer, effects on reproductive capacity, genotoxic effects, effects on the nervous system, stress, among others, and that currently in Iraq there are no exposure limits, it is necessary to establish the possible biological effects on behavioural changes caused by radiation and thus propose levels of regulation of exposure to UHF radiation.

2.4 Hypothesis

If it is known that electromagnetic radiations have different biological effects; then, treatment with 860 MHz non-ionizing radiation in mice will cause mice reprotuctive system.

2.5 Objectives

- To measure testosterone concentration and estrogenic hormone before and after exposure to the waves.
- To compare the effect on when irradiating a 860 MHz wave in mice.
- To determine the hormonal changes of corticosterone and testosterone in blood by irradiating an 860 MHz wave in mice.
- To investigate whether 860 MHz frequency Radiofrequency Radiation causes oxidative damage in testicular tissues of male rats.

3. MATERIALS AND METHODS

3.1 Animal Samples and Groups

Within the scope of the research, the experimental groups were formed from 35 healthy adult male Wistar Albino rats, 12-16 weeks old and weighing 230±15 g. Experiments on rats were carried out in the laboratory. Within the scope of the study, 35 rats were designed to form 5 groups. RF radiation application times are arranged according to Table 3.1. The first group is the control group and no application was made. RF radiation exposure was applied with a ESIME Zacatenco radiation equipment, field levels were measured with EMR 300. At the end of the 30-day experiment period, one of the testicular tissues belonging to the groups was preserved for oxidative damage examination, and the other testicular tissue for histological examination.

Paraffin blocks were obtained after the routine follow-up procedures were fixed in 10% neutral formaldehyde solution for at least 72 hours, and 4–5-micron thick sections were taken from the prepared paraffin blocks. Testicular tissues of the groups were examined by immunohistochemical methods. Immunohistochemical uptakes were evaluated for each antibody and for each group, and data were generated using Kruskal-Wallis and Mann-Whitney analysis (Zhang and Zhang 2009) to determine whether there was a significant difference in uptake intensities between groups. Parameters for oxidative damage were investigated in testicular tissue.

3.2 Equipment

- Analytical balance. Brand: Ohaus; Model: E4000
- GTEM cell (Transversal Electromagnetic in Giga Hertz), an amplifier and a signal generator.
- Test apparatus with two compartments
- Dark compartment
- Enzyme immunoassay kit for corticosterone. Brand: Detect X

- Testosterone kit. Brand: EIADSL-10-4000

3.3 Methodology Irradiation of Rats

The study was performed with 35 male rats, all were weighed and marked (Table 3.1). They were then randomly divided into five batches:

Table 3.1 Separation of 35 rats in to 5 groups

Group Name and Condition	**TREATMENT**
Control	No treatment
SUMMER RADIATION (RAD J)	Radiation 35 days at 860 MHz. 4 hrs daily in summer (June). (June 01 to July 05; 2022)
WINTER RADIATION (RAD F)	Radiation 35 days at 860 MHz. 4 hrs daily in winter (February). (February 01 to March 05, 2022)
RADIATION 15 DAYS (RAD D)	Radiation 15 days at 860 MHz. 4 hrs daily. (April 1 to April 14, 2022)
FALSE RADIATION (RDA F)	Isolation 15 days 4hrs daily. (April 1 to April 14, 2022)

The radiation was carried out in the Electromagnetic Compatibility Laboratory, the equipment used to irradiate the rats was a GTEM (Transversal Electromagnetic in Giga Hertz). Cell, an amplifier and a signal generator. A photograph of this equipment is shown in Figure 3.1.

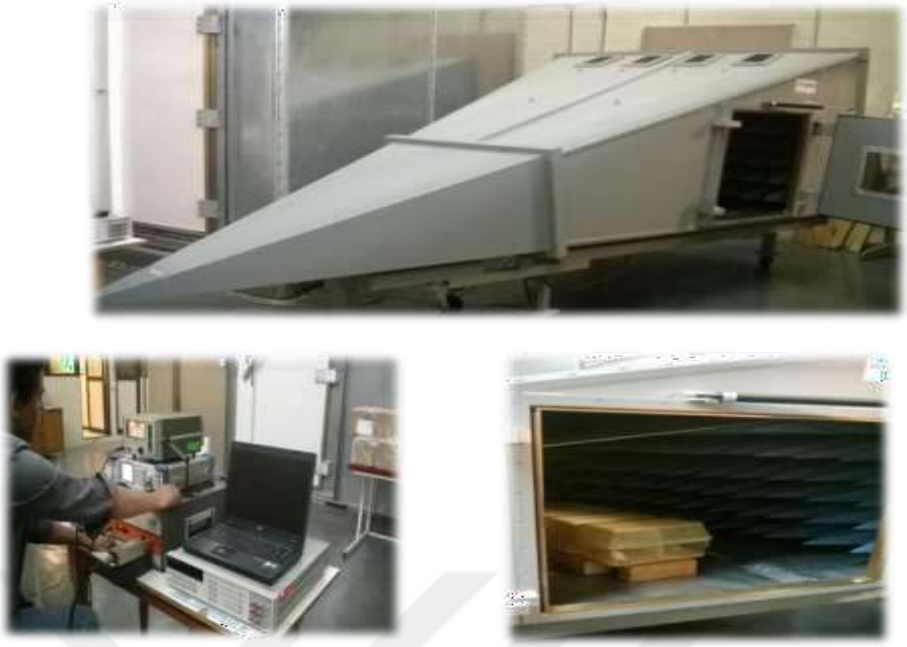


Figure 3.1 ESIME Zacatenco radiation equipment

The radiation batch (UHF) was subjected to a frequency of 860 MHz and a power of 0.5 W in the radiation equipment, for 4 hours/day for 35 days in winter and in summer, a second batch was subjected to this same radiation only for 15 days, taking care that the temperature was between 23-25°C and a humidity of 70%, without access to food or water.

A group of rats was used to submit it only to isolation for 4 hours a day without being exposed to radiation and a group of control rats which did not receive treatment.

3.4 Biochemical Analysis

3.4.1 Histological method

Before any microscopic examination could begin, all testicular tissue samples were fixed in 10% formaldehyde solution. Tissue samples were fixed, then transferred to cassettes and washed for 24 hours in running water. Tissues were dehydrated by being processed through a series of increasingly strong alcohol solutions (70%, 80%, 90%,

100%). After being polished in xylol, the tissues were embedded in molten paraffin. Sections of paraffin blocks (4-5 microns thick) were obtained, stained with haematoxylin and eosin, and evaluated in the LAS software to produce the corresponding figures.

3.4.2 Haematoxylin–eosin staining method

Preparation of dye solutions

1-Harris hematoxylin solution

- Hematoxylin 1 gr
- Alcohol 10 mL
- Potassium alum (aluminum potassium sulfate) 20 gr
- Distilled water 200 mL
- Mercury oxide 0.5 gr
- Glacial acetic acid 8 mL

2-Eosin solution

- Eosin 1 gr
- Distilled water 100 mL
- 1 small crystal of thymol

After the sections taken from the experimental groups were kept in an oven at 60 °C for 30 minutes, they were removed from xylol for 2x15 minutes and cleared of paraffin. Then, the slides were passed through decreasing series of alcohol (100%, 96%, 80%, 70%, 50%) and air-dried. After 10 minutes of washing in running water, they were stained in Harris Hematoxylin for 10 minutes and washed in running water for 10 minutes. It was dipped in 70% alcohol+2-3 drops of glacial acedic acid mixture and washed again in running water for 10 minutes. After the slides were kept in Eosin for 15

minutes and washed in running water for another 10 minutes, they were passed through a series of increasing grades of alcohol (50%, 70%, 80%, 96%, 100%), taken in xylol for 2x15 minutes and closed with entellan. In order to generate statistical data; 6 tubules were selected randomly for each subject, and the seminiferepithelial length was measured in 6 different regions in each tubule, and the data were recorded.

3.5 Determination of MDA in Tissue

MDA levels in the tissue were studied with the spectrophotometric method with the ready kit. Solutions Used: 10% tricarboxylic acid (TCA), 1% butyl hydroxy toluene (BHT), 0.67% thiobarbituric acid (TBA) in 95% alcohol. Tissue samples were homogenized with a weighed homogenizer in cold TCA (1 g tissue+9 mL 10% TCA) on ice. Then, the homogenate was centrifuged at 4.000 rpm for 15 minutes at +4°C, and the supernatant was taken and centrifuged again at 4.000 rpm for 8 minutes. 750 µL of the sample was taken, 750 µL of 67% TBA and 10 µL of 1% BHT were added to it, mixed thoroughly with vortex, and kept in a boiling water bath for 15 minutes, then cooled to room temperature and centrifuged at 4000 rpm. The optical density of the samples was read in the spectrophotometer at 535 nm, against the blank, by taking the supernatant. Tissue MDA levels were calculated in nmol per g tissue.

3.6 Determination of Glutathione in Tissue

Glutathione (GSH) in tissue was studied with the spectrophotometric method with the ready kit. Solutions used: 0.3 M NaH_2PO_4 DTNB-dithio nitro benzoic acid (0.4 mg/mL 1% sodium citrate). Tissue samples were weighed and homogenized with a homogenizer in cold TCA (1 g tissue+9 mL 10% TCA) on ice. Then, the homogenate was centrifuged at 4.000 rpm for 15 minutes at +4 °C, and the supernatant was taken and centrifuged again at 4.000 rpm for 8 minutes. 2 volumes of supernatant were mixed with 8 volumes of NaH_2PO_4 and 1 volume of DTNB solution. After waiting 5-10 minutes at room temperature, the absorbance of the mixture was read in the spectrophotometer at a wavelength of 412 nm versus the blank, and tissue GSH levels were calculated per µmol/g tissue.

3.7 Determination of NOx in Tissue

Total NOx amounts were studied with the ready kit using the spectrophotometric method. Tissues were homogenized with 5 layers of phosphate buffer (pH=7) and cooked at 2000 g for 5 minutes centrifuged. After the addition of NaOH to the supernatant, 5 min at room temperature wait, 5% $ZnSO_4$ is added, 300 g for 20 minutes centrifuged and read on the Elisa reader.

3.8 Determination of GPx in Tissue

It was studied with the ready kit using the spectrophotometric method. Tissue samples were washed with PBS solution containing 0.16 mg/mL heparin, pH 7.4. It was homogenized with 5-10 mL of homogenization solution (50 mM Tris-HCl, pH 7.5, 5 mM EDTA and 1 mM DTT) per gram tissue. The samples were centrifuged at 4 °C, 10.000 x g for 15 minutes, the supernatant was removed and stored at -80°C until they were studied.

3.9 Analysis of Results

The results were analysed with the statistical program Sigma Plot 12.0 and the tests that were carried out were ANOVA unifactorial, and ANOVA bifactorial followed by the test that best adapted (Shapiro-Wilk, T-student, Newman-Keuls), suggested by the program. A significant difference was considered with a $P \leq 0.05$.

4. RESULTS AND DISCUSSION

4.1 Results

Testosterone is one of the most important male hormones produced by the testes, and the pituitary gland and hypothalamus control the production of male hormones and sperm.

Testosterone is one of the most important male hormones in men. It is produced by Leydig cells in the testes. The pituitary gland and hypothalamus control the production of male hormones and sperm.

Luteinizing hormone and follicle-stimulating hormone produce testosterone, which are important hormones sent from the pituitary gland and act on the testicles.

The estrogen hormone has many functions in the body, as it maintains the reproductive system and female characteristics, and the estrogen hormone is present in males and females, but it is produced in larger quantities in the female bodies, as it is responsible for the physiological changes that contribute to puberty in mice, and in regulating the menstrual cycle, and childbearing.

The FSH hormone stimulates and releases testosterone (testicles) in male mice, where we notice through the results that there is no change in their proportions, that is, there is a slight change

As the LH hormone is associated with the FSH catalyst that helps in the reproduction process, as the lack of this hormone leads to a lack of sex hormones.

The LH hormone plays an important role in maintaining the health of the reproductive system in the body, and it also has an important role in controlling ovarian secretions of

estrogen and progesterone in female mice, and testicular secretions of testosterone in male mice.

We note from the aforementioned results that the effect of frequencies and waves on these variables did not change or a slight change was observed that did not appear to have an effect on the rats (Table 4.1).

Table 4.1 Hormone values of male mice after radio waves

No	Testesterone	Estrogen	FSH	LH
1	352	2.123	0.8	0.24
2	101.14	1.07	0.24	0.15
3	147.88	7.67	0.69	0.11
4	218.93	6.58	0.61	0.37
5	342.8	7.71	0.68	0.49
6	347.99	1.13	0.75	0.25
7	410.22	7.53	0.23	0.44
8	216.76	5.35	0.55	0.73
9	128.93	6.51	0.34	0.49
10	325.93	6.67	0.45	0.58
11	452.82	9.7	0.24	0.23
12	347.39	6.46	0.82	0.11
13	360.16	6.72	0.65	0.16
14	111.63	2.68	0.47	0.12
15	125.56	5.53	0.17	0.05
16	132.56	4.28	0.21	0.78
17	122.87	7.13	0.66	0.12
18	137.47	8.99	0.96	0.27
19	347.25	4.07	0.01	0.22
20	251.84	5.48	0.1	0.79
21	257.4	9.87	0.41	0.43
22	154.34	5.24	0.75	0.69
23	354.26	4.87	0.32	0.52
24	194.45	1.61	0.16	0.27
25	153.58	7.28	0.78	0.73
26	293.63	4.72	0.04	0.14
27	243.24	8.16	0.54	0.11
28	123.24	3.51	0.59	0.76
29	100.23	4.68	0.35	0.55
30	135.21	1.55	0.07	0.51
31	115.78	3.01	0.24	0.23
32	271.56	4.65	0.24	0.23
33	214.95	9.36	0.05	0.07
34	108.33	3.52	0.90	0.39
35	107.24	8.69	0.55	0.12

- The normal level of testosterone; 90-290

- The normal level of estrogen; 1.10-8.50
- The normal level of FSH; 0.10-0.75
- The normal level of LH; 0.12-0.70

All of these ratios were measured in pg/mL.

The results in the estrogen and testosterone analyzes showed a slight change in radio waves and frequencies to measure testosterone concentration and estrogenic hormone before and after exposure to the waves.

There was a slight change in the results of the study, but radio waves and frequencies did not affect significantly to determine the hormonal changes of corticosterone and testosterone in blood by irradiating an 860 MHz wave in mice.

4.2 Determination of Corticosterone and Testosterone

The results of the concentration of the hormones corticosterone and testosterone are presented in Figure 4.1 and Figure 4.2, where no significant difference was found between the treated groups with respect to the control.

Figure 4.1 shows a trend of decreased corticosterone concentration in the RAD J, RAD D and RDA F groups with respect to TG and a slight increase in the corticosterone concentration in the RAD F group (APPENDIX 1).

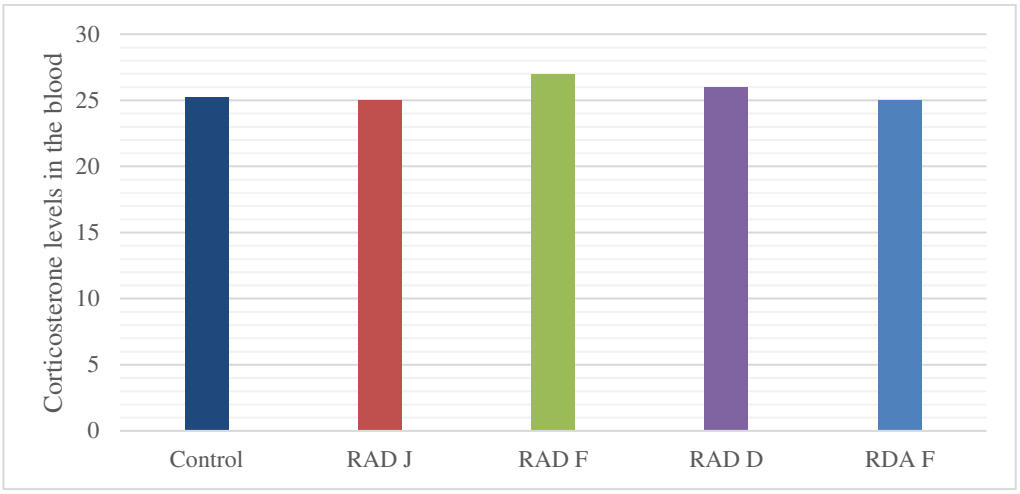


Figure 4.1 Blood corticosterone levels of rats after irradiation with a UHF frequency of 860 MHz (one-way ANOVA, (*p≤0.05))

Figure 4.2 shows an increasing trend in testosterone concentration in the 35-day treatment groups (RAD J, RAD F) with respect to TG and a decreasing trend in testosterone concentration in the RAD D and RDA F groups (APPENDIX 2).

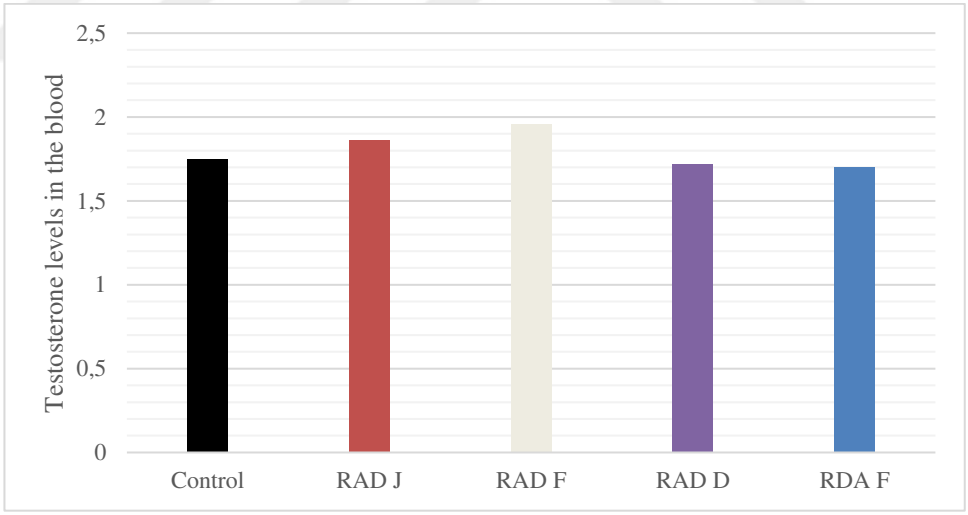


Figure 4.2 Testosterone levels in the blood of rats after irradiation with a UHF frequency of 860 MHz (one-way ANOVA, (*p≤0.05))

Body Temperature Following Exposure

All rats RAD J; RAD F; RAD D and RDA F exposed to power level of Radiation (860 MHz) survived to the end of the studies. In RAD J rats exposed to radiation, mean body

temperatures were significantly higher in the groups exposed to RAD F compared to controls. Overall, mean body temperatures were elevated at more individual time points with increasing RAD J compared to time-matched controls.

Temperature Laboratory

High temperature in the laboratory slows down the action of the waves that the mice are exposed to, as these aggregates were exposed to a temperature of 37 degrees, and no change was shown, and they were exposed to a temperature of 37.5 and 38 degrees. Anti-waves and frequencies, also reduces impacts on mice.

As the study conducted for these groups showed that they usually raise mice at temperatures much lower than the degrees during which they observed a decline in radio waves, explaining that this did not affect the results of their study, or a slight change was observed in the results.

It is known that mice prefer temperatures closer to warmth in general, and that if they were given the choice, they would choose to live in a medium with a temperature of 37 to 37.5 degrees Celsius. Mice are kept in most laboratories at a temperature ranging 37 degrees Celsius for several reasons, among these reasons is that the mice changed the system of converting the waves and frequencies they were exposed to into energy for them to continue to maintain their normal temperature, which cost them a lot of energy.

To see how much this affects the mice's ability to counteract the frequencies, the temperatures need to be between 37.5 to 39 degrees Celsius, or even higher, 38 to 39 degrees Celsius.

They found that the frequencies occurred more slowly when the mice were living at a higher temperature, and that the frequencies and waves they were exposed to were lower when the temperature was raised.

As the temperature in the laboratory was within the limits of 37 and 38, and no change in the waves appeared.

Studies have shown that mice that live at higher temperatures form frequencies and waves that occur less to mice, and that mice that live in a lower temperature have more waves and frequencies.

Where the study concluded by taking the effect of the temperature of the environment in which a person lives when searching for the effect of waves and frequencies.

4.3 Biochemical Analysis

4.3.1 Hematoxylin-eosin staining findings

In the Hematoxylin-Eosin staining of the testicular tissue, the Control and Sham Control groups were observed with normal structures of the seminiferous tubules of the tissue and the interstitial connective tissue located between these tubules. Spermatogenic cells located in the germinal epithelium of the seminiferous tubule; spermatogonia with dense nuclei located in the basal, Spermatocytes I (Primary Spermatocytes) attracting attention with their large appearance in the upper row, and spermia in the other layers with their tails oriented towards the lumen. It was observed that the lumen of the seminiferous tubule was densely filled with spermium. Again, among the spermatogenic cells, pyramidal shaped Sertoli cells extending from basal to apical were observed with their normal structures. Numerous capillaries and interstitial (Leydig) cells in groups were seen in the interstitial space between the tubules with their normal structures (Figure 4.3) (Figure 4.4).

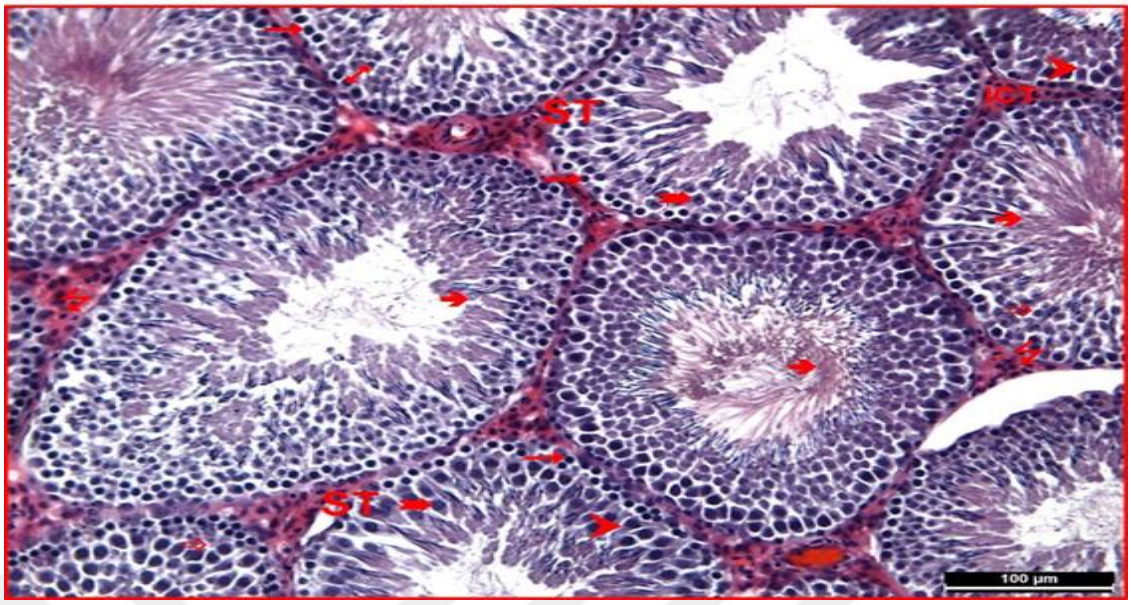


Figure 4.3 In the testicular tissue of the control group, seminiferous tubule (ST), interstitial connective tissue (ICT), germinal epithelium (↬), spermatogonium (→), primary spermatocyte (➟), spermium (➔), Sertoli cell (➤), capillaries (⇨), and Leydig cells (⇳) is monitored (Hematoxylin-Eosin x200)

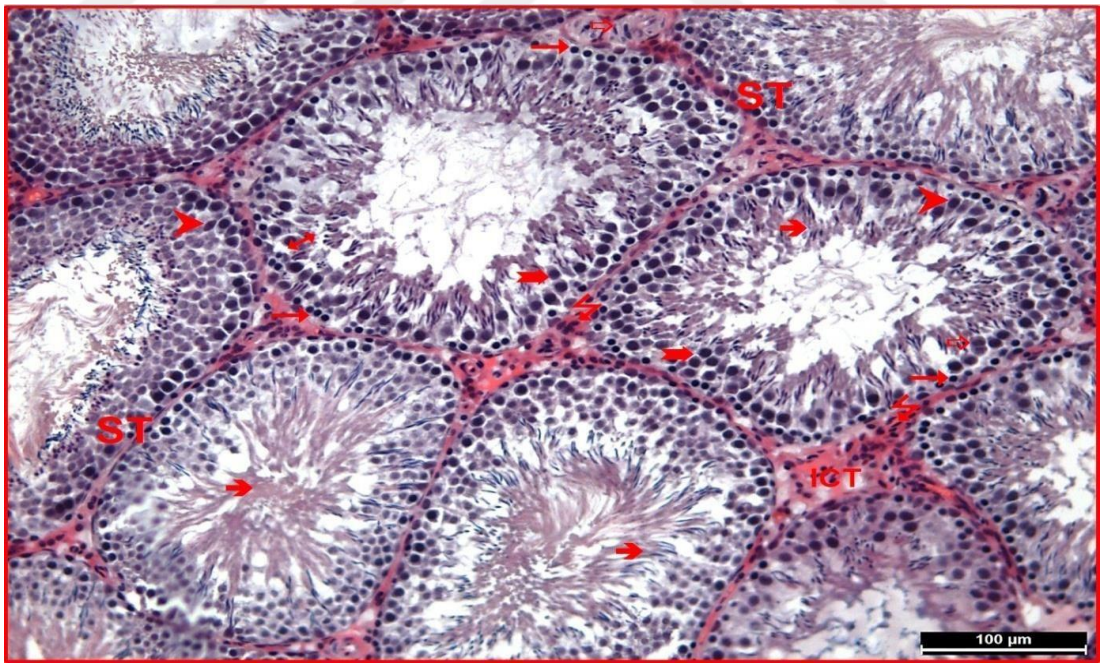


Figure 4.4 Seminiferous tubule (ST), interstitial connective tissue (ICT), germinal epithelium (↬), spermatogonium (→), primary spermatocyte (➟), spermium (➔), Sertoli cell (➤), capillaries (⇨), and Leydig cells (⇳) is monitored (Hematoxylin-Eosin x200)

In the Hematoxylin-Eosin staining of testicular tissue belonging to the Control Melatonin and Sham Melatonin groups, it was observed that the general structure was identical to the Control and Sham Control groups. Apart from the control groups, it was noted that spermatocytes were relatively higher in some seminiferous tubules in the melatonin-administered groups, and the presence of relatively dense spermium in the lumens of some tubules. This finding was interpreted as melatonin administration may have increased sperm production. In the examinations of the Sham Control group, it was also noted that some capillaries in the interstitial area were dilated (Figure 4.5) (Figure 4.6).

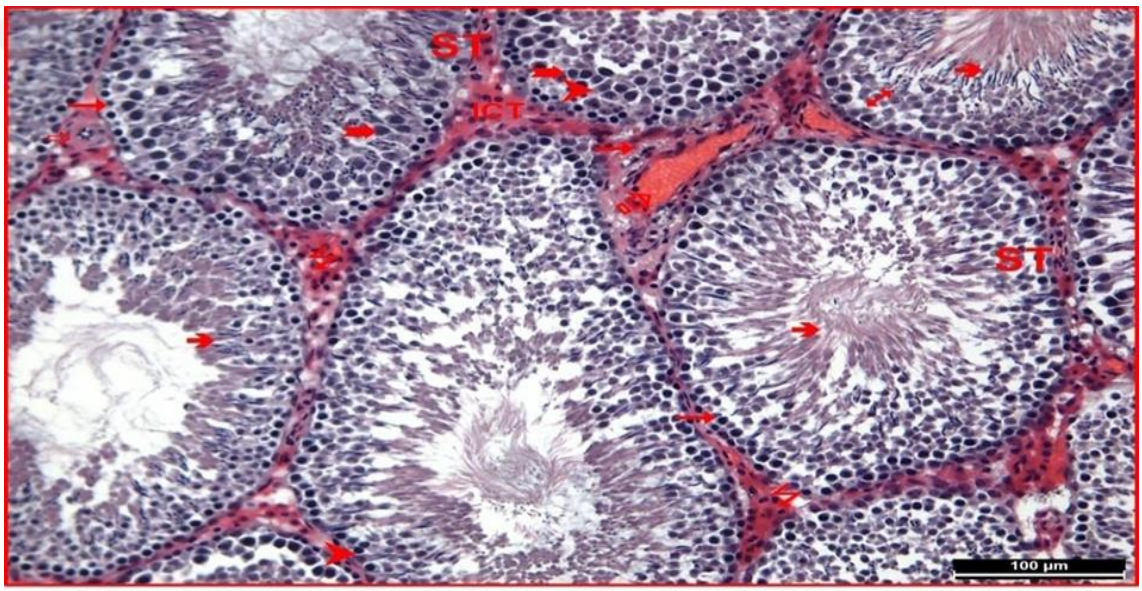


Figure 4.5 Control Melatonin group seminiferoustubule (ST), interstitial connective tissue (ICT), germinal epithelium (↦), spermatogonium (→), primary spermatocyte (➡), spermium (➔), Sertoli cell (➤), capillaries (⇨), and Leydig cells in testicular tissue (⇦) is observed (Hematoxylin-Eosin x200).

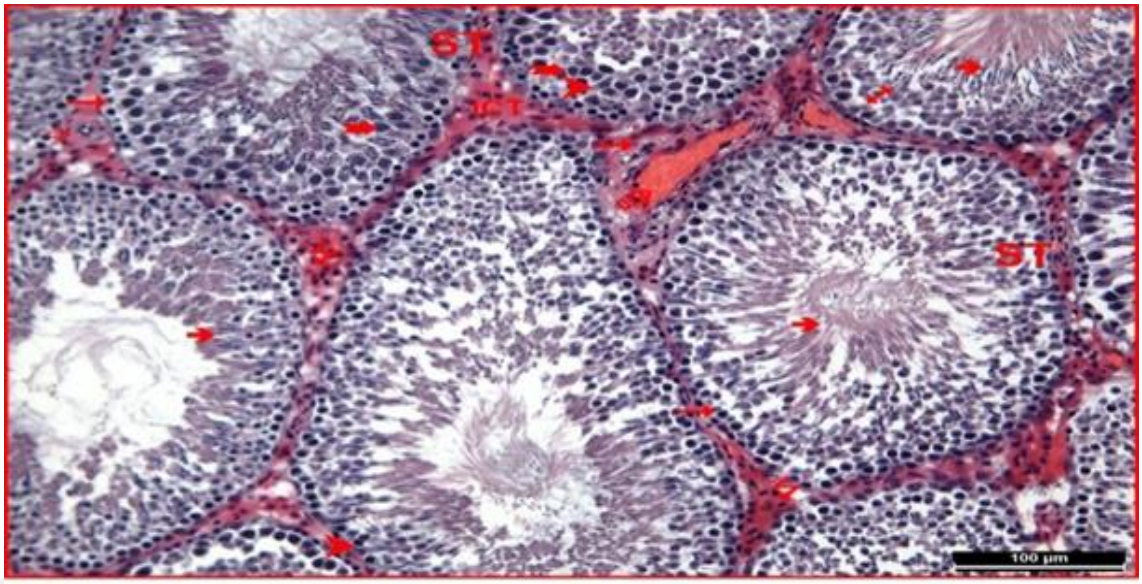


Figure 4.6 In the ShamMelatonin group testicular tissue, seminiferous tubule (ST), interstitial connective tissue (ICT), germinal epithelium (↦), spermatogonium (→), primary spermatocyte (➡), spermium (➔), Sertoli cell (➤), capillaries (⇨), dilatecapillaries (⇪) and Leydig cells (⇖) are observed (Hematoxylin-Eosin x200).

In the examinations of the RF radiation group, the seminiferous tubules did not show any deformity, but the presence of intense edema in the germinal epithelium of most tubuldeseminiferous tubules was noted. It was noted that the normal impression and organization of the epithelium disappeared in the tubules where edema was intense, and some spermatogonia preserved their basal positions, but other than that, other cells of the spermatogenic series came together out of their normal arrangement and emptied into the lumen. It was clearly distinguished that the intercellular connections were disrupted in the tubules. It was also noted that spermium in the lumens of some tubules was relatively reduced compared to other groups. Intensive vacuolization was observed in the examinations performed in the interstitial area of this group. Again in this area, it was observed that the cell population and connective tissue elements decreased relatively compared to other groups and gained a hyalinized appearance (Figure 4.7).

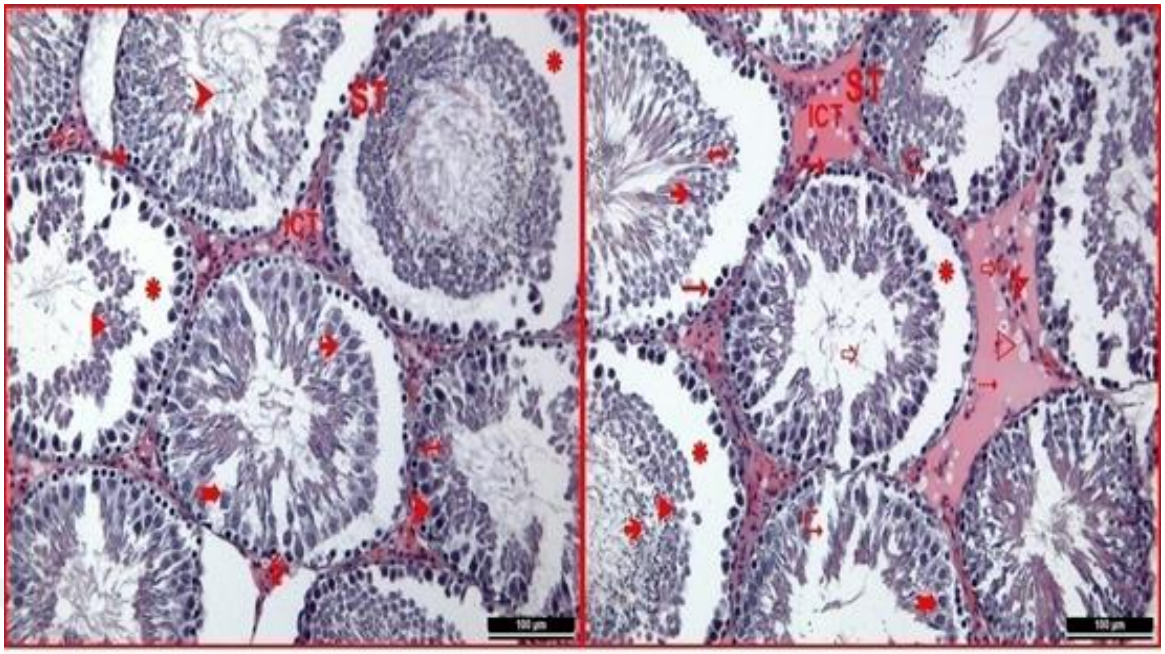


Figure 4.7 In RF radiation group testicular tissue, seminiferoustubule (ST), interstitial connective tissue (ICT), germinal epithelium (↔), spermatogonium (→), primary spermatocyte (➸), spermium (➔), Sertoli cell (➤), capillaries (⇨), dilatecapillaries (⇪), Leydig cells (⇳), edema (➸), cells of the spermatogenic series emptied towards the lumen (▶), areas where intercellular connections are broken (↳), vacuolization (▷) and hyalinization (⇢) are observed (Hematoxylin-Eosin x200).

In the Hematoxylin-Eosin staining of testicular tissue belonging to the RF radiation and Melatonin group, it was described as the most striking finding that the general appearance was similar to the Control and Melatonin groups. On the other hand, it was observed that impaired connections between edema and spermatogenic series cells continued to be observed in some tubules. Again, edema was observed in the interstitial area in some regions. However, it was noted that the cell population in this area increased relatively compared to the RF radiation group (Figure 4.8).

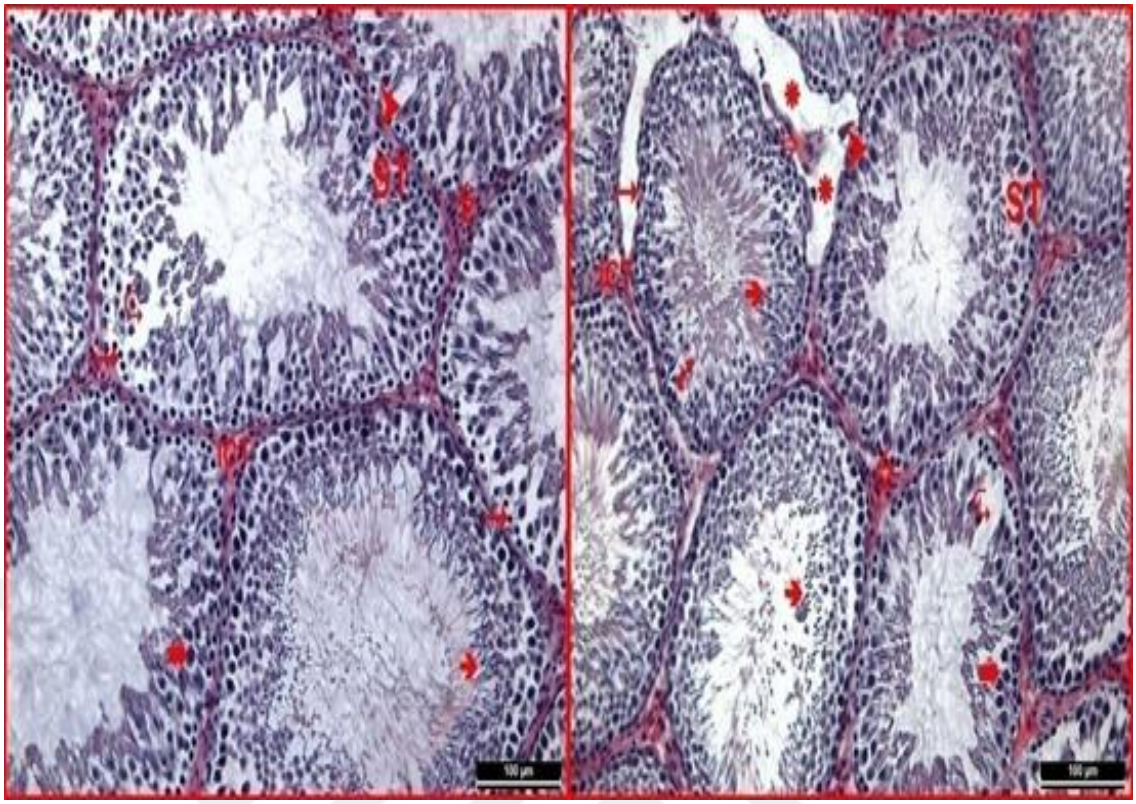


Figure 4.8 RF radiation and Melatonin group in testicular tissue, seminiferoustubule (ST), interstitial connective tissue (ICT), germinal epithelium (↔), spermatogonium (→), primary spermatocyte (➡), spermium (➔), Sertoli cell (➤), capillaries (⇨), Leydig cells (⇦), edema (➡) and regions where intercellular connections are disrupted (↪) are observed (Hematoxylin-Eosin x200)

As a result, it was determined that radiation application caused degeneration in testicular tissue and structural disorder in testicular morphology, melatonin application alone had a positive effect on sperm production in healthy rats without radiation application, and melatonin given with radiation application had a partial protective effect. It was concluded that the use of melatonin application as a preventive as well as its use as a therapeutic agent can provide a much better protective effect on the damage caused by radiation application.

4.4 Statistical Findings

Kruskall-Wallis test was used in the statistical evaluation of the data obtained from the study, and in case of a significant difference, pairwise comparisons were made with the Mann 48 Whitney U test in order to determine which groups caused the difference. Statistical significance level was taken as $p \leq 0.05$. Statistical analyses of the available data were done with SPSS 20.0. The table below shows the levels of MDA, GSH, NO and GSH-Px in testicular tissue (Figure 4.9) (Figure 4.10).

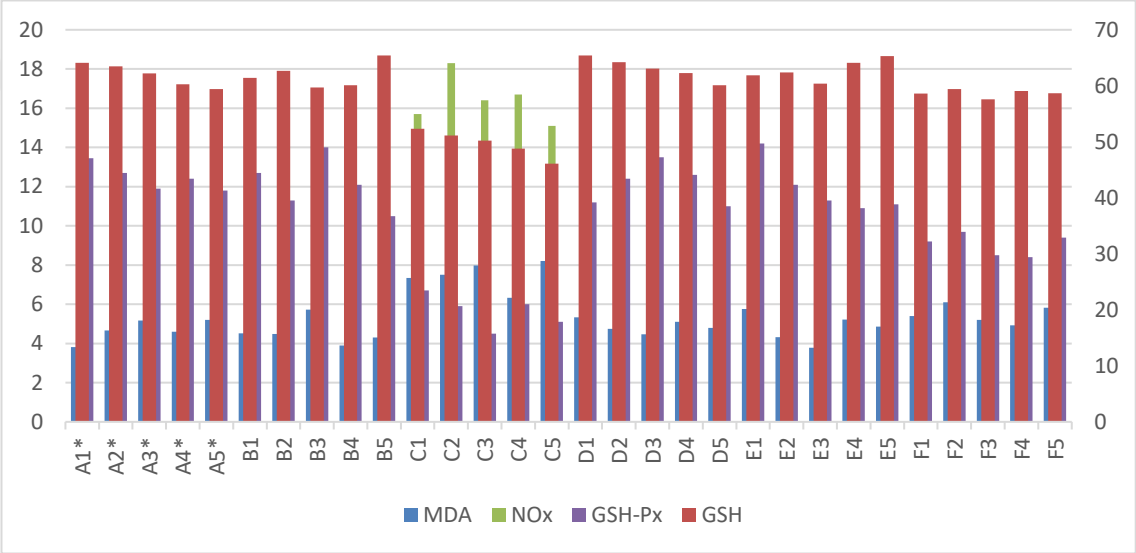
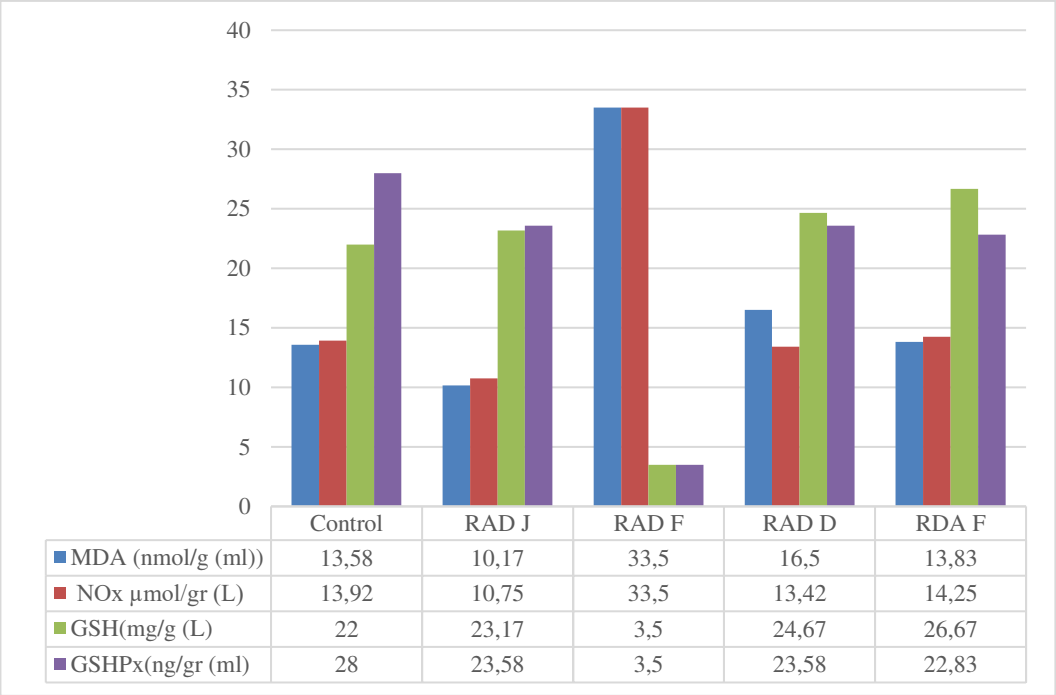


Figure 4.9 MDA (nmol/g (mL)) GSH (mg/g (L)), NOx (µmol/gr (L)), and GSH-Px levels (ng/gr (mL)) in testicular tissue [*A- Control; B-RAD J; C-RAD F; D-RAD D; E-RDA F]



	Control	RAD J	RAD F	RAD D	RDA F
MDA (nmol/g (ml))	13,58	10,17	33,5	16,5	13,83
NOx µmol/gr (L)	13,92	10,75	33,5	13,42	14,25
GSH(mg/g (L)	22	23,17	3,5	24,67	26,67
GSHPx(ng/gr (ml)	28	23,58	3,5	23,58	22,83

Figure 4.10 Testicular tissue mean biochemical parameters

In the light of the biochemical data obtained in our study, when the antioxidant levels in the testicular tissue were compared according to the groups, the MDA and NOx values of the control and sham groups were similar, while a significant increase was observed in the RF group, but the same increase was not observed in the RFR melatonin group. This is an indication that RF radiation causes oxidative damage in rat testis cells. GSH and GSH Px levels decreased in the RF group. These values differed less between the control group, sham group and melatonin group.

4.5 Discussion

The biological manifestations detected in the nervous system in relation to EMF exposure can originate from physiological responses to harmful effects, depending on the characteristics and intensity of the field.

Currently there is controversy about the harmful effects that electromagnetic radiation can generate in living beings, scientific studies vary in the results according to the magnitude and time of exposure to radiation, but there is no consensus on the alterations found.

In the study, rats in the experimental group (n=10) were exposed to EMR for two hours, while rats in the control group (n=10) spent two hours without receiving EMR under the same conditions. The rats in the experimental group were evaluated in two effect groups as acute (group 1) and chronic (group 2) effects, expressing the effects of EMR on waves. Recording of rat with the OF test was initiated within five minutes (group 1) or 24 hours (group 2) after EMR exposure.

In this study, in addition to the acute effects, delayed effects of radiation were also observed in male and female rats one day after exposure. In addition, in the analyzes of glucocorticoid plasma levels.

In the acute phase (increases in hormone levels), co-directed shifts were also observed in female and male rats. Delayed effects (24 hours) of EMR resulted in reductions in glucocorticoid levels in male rats only. In the evaluation of the effects of EMR on antioxidant defense system (ADS: anti-oxidant defense system) parameters, it was determined that lipid hydroperoxide levels decreased in male rats after 20 minutes of EMR compared to the control. An increase in SOD-like plasma activity was detected at 24 hours after EMR in female rats. These results proved that EMR exposure and sex differences play an important role in the rate of development of the ADS reaction response in experimental animals. However, in the acute period after EMR exposure,

the mechanisms of the symptoms observed in the ADS warning against increased activity in the stress-producing system have not been clarified. In conclusion, it was shown in the study that long-term exposure (at least 24 hours) to 905 MHz EMR had significant effects on exploratory activities, as well as on the activities of stress-inducing and stress-limiting systems in rats.

The determination of corticosterone and testosterone through the Elisa test allows knowing the levels of these hormones in the serum of the rats, these were determined because they are involved in the stress circuit. and depression in rats. However, no differences were found in either of the two hormones, an increase in corticosterone was expected in showed as well as in the sham radiation group.

On the other hand, when corticosterone concentration increased, a reduction in testosterone concentration was expected, since stress lowers this hormone through an enzyme called 11ßHSD-1. However, testosterone levels remained similar with respect to the control group, it is considered that there was no significant difference in the concentration of corticosterone and testosterone because the neurotransmitter GABA inhibits cortisol secretion, lowers acetylcholine and CRH; In addition to the fact that cortisol itself inhibits its own synthesis, by increasing glucocorticoids, they bind to hypothalamic and hippocampal receptors, they work by acting at the level of transcription and suppression of gene expression for CRH or the sample was not taken at the time the treatment was concluded, but until the end of the behavioral tests, which could be the reason that the concentration of the hormones returned to normal values. It is suggested that the samples for the determination of the hormones be carried out at the end of the treatment before the behavioural tests.

In the Hematoxylin-Eosin staining of the testicular tissue, the Control and RAD J groups showed normal structures of the seminiferous tubules of the tissue and interstitial connective tissue located between these tubules, germinal epithelium, spermatogenic cells, basal densely nucleated spermatogonia, Sertoli cells and Leydig cells. At the same time, it was observed that the general structure was similar to Control and RAD J groups in Hematoxylin-Eosin staining of testicular tissue belonging to

Control Melatonin and RAD J groups. Apart from the control groups, it was noted that the primary spermatocytes were relatively higher in some seminiferous tubules in the melatonin-administered groups, and the presence of relatively dense spermium in the lumens of some tubules. This finding was interpreted as melatonin administration may have increased sperm production.

In the examinations of the RF radiation group, however, the seminiferous tubules did not show any deformity, but the presence of intense edema in the germinal epithelium of the seminiferous tubule in most tubules was noted. It was observed that the normal impression and organization of the epithelium disappeared in the tubules where edema was intense, some spermatogonia kept their basal places, but other cells of the spermatogenic series came together out of their normal arrangement and emptied into the lumen, and the intercellular connections in the tubules were clearly distinguished. It was also noted that the spermium in the lumens of some tubules was relatively reduced compared to other groups.

In addition, intense vacuolization was observed in the examinations made in the interstitial area of this group. Compared to other groups, it was observed that the cell population and connective tissue elements decreased relatively and gained a hyalinized appearance. In a previous study on guinea pigs, it was shown that the number of spermatozoa decreased in subjects exposed to low-dose RAD Fadiation for 20 minutes and 60 days a day (Gülyüz *et al.* 2012). Similarly, rats who received low-dose RAD Fadation for 1 hour a day for 7 weeks showed a decrease in sperm count (Bin-Meferij and El-Kott 2015). In a study conducted on humans, it was found that the sperm count decreased with the increase in the duration of mobile phone use (Agarwal *et al.* 2008). On the other hand, there are studies in the literature with different results; For example, in a study it was shown that there was no change in sperm count in rats exposed to 845 MHz RAD Fadiation for 45 minutes a day for 12 weeks (Agarwal *et al.* 2009) has been shown to have no adverse effects. In a study conducted on humans, it was shown that when human semen was exposed to cell phone radiation in vitro for 1 hour, there was no difference in sperm concentration compared to the control.

A decrease in the seminiferous tubule epithelial cell population was observed in testicular tissues of rats exposed not only to RF radiation but also to microwave radiation (Chauhan *et al.* 2017). In rats exposed to Wi-Fi radiation for 1 hour a day for a month, there was no significant difference in seminiferous tubule diameters and the number of pycnotic and karyotic cells compared to the control group decrease in the number of testicular tissue and an increase in apoptosis was observed (Saygin *et al.* 2011). As a result of 50 Hz Very Low Frequency radiation exposure in mice, it was observed that spermatogonial cells were separated from the germinal epithelium, the amount of mature spermatozoa in the seminiferous tubule decreased and some Sertoli cells were flattened (Hamdi *et al.* 2011). In another study conducted with 900 MHz RF radiation, separation of the basement membrane in the seminiferous tubules of rats, vacuoles in the seminiferous tubule epithelium and basement membranes, edema in the intertubular area, apoptotic cells between the spermatogonial cell lines were observed (Odacı and Özyılmaz 2015). As a result of exposure, decrease in seminiferous tubule diameters, enlargement in the lumen, necrosis in the tubule epithelium were observed (Chauhan *et al.* 2017).

Oxidative stress resulting from excessive ROS production caused by RAD Fadiation exposure may be the cause of tissue damage (Koç *et al.* 2013) was found to be lower than (Odacı and Özyılmaz 2015). In a study in which human semen was exposed to in vitro cell phone radiation for 1 hour, it was shown that the level of ROS was higher in the semen exposed than the control. It was found that the MDA level in the testis increased and the GSH level decreased in rats exposed to low-dose EMA for 1 hour a day for four weeks compared to the control group (Mailankot *et al.* 2009). In a study examining the effects of mobile phone-induced electromagnetic radiation on oxidant and antioxidant status in rat blood and testis tissues, it was shown that CAT activity in blood and testis tissues increased up to 3 times, and GSH and GSH-Px levels decreased 3-5 times. It has been shown that vitamins C and E improve EMA-induced oxidative stress in the testis and restore CAT, GSH and GSH-Px levels to physiological levels (Al-Damegh 2012). In 3 different studies conducted in 2005 and 2006, it has been shown that antioxidants such as melatonin, CAPE, E, and C vitamins prevent oxidative stress caused by RAD in animal tissues (Ozguner *et al.* 2006).

The mentioned studies generally show that there is an inverse ratio between RAD exposure and sperm concentration, motility, and normal morphology parameters. The reason for this may be thought that RAD increases oxidative stress in semen by acting on plasma membrane enzymes. It is also clear that these abnormalities are dependent on the duration of exposure. It is understood that subjects exposed to long-term RAD Fadiation suffer more damage.

The future direction of this work is reflected in the investigation of the effect on DNA by the comet test, brain morphology, testicular morphology, spermatogenesis and teratogenesis.

5. CONCLUSIONS AND RECOMMENDATION

- Electromagnetic radiations have different biological effects then, treatment with 860 MHz non-ionizing radiation in mice.
- The effect on when irradiating a 860 MHz wave in mice and testosterone and estrogenic hormone before and after exposure to the waves.
- The hormonal changes of corticosterone and testosterone in blood by irradiating an 860 MHz wave in mice.
- Investigate whether 860 MHz frequency Radiofrequency Radiation causes oxidative damage in testicular tissues of male rats.

- There was no change in the concentration of testosterone and corticosterone with respect to the control group.
- In previous studies, it has been stated that radiation causes oxidative damage. Oxidative damage is thought to depend on the duration, frequency, strength and type of radiation exposure. Our study also showed that exposure to radiation for a certain period of time can cause oxidative stress in tissues and cause structural damage in tissues. Also, Melatonin has been used to see if the negative effects of radiations are eliminated or reduced. In the light of the data we obtained from NOx, GSPH, GSPHx and MDA levels of testicular tissue, it was concluded that melatonin increased antioxidant levels despite oxidative damage in testicular tissue. With this study, it is hoped that the effects of radiation will be better understood, the effects of antioxidants on male infertility and the treatment methods to be developed in this regard will shed light on new studies.

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APPENDICES

APPENDIX 1. Procedure for the determination of corticosterone

APPENDIX 2. Procedure for the determination of testosterone

APPENDIX 1. Procedure for the determination of corticosterone

- Reagents and samples have reached room temperature and are thoroughly mixed by gentle inversion before use.
- The microtiter strips used were marked.
- The problem sample was prepared by adding the same amount of dissociation agent and a 1:50 dilution was made with assay buffer.
- 50 µL of each standard and problem sample were pipetted into the corresponding wells.
- 75µL of assay buffer in non-specific binding (NSB) wells.
- 50 µL assay buffer in the wells to act as maximum binding wells.
- 25 µL of corticosterone conjugate solution was added to each well using a semi-automated dispenser.
- 25 µL of corticosterone antibody was added to each well using a semi-automated dispenser except for the NSB well.
- Wells were incubated, shaking at high speed (500-700 rpm) on an orbital microplate shaker, for one hour at room temperature.
- Each well was aspirated and washed 4 times with 30 µL of wash solution.
- 100 µL of TMB Chromogen Solution was added to each well using a semi-automated dispenser.
- Wells were incubated, shaking at high speed (500-700 rpm) on a microplate orbital shaker, for 30 minutes at room temperature. Direct exposure to sunlight was avoided.
- 50 µL of stop solution was added to each well using a semi-automated dispenser.
- The absorbance of the solution in the wells was read after 30 minutes, programmed at 450 nm.
- With the data obtained, a type curve was made and subsequently the values of the corticosterone concentrations of the problem samples were obtained.

APPENDIX 2. Procedure for the determination of testosterone

- Reagents and samples have been brought to room temperature (~25°C) and mixed thoroughly by gentle inversion before use.
- The microtiter strips used were marked.
- 50 µL of each standard, control and problem sample were pipetted into the corresponding wells.
- Enzyme conjugate solution was prepared by diluting enzyme conjugate concentrate in conjugate diluent.
- 100 µL of enzyme conjugate solution was added to each well using a semi-automated dispenser.
- 100 µL of testosterone antiserum was added to each well using a semi-automated dispenser.
- Wells were incubated, shaking at high speed (500-700 rpm) on an orbital microplate shaker, for one hour at room temperature (~25°C).
- Each well was aspirated and washed 5 times with the washing solution, using an automatic microplate washer. Blotted dry by inverting the plate on absorbent material. NOTE: The use of an automated microplate washer is highly recommended. Not performing a complete wash negatively affects the precision of the assay. If a microplate washer is not available, (a) fully aspirate the liquid from each well, (b) dispense 0.35 mL of wash solution into each well, and (c) repeat steps (a) and (b) five times.
- 100 µL of TMB Chromogen Solution was added to each well using a semi-automated dispenser.
- Wells were incubated, shaking at high speed (500-700 rpm) on an orbital microplate shaker, for 30 minutes at room temperature (~25 °C). Direct exposure to sunlight was avoided.
- 100 µL of stop solution (0.2 M sulfuric acid) was added to each well using a semi-automatic dispenser.
- The absorbance of the solution in the wells was read after 30 minutes, using a microplate reader programmed at 450 nm.
- With the data obtained, a type curve was made and later the values of the testosterone concentrations of the problem samples were obtained.