Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8648
Doi: 10.4025/actascihealthsci.v43i1.53934

Effects of the 27.12 MHz magnetic field emitted by short-wave equipment on spermatogenesis

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ABSTRACT. Studies have shown the influence of magnetic fields on several biological systems. Some of these fields, such as shortwave (SW) magnetic fields, have been used for therapeutic purposes. However, the possible adverse effects caused by this treatment still need to be better understood. The present study aimed to ascertain whether a single daily exposure (15 min.), for 15, 30 and 60 days, to SW magnetic field (27.12 MHz) can impede spermatogenesis. Exposure to SW magnetic fields did not change the weight of body, testis, epididymis, prostate, seminal vesicle or the gonadosomatic index. The plasma testosterone levels and testicular component volumes (seminiferous tubules, lumen, lamina propria, connective tissue, blood vessels, intertubular tissue) also remained unchanged. Histopathological analysis and spermatogenesis markers showed no changes after exposure to SW magnetic fields. However, some analyses showed changes in the lamina propria, daily sperm production, individual volume and population of Leydig cells. In conclusion, exposure to SW magnetic field for up to 60 days seems to be safe for spermatogenesis, but this exposure caused minor testicular changes that need to be better understood in the long term. This is of particular interest to health professionals who handle such SW devices for long periods of time.

Keywords: magnetic fields; non-ionizing radiation; shortwave; spermatogenesis; testis; testosterone.

Received on May 27, 2020.
Accepted on October 9, 2020

Introduction

Advances in telecommunications technology and electronic equipment have led to increased exposure of society to electric and magnetic fields of various frequencies and intensities (Tenorio et al., 2014; Saliev, Begimbetova, Masoud, & Matkarimov, 2019). In addition to the electromagnetic exposure generated by computers, electrical networks, industrial equipment, radio systems, cellular and wireless telephones; exposure also occurs during medical procedures, such as magnetic resonance imaging, electrotherapy, microwave, radiofrequency and thermotherapy using shortwave frequencies (with or without the diathermy effect) (Murray & Pethica, 2016; Storch, Dickreuter, Artati, Adamski, & Cordes, 2016; Ye, Chen, Wang, & Shen, 2017). In addition to patient exposure to therapeutic and diagnostic electric and magnetic fields, healthcare professionals who use this equipment are exposed for longer, including in the pelvic region, where the reproductive organs are located (Tenorio et al., 2014; Montenegro et al., 2017). Although much research is being carried out to clarify the biological effects of different types of electric and magnetic fields, it is still unclear the full extent of these effects on the human organism (Tenorio et al., 2012; Storch et al., 2016; Çetkin et al., 2017; Sage & Burgio, 2018).

Shortwave magnetic fields (SW) are used by physiotherapists to treat diseases because of the degree penetration in the human body and effects, such as deep heating, caused by the interaction between the SW field and molecules of water in the body. This shortwave is a type of radiofrequency nonionizing radiation and the frequency commonly used is 27 MHz with a wavelength of 11 m (Shah & Farrow, 2007; Sedhom, Elnaggar, & Shokri, 2017). The SW field has already been used to facilitate treatment of pain associated with musculoskeletal disorders and degenerative joint disorders, as well as to stimulate the proliferation of fibroblasts and chondrocytes and thereby reduce the inflammatory process, causing analgesia and vasodilation (Guo, Kubat, Nelson, & Isenberg, 2012; Rawe, 2014; Montenegro et al., 2017; Sedhom et al., 2017).

Infertility can affect 15% of human couples and nearly 50% occurs as a result of factors relating to males. In general, 8% of men have fertility problems and 10% of these have issues affecting their fertility potential which can be reversed (Pal et al., 2017; Tenorio et al., 2019). Studies have shown changes in parameters of male fertility to result from exposure to electric and magnetic fields (Kim et al., 2009; Tenorio et al., 2012; Bahaodini, Owjfard, Tamadon, & Jafari, 2015; Houston, Nixon, King, De Iuliis, & Aitken, 2016; Çetkin et al., 2017). However, other studies have found no changes in male fertility after exposure to such fields (Kumari et al., 2017).

As the use of the SW magnetic field has been recommended for the treatment of diseases, the present study aimed to ascertain whether exposure to the SW magnetic field generated by shortwave equipment can change the spermatogenesis and fertility parameters in adult rats.

Material and methods

The present study used 90-day-old adult Wistar rats (Rattus norvegicus, var. Albinus) from the bioterium of the Department of Animal Morphology and Physiology (DMFA) of the Federal Rural University of Pernambuco (UFRPE). The animals were kept in a controlled temperature environment (23 ± 2ºC), in a 12-hour light-dark cycle, with water and balanced feed (Purina Labina, Paulínea, Brazil) *ad libitum*.

The animals were randomly selected to form the experimental groups and the control group (n = 8). Animals were exposed to the 27.12 MHz magnetic field for 15, 30 and 60 days, with a single daily exposure of 15 minutes. The experimental protocol was approved by the Ethics Committee of the Federal Rural University of Pernambuco (230820094492014 DMFA-UFRPE), in accordance with the basic principles for research using animals.

Exposure to the magnetic field

The magnetic field was irradiated using commercial shortwave equipment (Thermowave, Bioset, Brazil) by way of silicon-coated coils placed in series 3 cm below the animals to ensure that the magnetic field oscillated as little as possible. The duration of exposure to the magnetic field was 15 minutes, a time frame that lies within the parameters recommended by the manufacturer of the shortwave equipment. The experimental groups were exposed to 27.12 MHz non-ionizing sinusoidal magnetic fields (varying in time). The magnetic fields were measured and monitored using a teslameter (Phywe, Germany). The magnetic flux density varied between 30 and 60 mT inside the containers where the animals were exposed to the magnetic field. Wide variations in magnetic flux intensity also occur in therapeutic treatments due to the distance between the body and the coil generating the magnetic field.

To reduce stress, the rats were individually exposed to the SW field in cylindrical plastic containers (18 x 8 cm) allowing only limited movement. The animals were previously adapted to these containers for 10 days. During the same period, the control animals were similarly conditioned and kept in a similar environment and container.

Spermatogenesis markers

At 15, 30 and 60 days of experimental treatment, the animals were heparinized (125 IU 100 g^{-1}) and anesthetized with xylazine (10 mg kg^{-1}) and ketamine (115 mg Kg^{-1}). The animals were perfused intracardiacally with 0.9% NaCl solution, plus sodium heparin (50 IU L^{-1}) for 10 minutes. After lavage of the vascular system, the animals were perfused with 4% glutaraldehyde in a 0.1M phosphate buffer (pH 7.3) for 10 minutes. Testis, epididymis, prostate and seminal vesicle were removed and weighed using a scale (BEL Engineering MARK 500, Brazil). The net weight of the testes and the gonadosomatic index (GSI) were calculated according to Tenorio et al. (2011).

The testicles were cut into fragments of 2 mm thickness and fixed again in glutaraldehyde solution, dehydrated in a graded alcohol series and embedded in plastic resin of glycol methacrylate (Leica, Germany). Cross-sections of the testis (4 µm) were stained using 1% toluidine blue/sodium borate. Images were captured using optical microscope (Leica DM500, Germany) and camera (Leica ICC50, Germany) connected to a computer.

Testicular cross-sections were analyzed morphometrically in accordance with Siqueira Bringel et al. (2013) and analysis of Leydig cells were according to Silva et al. (2014). The volume density of testicular components was measured using point counting by systematic allocation of 441 intersection points on the testicular

images. Fifteen fields were randomly analyzed, totaling 6615 points for each animal. The volumes (µl) of testicular components were obtained by multiplying the count by the net weight of the testis. Diameters of 30 rounded seminiferous tubules were measured per animal. The total length of seminiferous tubules (TLST) per testis was obtained by dividing the seminiferous tubule volume by π and the radius of seminiferous tubules divided by 2.

The numbers of germ and Sertoli cells (GSCC) were counted in 10 tubules showing stage VII of the seminiferous epithelium cycle (acrosomal method). The nuclei of spermatocyte I in preleptotene and pachytene, round spermatids and Sertoli cells were counted. These crude counts (CC) were corrected for cross section thickness (S) and mean nuclear or nucleolar diameter (ND):

$$GSCC = CC \times \frac{S}{S + \sqrt{\left(\frac{ND^2}{2}\right) - \left(\frac{ND^2}{4}\right)}}$$

The Sertoli cell population (SCP) was determined using the corrected count of Sertoli cells per tubule section (CSC), section thickness (S) and the total length of seminiferous tubules (TLST):

$$SCP = \frac{TLST \times CSC}{S}$$

Daily sperm production per testis was calculated as:

$$DSP = \frac{NSCT \times RSC \times RFS\,VII}{SDVII}$$

DSP = Daily sperm production; NSCT = Total number of Sertoli cells per testis; RSC = Round spermatid counts; RFS VII = Relative frequency of stage VII; SDVII: Stage VII duration in days.

Daily sperm production per gram of testis (DSP g^{-1}) was obtained from the ratio between daily sperm production per testis and testicular net weight.

Plasma testosterone analysis

Blood samples were collected by puncture at the convergence of cranial and caudal cava veins, desorbed by centrifugation, packed in plastic containers (2 per sample) and stored in a freezer at -20ºC. Testosterone was measured by the enzyme-immunoassay method (ELISA - Enzyme Linked Immuno Sorbent Assay), with absorbance reading at 405 nm, as previously described by Tenorio et al. (2012). The samples were read in duplicate, maintaining the coefficient of intra- and inter-assay variation lower than 10%.

Statistical analysis

The Shapiro-Wilks test was used to check the normality of the data. Depending on the result of this, a parametric test T, or nonparametric Mann-Whitney test was used. Results were expressed as mean (±) standard deviation. A p value < 0.05 was considered statistically significant.

Results

Body weight and weight of androgen-dependent organs

Body weight analysis showed no significant change after exposure to the SW magnetic field for 15 minutes daily during the 60 days of the experiment (Figure 1). It is worth mentioning a tendency for body weight to decrease over the 30 days of exposure to the SW field. This was not statistically significant but may indicate a possible long-term effect.

The weight of androgen-dependent organs of rats exposed to the SW magnetic field for 15, 30, and 60 days also remained unchanged (Table 1).

Spermatogenesis markers

The volumes of testicular components: seminiferous tubule, seminiferous epithelium, tubular lumen, connective tissue, blood vessels, lymphatic space and intertubular tissue did not show significant changes after exposure to SW magnetic field for 15, 30 and 60 days. Only the volume of the lamina propria showed an increase in the animals exposed to the SW field for all the periods covered by the experiment (Table 2).

Most of the spermatogenesis parameters did not show significant changes after 15, 30 and 60 days of exposure to the SW magnetic field (Table 3). However, daily sperm production (DSP) and sperm production per gram of testis (SP g^{-1}) increased in all experimental periods, including a statistically significant increase after 15 days of exposure to the SW field.

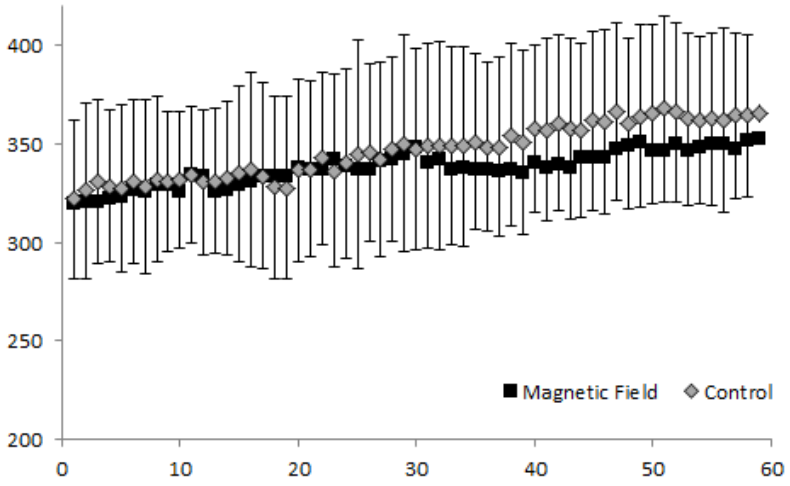

Figure 1. Body weight of control group adult rats and those exposed to shortwave magnetic field (27.12 MHz) 15 minutes daily for 60 days.

Table 1. Weight of androgen-dependent organs (g) and testicular net weight in adult control group rats and those exposed to shortwave magnetic field (27.12 MHz) 15 minutes daily for 15, 30 and 60 days. GSI = Gonadossomatic Index.

15 days	Control	Magnetic Field	p
Testicle	1.62 ± 0.07	1.58 ± 0.06	0.27
Testicle net weight	1.52 ± 0.06	1.48 ± 0.06	0.28
Epididymis	0.63 ± 0.05	0.62 ± 0.04	0.87
Prostate	0.75 ± 0.30	0.77 ± 0.13	0.27
Seminal Vesicle	1.49 ± 0.25	1.53 ± 0.16	0.72
GSI (x 10^{-3})	4.70 ± 0.53	4.60 ± 0.59	0.95
30 days	Control	Magnetic Field	p
Testicle	1.63 ± 0.25	1.69 ± 0.20	0.67
Testicle net weight	1.53 ± 0.23	1.58 ± 0.19	0.87
Epididymis	0.71 ± 0.12	0.74 ± 0.10	0.91
Prostate	0.74 ± 0.19	0.83 ± 0.13	0.33
Seminal Vesicle	1.81 ± 0.43	1.88 ± 0.28	0.95
GSI (x 10^{-3})	4.70 ± 0.40	4.60 ± 0.51	0.93
60 days	Control	Magnetic Field	p
Testicle	1.61 ± 0.16	1.56 ± 0.24	0.53
Testicle net weight	1.50 ± 0.15	1.46 ± 0.23	0.52
Epididymis	0.74 ± 0.05	0.78 ± 0.10	0.53
Prostate	0.81 ± 0.15	0.63 ± 0.14	0.10
Seminal Vesicle	2.15 ± 0.30	2.02 ± 0.35	0.53
GSI (x 10^{-3})	4.30 ± 0.44	4.40 ± 0.29	0.92

Leydig cells analysis

The individual volume of Leydig cells increased in the animals exposed to the SW magnetic field for 15 and 30 days. On the other hand, animals irradiated for 60 days showed a reduction in the volume of individual Leydig cells (Table 4). Leydig cells exposed to the SW field also showed a reduction in the total cell population and population per gram of testis after 15 and 30 days, as well as an increase in the total cell population and population per gram of testis after 60 days of exposure (Table 4).

It is worth noting that the control group showed constant results in the Leydig cell parameters over 15, 30 and 60 days, with a total of 24 control animals showing constant results for Leydig cells, while those exposed to the magnetic field showed significant changes in Leydig cells throughout the experimental period.

Table 2. Volume (µL) of testicular components in adult control group rats and those exposed to shortwave magnetic field (27.12 MHz) 15 minutes daily for 15, 30 and 60 days.

15 days	Control	Magnetic field	p
Seminiferous tubules	385.82 ± 7.13	386.24 ± 9.19	0.79
Seminiferous epithelium	350.29 ± 9.62	352.38 ± 5.88	0.87
Lumen	33.50 ± 9.74	30.71 ± 4.37	0.38
Lamina propria	2.01 ± 0.77	3.14 ± 0.46	0.00*
Connective tissue	1.17 ± 0.50	1.43 ± 0.93	0.87
Blood vessels	8.40 ± 3.51	6.73 ± 3.86	0.50
Lymphatic space	27.77 ± 4.52	30.46 ± 5.66	0.44
Intertubular Tissue	55.18 ± 7.13	54.60 ± 9.18	0.72
30 days	Control	Magnetic field	p
Seminiferous tubules	380.55 ± 14.09	376.08 ± 11.28	0.44
Seminiferous epithelium	351.31 ± 15.04	340.69 ± 13.19	0.19
Lumen	28.12 ± 12.25	33.95 ± 5.80	0.44
Lamina propria	2.21 ± 0.46	2.83 ± 0.51	0.04*
Connective tissue	3.84 ± 1.91	3.57 ± 1.02	0.95
Blood vessels	9.15 ± 4.62	11.09 ± 5.05	0.38
Lymphatic space	31.61 ± 8.76	35.20 ± 6.63	0.34
Intertubular Tissue	60.45 ± 14.09	65.10 ± 11.26	0.44
60 days	Control	Magnetic field	p
Seminiferous tubules	392.21 ± 7.66	389.13 ± 18.80	0.95
Seminiferous epithelium	359.43 ± 12.38	361.25 ± 24.98	0.39
Lumen	29.50 ± 9.96	22.83 ± 8.65	0.33
Lamina propria	2.28 ± 0.61	4.04 ± 1.73	0.01*
Connective tissue	2.24 ± 1.00	3.09 ± 1.70	0.35
Blood vessels	4.28 ± 2.79	3.88 ± 2.66	0.86
Lymphatic space	25.76 ± 3.42	27.51 ± 17.84	0.28
Intertubular Tissue	48.79 ± 7.66	51.69 ± 18.70	0.86

*Statistically significant ($p < 0.05$).

Table 3. Spermatogenesis parameters in adult control group rats and those exposed to the shortwave magnetic field (27.12 MHz) for 15 minutes daily for 15, 30 and 60 days. SCI = Sertoli cell index, DSP = Daily sperm production, SP g^{-1} = sperm production per gram of testis.

15 days	Control	Magnetic field	p
Tubular length (m)	14.96 ± 2.30	16.50 ± 2.53	0.28
Tubular diameter (µm)	338.61 ± 21.35	291.07 ± 81.05	0.10
Epithelium height (µm)	114.81 ± 19.88	146.96 ± 33.05	0.10
Tubular area (μm^2) (x 10^5)	90.00 ± 1.10	71.00 ± 2.80	0.13
Epithelial area (μm^2) (x 10^3)	79.60 ± 1.28	75.40 ± 1.06	0.72
Sertoli cell / cross-section	5.43 ± 0.36	5.51 ± 0.39	0.44
Sertoli cell population (x10^6)	20.00 ± 3.20	51.00 ± 8.20	0.19
SCI	8.10 ± 1.56	8.40 ± 0.79	0.32
DSP (x 10^6)	12.03 ± 2.50	15.07 ± 2.10	0.04*
SP g^{-1} (x 10^5)	8.01 ± 1.40	9.60 ± 1.50	0.03*
30 days	Control	Magnetic field	p
Tubular length (m)	15.57 ± 3.83	16.49 ± 2.72	0.44
Tubular diameter (µm)	331.21 ± 16.90	324.50 ± 14.57	0.38
Epithelium height (µm)	106.30 ± 3.78	102.73 ± 5.62	0.23
Tubular area (μm^2) (x 10^5)	86.00 ± 8.40	82.00 ± 7.40	0.39
Epithelial area (μm^2) (x 10^3)	78.60 ± 7.28	77.80 ± 7.93	0.10
Sertoli cell / cross-section	5.67 ± 0.44	5.69 ± 0.38	0.75
Sertoli cell population (x10^6)	22.00 ± 6.00	23.00 ± 3.20	0.64
SCI	8.53 ± 1.66	9.04 ± 1.15	0.57
DSP (x 10^6)	13.20 ± 3.00	14.30 ± 3.90	0.45
SP g^{-1} (x 10^5)	8.90 ± 1.10	9.90 ± 1.60	0.23
60 days	Control	Magnetic field	p
Tubular length (m)	15.18 ± 1.85	14.04 ± 2.23	0.39
Tubular diameter (µm)	335.97 ± 18.88	341.97 ± 13.66	0.69
Epithelium height (µm)	111.73 ± 9.13	103.74 ± 4.63	0.15
Tubular area (μm^2) (x 10^5)	88.00 ± 1.00	91.00 ± 7.20	0.66
Epithelial area (μm^2) (x 10^3)	82.10 ± 1.01	81.90 ± 4.55	0.90
Sertoli cell / cross-section	5.72 ± 0.29	5.78 ± 0.37	0.68
Sertoli cell population (x10^6)	21.00 ± 2.80	20.00 ± 4.30	0.46
SCI	8.38 ± 1.15	9.21 ± 1.54	0.33
DSP (x 10^6)	13.05 ± 2.10	14.08 ± 2.70	0.25
SP g^{-1} (x 10^5)	9.02 ± 1.40	9.50 ± 1.70	0.53

*Statistically significant ($p < 0.05$).

Table 4. Volume and population of Leydig cells in adult control group rats and those exposed to shortwave magnetic field (27.12 MHz) 15 minutes daily for 15, 30 and 60 days.

15 days	Control	Magnetic field	p
Individual volume (μm^3)	826.26 ± 113.98	1205.2± 572.51	0.03*
Total cell population ($x10^7$)	7.30 ± 1.19	5.01 ± 1.44	0.04*
Cell population per gram ($x10^7$)	5.02 ± 1.32	3.38 ± 0.96	0.00*
30 days	Control	Magnetic field	p
Individual volume (μm^3)	884.35 ± 221.73	1163.87 ± 307.84	0.00*
Total cell population ($x10^7$)	6.70 ± 1.70	5.04 ± 1.88	0.05*
Cell population per gram ($x10^7$)	4.20 ± 0.73	3.16 ± 1.05	0.03*
60 days	Control	Magnetic field	p
Individual volume (μm^3)	859.62 ± 78.98	547.79 ± 78.66	0.00*
Total cell population ($x10^7$)	6.65 ± 1.51	11.01 ± 4.56	0.04*
Cell population per gram ($x10^7$)	4.38 ± 0.77	7.35 ± 2.13	0.00*

* Statistically significant ($p < 0.05$).

Plasma testosterone

The analysis of plasma testosterone showed no statistical difference between the animals exposed to the SW magnetic field and their respective controls. Although there was a tendency for plasma testosterone levels to increase in animals exposed to the magnetic field, especially after 15 days, this was not statistically significant (Figure 2).

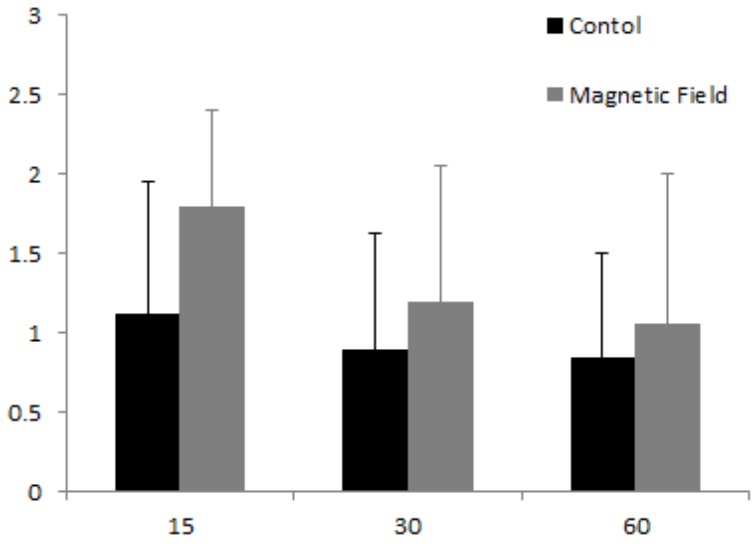

Figure 2. Plasma testosterone (ng mL^{-1}) in control group adult rats and those exposed to shortwave magnetic field (27.12 MHz) 15 minutes daily for 15, 30 and 60 days.

Histopathological analysis

Seminiferous tubules in control and exposed animals showed normal spermatogenesis, including the various stages of germ cell maturation, such as spermatogonia, spermatocytes and spermatids. Spermatozoa were also observed in the lumen of the seminiferous tubules and a normal process of spermiation was observed in the epithelium. Seminiferous tubules also

showed normal germ cell associations forming the stages of the seminiferous epithelium cycle.

The animals exposed to the magnetic field and their respective controls showed few differences in the testicular parenchyma (Figure 3). No pathological lesions, such as vacuolations, the formation of giant syncytial cells, degeneration or desquamation of germ cells, neoplasms, inflammatory infiltrate, changes in intertubular concentrations of blood vessels or the size and appearance of the seminiferous tubules, were observed. The animals exposed to the magnetic field showed only thickening of the lamina propria (Figure 3C).

The intertubular tissue showed an increase in the size of Leydig cells at 15 and 30 days of exposure to the magnetic field compared to the control, as well as Leydig cells showed reduced size in the animals exposed to the SW field for 60 days (Figure 4).

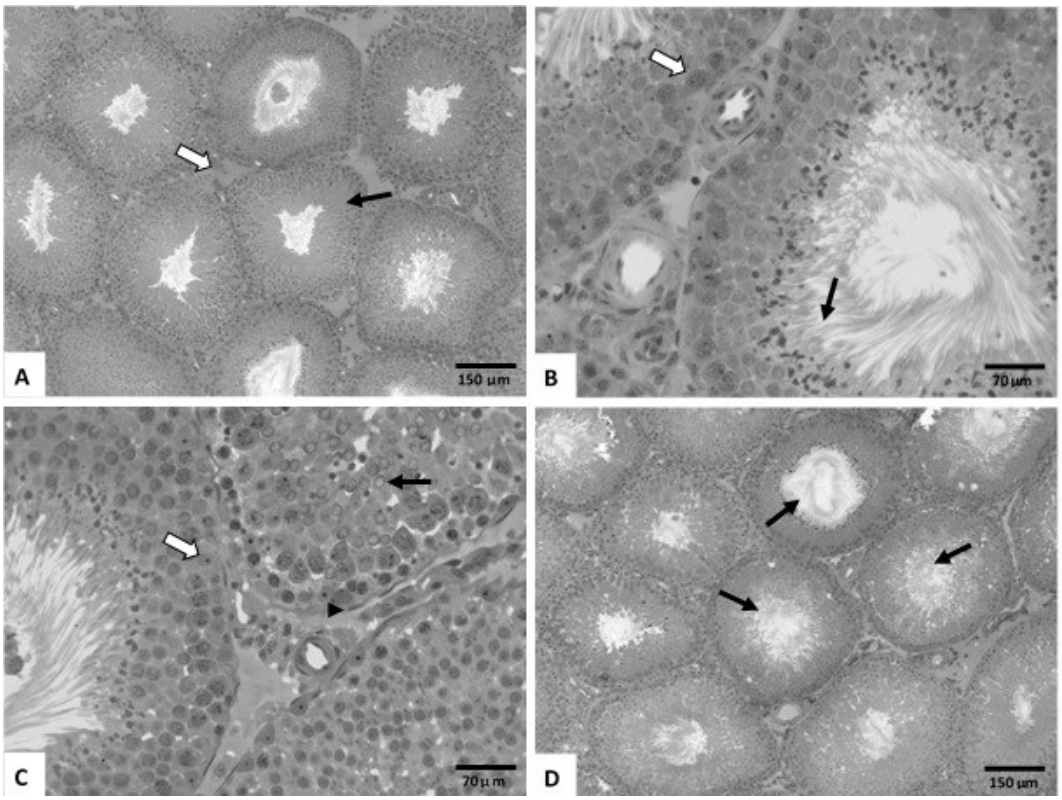

Figure 3. Photomicrographs of testicular parenchyma in adult control rats (A) and those exposed to 27.12 MHz shortwave (SW) magnetic field for 15 (B), 30 (C) or 60 days (D). A) Normal seminiferous tubule cross-section (black arrow) and intertubular tissue (white arrow) in testis of control rat. B) Seminiferous tubule showing spermatozoa in spermiation with tails extended towards the tubular lumen in animals exposed to SW fields for 15 days (black arrow). Note spermatocytes I in pachytene (white arrow). C) Seminiferous tubule showing rounded spermatid (black arrow), Sertoli cell (white arrow) and thickening of the lamina propria (arrow head) in an animal exposed to the SW field for 30 days. D) Seminiferous tubule with spermatozoa inside the lumen in animals exposed to SW fields for 60 days (black arrow).

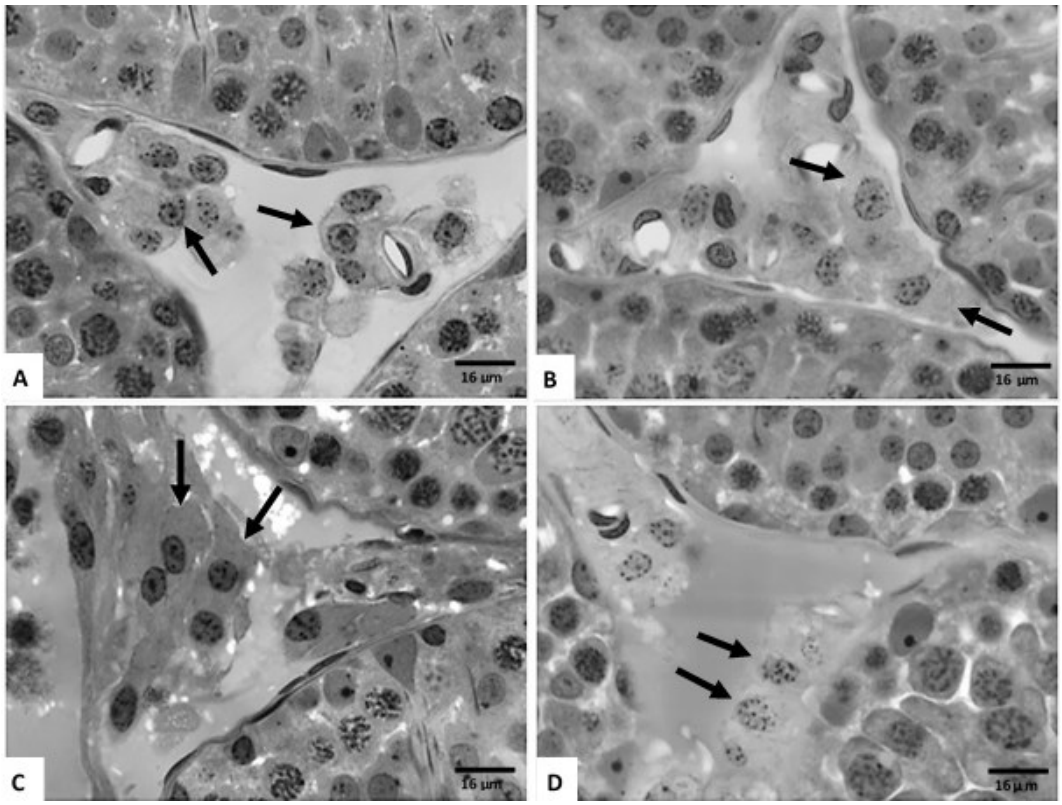

Figure 4. Photomicrographs of the intertubular tissue of testes in adult control rats (A) and those exposed to 27.12 MHz shortwave (SW) magnetic field for 15 (B), 30 (C) or 60 days (D). Intertubular tissue showing normal Leydig cells in control animals (A) and enlarged (B and C) or reduced-sized (D) Leydig cells in animals exposed to SW magnetic fields.

Discussion

The potential advantages of therapy using electromagnetic fields include non- invasiveness, safety, low toxicity for non-diseased cells and the possibility of combination with other available therapies (Vadalà et al., 2016). However, the full extent of the effect on the human organism is not yet clear (Storch et al., 2016; Sage & Burgio, 2018).

The present study did not find statistically significant changes in body weight resulting from exposure to an SW magnetic field for 60 days. This result corroborates previous studies that likewise found no changes in body weight, such as Kumari et al. (2017) evaluating intermediate frequency magnetic field (7.5 kHz and 120 μT); and Chung, Kim, Myung and Lee (2003) analyzing low frequency electromagnetic fields. However, it is noteworthy that some studies observed changes in body weight due to long-term exposure to low frequency electromagnetic fields (Gerardi et al., 2008; Qi et al., 2015).

This may corroborate the tendency to decrease body weight observed at the end of the present study after exposure to an SW magnetic field for more than 30 days. Long-term exposure to SW fields may be of particular importance for healthcare professionals who constantly handle this equipment and are exposed to SW fields for long periods.

Testicular weight is fundamental for evaluation of reproductive function and is related to sperm production and a reduction in testicular weight indicates germ cell loss (Lanning et al., 2002; França, Avelar, & Almeida, 2005). In the present study, there was no statistically significant effect on the weight of the testis, epididymis, prostate or seminal vesicle after exposure to the SW magnetic field, corroborating the results previously described by Çelik et al. (2012), Tenorio et al. (2012), Bahaodini et al. (2015) and Kumari et al. (2017) after exposure to intermediate frequency magnetic field, low frequency electromagnetic fields and cell phone fields.

Despite research evaluating varying frequencies and intensities of electric and magnetic fields have observed significant changes in testicular components (Kim et al., 2009; Saygin et al., 2011; Tenorio et al., 2011, 2012, 2014; Gye & Park, 2012; Bahaodini et al., 2015; Asghari, Khaki, Rajabzadeh, & Khaki, 2016; Çetkin et al., 2017); the present study did not observe major changes in testicular components. Previous studies have likewise not observed changes in germ cells after exposure to fields at various frequencies, such as 50 Hz, 7.5 kHz and 915 MHz (Dasdag et al., 2003; Çelik et al., 2012; Duan et al., 2014; Kumari et al., 2017). Although there are several studies showing testicular damage as well as not showing this damage after exposure to electric and magnetic fields, the factors justifying these differences remain unclear. According to Tenorio et al. (2012), factors influencing the electromagnetic effects on testicular components include variation in frequency and intensity of the field, species and strain of the animals, variation in exposure time, direction of radiation incidence and individual sensitivity.

A marked difference between our methodology and those of studies showing adverse effects on reproduction was the duration of exposure to the magnetic field, exposure time seems to be important for treatment safety. In the present study, similar to treatment using SW, there was a single daily exposure of 15 minutes; a short and non-repetitive exposure akin to that used in treatments using SW magnetic field equipment. Longer and repeated exposure needs to be further analyzed as this poses a risk to healthcare professionals who handle SW equipment constantly and for long periods.

Although the present study did not observe changes in the seminiferous tubule epithelium, we did find an increase in lamina propria (tunica propria) volume in the testicles of animals exposed to the SW magnetic field. The lamina propria consists of layers of myofibroblasts with intermingled connective tissue ground substance surrounding the seminiferous tubules. When spermatogenesis is impaired, the peritubular tissue commonly becomes thickened by connective tissue (Holstein, Schulze, & Davidof, 2003). The thickening of the lamina propria is commonly observed in damaged testes, so, although the present study did not observe pronounced deleterious effects on the seminiferous tubules, the peritubular tissue may be altered due to exposure to the SW field. In a previous study, Çelik et al. (2012) reported normal seminiferous tubules in rats exposed to the electromagnetic field of a cell phone. However, these authors also reported increased thickness of the lamina propria and the collagen fiber contents, corroborating the lamina propria changes observed in the present study. Tenorio et al. (2014) observed a similar increase in lamina propria in rat testicles exposed to a 60 Hz and 1 mT magnetic field for 30 days. We have not found previous studies reporting increased lamina propria due to SW field exposure (27.12 MHz).

Intertubular tissue also contains testosterone-producing Leydig cells (Huhtaniemi, 2015). The present study observed changes in the quantity and size of Leydig cells after exposure to the SW field for 15, 30 and 60 days. Saygin et al. (2011), Tenorio et al. (2011) and Ozguner et al. (2002) also observed changes in the Leydig cells of testis tissue in rats exposed to 2.45 GHz, 60 Hz and 50 Hz electromagnetic fields respectively. We have not found previous studies reporting changes in the quantity and size of Leydig cells due to SW field exposure (27.12 MHz).

The increase in Leydig cell size found in the present study may have occurred to compensate for the decrease in Leydig cell quantity as a way of maintaining normal testosterone levels. Ordinarily, testosterone level is directly correlated with Leydig cell size (França et al., 2005). Given the 40 and 13% of Leydig cell volume occupied by the smooth endoplasmic reticulum and mitochondria involved in steroidogenesis (Tenorio et al., 2011), the increase in the Leydig cell population and individual volume observed in the present study may compensate for adverse effects and maintain normal levels of testosterone.

According to Forgács et al. (2004), magnetic field exposure is able to increase the steroidogenic responsiveness of Leydig cells to LH; however, serum concentrations of testosterone were unaltered, probably because of the feedback mechanisms of the hypothalamic-pituitary-gonadal axis. In the present study, plasma testosterone levels did not change statistically between control groups and those exposed to the SW field. However, the results show a 35, 27 and 20% increase tendency in animals exposed to SW field for 15, 30 and 60 days. These may be related to changes in Leydig cells and endocrine compensatory mechanism factors.

The full extent of the effects of electromagnetic fields on steroidogenesis and testosterone is currently unclear. As in the present study, Duan et al. (2014), Kim et al. (2009), and Çetkin et al. (2017) did not observe changes in testosterone in animals exposed to 50, 60 Hz and 915 MHz fields respectively. On the other hand, changes in testosterone concentrations due to exposure to electric and magnetic fields have been reported in previous studies (Kesari, Kumar, Nirala, Siddiqui, & Behari, 2013; Bahaodini et al., 2015; Asghari et al., 2016; Ebrahim, Azab, Albasha, & Albishti, 2016).

As secondary messengers of signal transduction in Leydig cells, mainly cAMP and Ca^{2+}, play a major role in steroidogenesis and testosterone production (Tenorio et al., 2019), one possible mechanism of action of the magnetic fields on Leydig cells may be associated with alterations in cAMP content and calcium signaling induced by exposure to a magnetic field, since these cell signaling (cAMP and Ca^{2+}) can be changed by magnetic field exposure (Forgács et al., 2004; Jung & Kim, 2017; Buckner, Buckner, Koren, Persinger, & Lafrenie, 2018).

One interesting finding of the present study was the 17, 10 and 7% increase in sperm production in animals exposed to the SW magnetic field for 15, 30 and 60 days respectively. This increase in sperm production was more pronounced at the beginning of exposure (15 days). We did not find previous studies reporting an increase in daily sperm production due to SW field exposure (27.12 MHz). Interestingly, Kumari et al. (2017) also observed a stimulating effect of the intermediate frequency magnetic field (7.5 kHz), demonstrated by increased sperm motility. The increase in sperm production observed in the present study may occur as a result of a direct effect of the magnetic field on germ cells, as there are studies showing an increase in cell proliferation caused by exposure to electromagnetic fields (Nurković et al., 2017). It is noteworthy that previous studies generally report magnetic fields causing a reduction in male fertility parameters for low frequency fields (Tenorio et al., 2012, 2014; Bahaodini et al., 2015; Lee, Park, & Kim, 2016) and radiofrequency (Yan et al., 2007; Kesari, Kumar, & Behari, 2011; Odacı et al., 2016; Chauhan, Verma, Sisodia, & Kesari, 2017), or no effect on male fertility (Dasdag et al., 2003; Ribeiro et al., 2006).

Physiotherapists use different forms of electromagnetic energy for therapeutic purposes, including radiofrequency non-ionizing radiation shortwave devices (Shah & Farrow, 2007). According to Koutsojannis, Andrikopoulos, Adamopoulos and Seimenis (2018), there is considerable concern about electromagnetic radiation from shortwave (SWD) equipment used in physiotherapy. Although patients are only exposed to SW fields during treatment, healthcare professionals handling these devices are exposed for much longer, including in their pelvic area near the testicles (Tenorio et al., 2014; Montenegro et al., 2017). There is therefore a need for further studies addressing the long-term effects of these SW magnetic fields.

Conclusion

The present study verified whether a single daily exposure (15 min. for 60 days) to the SW magnetic field generated by shortwave equipment can change the spermatogenesis and fertility parameters. Despite minor

changes in the lamina propria, Leydig cells and sperm production, the present study did not observe prominent damage to testicle that effectively compromised male fertility and seems to be safe for spermatogenesis.

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