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CIÊNCIAS BIOLÓGICAS:

Gênese na formação multidisciplinar

Alana Maria Cerqueira de Oiveira
(Organizadora)

Atena Editora
Ano 2022

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APRESENTAÇÃO

O Livro “Ciências biológicas: Gênese na formação multidisciplinar 2”, traz ao leitor vinte capítulos de relevada importância na área de Genética, Citogenética, Imunologia, Parasitologia, Química medicinal, Saúde pública e Ecologia. Entretanto, caracteriza-se como uma obra multidisciplinar que engloba diversas áreas da Ciências biológicas.

Os capítulos estão distribuídos em temáticas que abordam de forma categorizada e multidisciplinar a Ciências biológicas , as pesquisas englobam estudos de: mapeamentos genético, citogenético, sequenciamento, genética e educação ,análises forenses , doenças genética, eugenesia clássica, engenharia genética, análise por PCR, cultura de células de linfoma e leucemia, saúde mental, resposta imune, vacinação contra a covid-19, vírus Sars-Cov-2, métodos de extração de lipídios ,levantamento taxonômico, morfologia vegetal, eficiência de inseticidas , química medicinal, cromatografia líquida de alta eficiência (CLAE), espectroscopia de infravermelho (IV) e espectrometria de massas (EM), problemática ambiental e de saúde pública, poluentes emergentes e biodiesel.

A obra foi elaborada primordialmente com foco nos profissionais, pesquisadores e estudantes pertencentes às áreas de Ciências biológicas e Ciências da Saúde e suas interfaces ou áreas afins. Entretanto, é uma leitura interessante para todos aqueles que de alguma forma se interessam pela área.

Cada capítulo foi elaborado com o propósito de transmitir a informação científica de maneira clara e efetiva, em português, inglês ou espanhol. Utilizando uma linguagem acessível, concisa e didática, atraindo a atenção do leitor, independente se seu interesse é acadêmico ou profissional.

O livro Ciências biológicas: Gênese na formação multidisciplinar 2”, traz publicações atuais e a Atena Editora traz uma plataforma que oferece uma estrutura adequada, propícia e confiável para a divulgação científica de diversas áreas de pesquisa.

Alana Maria Cerqueira de Oliveira

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BIOLOGICAL EVALUATION OF A THERAPEUTIC DEVICE THAT IS BASED IN PULSED-ELECTROMAGNETIC FIELDS AND STATIC MAGNETIC FIELDS ON A MURINE MODEL

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ABSTRACT: There is number of publications trying to demonstrate the efficacy of magnetic fields treatments in medicine. By the way, pulsed electromagnetic fields (PEMF's) and static magnetic fields (SMF's) technologies have been used on several pathological conditions with promising results. More recently, these magnetic fields have been also used to treat several types of cancer. However, there are conflicting results and remains continuing concern that exposure to electromagnetic devices may cause deleterious health effects. The current study was aimed to further evaluate the potential cytogenotoxic effects induced in rats exposed in a device that uses PEMF's combined with SMF's. Thirty sexually mature 14-week-old male and female Sprague Dawley rats were distributed into three groups: (a) 5 males and 5 females independently exposed to PEMF's combined with SMF's, (b) animals treated with SMF's only, and (c) non-exposed animals. Micronucleus test in rat bone-marrow and male germ cells analysis were performed following standardized procedures. The observed results showed a lack of alterations on micronuclei frequency, on scored polychromatic erythrocytes percentage, and on sperm counts and morphological characteristics of male germ cells in animals exposed to magnetic fields device compared to unexposed animals. These results suggest that magnetic fields generated in this therapeutic device did not have any detectable cytotoxic or genotoxic effect in exposed rats. In view of these findings and the contradictory reports in the literature, it is necessary to carry out more research to help to clarify the controversy regarding cytogenotoxic risk associated with medical magnetic fields exposures.
KEYWORDS: PEMF-medical device, static magnetic fields device, cytotoxicity, genotoxicity, micronuclei, sperm abnormalities.

RESUMEN: Hay una diversidad de publicaciones que intentan demostrar la eficacia de los tratamientos con campos magnéticos en el área médica. Las tecnologías que usan campos electromagnéticos pulsados (PEMF, por sus siglas en inglés) y campos magnéticos estáticos (SMF, por sus siglas en inglés) han sido utilizadas en varias condiciones patológicas con resultados prometedores. Más recientemente, los tratamientos magnéticos también se han utilizado para tratar varios tipos de cáncer. Sin embargo, hay resultados contradictorios reportados en la literatura y sigue existiendo preocupación entre la comunidad científica de que la exposición a dispositivos electromagnéticos pueda causar efectos nocivos para la salud. El presente estudio, tuvo por objeto evaluar los posibles efectos cito-genotóxicos inducidos en ratas expuestas en un dispositivo terapéutico que utiliza PEMF en combinación con SMF, originalmente diseñado para el tratamiento de diversos tipos de cáncer en humanos. Para tal efecto, 30 ratas Sprague Dawley, machos y hembras, sexualmente maduras y de 14 semanas de edad se distribuyeron en tres grupos de estudio: (a) 5 machos y 5 hembras expuestas de forma independiente a PEMF combinados con SMF, (b) animales tratados solo con SMF y (c) animales no expuestos. Se realizaron pruebas citogenéticas; como la prueba de micronúcleos en médula ósea, y el análisis de células germinales masculinas siguiendo protocolos previamente estandarizados. Los resultados observados, mostraron ausencia de alteraciones en la frecuencia de micronúcleos, en el porcentaje de eritrocitos policromáticos, en el recuento de espermatozoides y las características morfológicas de las células germinales masculinas en animales expuestos al dispositivo terapéutico, en comparación con los animales no expuestos. Estos resultados sugieren que los campos magnéticos generados en esta máquina de uso médico no tuvieron ningún efecto citotóxico o genotóxico detectable en

las ratas expuestas. En vista de estos hallazgos, y de los informes contradictorios publicados en la literatura, se hace pertinente y necesario llevar a cabo más estudios sobre la inocuidad de estos dispositivos utilizando otros tipos celulares y una variedad de pruebas citogenéticas.
PALABRAS CLAVE: Campos magnéticos, genotoxicidad, micronúcleos, espermatozoide, mutación.

1 | INTRODUCTION

Several techniques that involve electromagnetic fields (EMF's) procedures have been used for therapeutic purposes, but have also been correlated with deleterious health effects. The bio-effects of exposure to electromagnetic fields have been studied for many years. However, among scientific community, there is still no agreement on whether these effects are physiologically significant (Macklis, 1993; Miller and Green, 2010; Binhi, 2012).

Interest in the health effects of EMF's on human populations was rekindled by a series of epidemiological studies carried out during the late 1970's and early 1980's (Jauchem and Merritt, 1991). By the way, Milham (1982) analyzed the occupational grouping of cancer deaths in adult white men who died between 1950 and 1979; an increased mortality ratio for leukemia was found. More recent studies in adult human beings have suggested increased occupationally associated EMF's risks for breast cancer, abnormal pregnancies, chromosomal abnormalities, congenital deformities, and several other health conditions (Ahlbom et al., 2001; Pearce et al., 2007; Hug et al., 2010). Based in these studies and others, there is no doubt that life bodies can be affected by EMF's at several levels. Actually, these fields are the most probable candidate to affect cellular interactions and it is possible to consider a diversity of cellular effects induced of both, electric and magnetic fields (Cifra et al., 2011).

With regard to PEMF's, they have been used for the past 40 years to treat therapeutically resistant problems of the musculoskeletal system. Recently, diverse approaches using PEMF's procedures were tested for therapeutic effects in several types of cancer (Haro et al., 2005; Grosel et al., 2006).

While the issue of potential genotoxic and cytotoxic effects of EMF's is controversial, there are many commercial machines that use several types of magnetic fields that are unproven or have not been properly tested. In view of this, we have aimed the present study to further evaluate the cytological effects induced in rats exposed in a patented medical device (Davidson, 2001), originally designed to treat several types of cancer in human beings. This machine uses PEMF's and SMF's. For the present study, we decided to confine our *in vivo* experimental investigation to single parameters, i.e., the analysis of micronuclei in bone marrow, sperm analysis, and morphological characteristics of male germ cells in exposed Sprague-Dawley rats.

2 | MATERIALS AND METHODS

Animals

14-week-old, sexually mature, male and female rats (Sprague-Dawley line) were used. Animals weighing 250-300 g for females, and 400-450g for males were born and raised in our breeding colony. After 10 days quarantine period, animals were then randomly distributed into experimental and control groups. At the time of experiments the animals were housed at 25º C in standardized acrylic cages (five animals per cage) under a 12-12 h light/dark cycle, with free access to standard diet and tap water. This research project fulfilled all requirements of the University's Animal Care and Use for Research Protocol, which is based on the National Guidelines for Ethics and Biosafety under the General Law of Health for issues regarding Health Research, Ministry of Health, Mexico City.

Magnetic Field Machine and Measurements

Magnetic fields were generated by a patented machine that combine PEMF's and SMF's exposure according to Davidson (2001). In summary; this machine comprised a quantity of permanent neodymium magnets arranged in a side-by-side relationship. Each permanent magnet being adjacent to the magnetic north pole and magnetic south pole of an adjacent permanent magnet, respectively. Thus, the plurality of these magnets forms a ring of permanent magnets. The device further includes an electrically conductive wire wound substantially around the ring, and tubing wrapped around the ring of permanent magnets between windings of the wire. A cooling device introduces a flow of coolant through the tubing. This machine also includes a control circuit, connected to the wire, for selectively generating a coil current for passing through the wire. The current has an AC component and a DC component. According to the manufacturer, the frequency of the AC component is programmable and is set to substantially match a resonant frequency associated with the organisms to be treated. Thus, the coil current creates an electromagnetic field that interacts with the magnetic field generated by the ring of permanent magnets to generate a complex field that causes ionic collisions within the cells to be treated. This device was originally designed to treat several types of cancer in humans (Davidson, 2001).

Magnetic flux density (rms) was measured in the middle of the ring where the magnetic fields were homogeneous using an axial Hall-effect probe (Bell FW 6010 teslameter, Orlando Fl.) Moreover, an oscilloscope (BK-Precision model 2120) was coupled to the system to monitor the resultant field. A pulsed 120 Hz square wave form electromagnetic field was then generated, with a maximum peak of 17.6 mT (rms) at the center of the exposure zone where cages containing animals were placed. On the other hand, the SMF's were measured with the teslameter, setting the apparatus in DC mode, this value was a maximum peak of 8.6 mT at the middle of the exposure zone. Furthermore, the local geomagnetic field was measured by using an axial high sensitivity Hall probe (Integrity Design IDR-321 geomagnetometer,

Essex Jct., VT9) and the average value was 20 μT within the exposure zone. The local temperature in the exposure zone was also measured setting the Bell FW 6010 teslameter in Temp mode. The measured temperature value was an average of 25.3 ± 0.5 ºC when the machine was on, and 24.9 ± 0.3 ºC when the machine was off, no statistically significant differences were observed between two conditions (Kolmogorov-Smirnov test for normality, followed by paired *t*-test).

Experimental Protocol

Three experimental regimens were considered for bioassays: (*a*) 5 male and 5 female rats, independently exposed to 120 Hz PEMF's combined with SMF's for three consecutive days, two exposures of 50 min each with no-exposure intervals of 1 h between exposures, *(b*) 5 male and 5 female rats, independently exposed only to SMF's, without current in the coil, and (*c*) non-treated animals, including 5 males and 5 females without any magnetic exposure. During the no-exposure intervals, the cages containing the animals were allocated in the same exposure room where any detectable EMF was measured. In the case of micronuclei analysis, both male and female animals were used. For germ-cell tests, only males were analyzed.

Micronucleus Test

Animals were killed at the end of exposure time. Following sacrifice, Acridine Orange fluorescent staining was applied to the micronucleus (MN) test, according to the previous described procedure (Hayashi et al., 1983). In summary; bone marrow from both femurs was flushed with 0.5 mL of fetal calf serum into a microfuge tube using a 1mL syringe fitted with a 22 G needle. The cells were concentrated by gentle centrifugation at 600x *g* for 1-3 min and a small drop of resuspended cells was placed on a clean microscope slide to make a thin smear. All smears were air-dried, fixed in absolute methanol and stained using Acridine Organge (Fisher Sci. Co., Fair Lawn, NY)

Coded slides were examined under x1000 magnification using a fluorescence microscope equipped with appropriate filters. Immature, polychromatic erythrocytes (PCE's) were identified by their orange-red color, mature erythrocytes by their green color and the MN by their yellowish color. For each rat, 500 erythrocytes were examined to obtain the percentage of PCE's. In addition, for each rat, 2000 consecutive PCE's were examined to determine the incidence of MN. Decoding of the slides was done after completing the microscopic analysis.

Male Germ Cells and Sperm Morphology Analysis

For sperm counts, orchidectomy was performed by open castration method. A midline or pre-scrotal incision was made and the testes were milked out on the incision site. The testicles were then exposed by incising the *tunica vaginalis*. The spermatic cord was exposed, ligated and incised. Semen samples were thereafter collected from the cauda

epididymis according to a previously described procedure of Oyeyemi and Ubiogoro (2005). These samples were then analyzed immediately after collection. The spermatozoa were scored by using an improved Neubauer chamber as described by Pant and Srivastava (2003).

For sperm morphology analysis, smears were prepared from epididymis following the previously described method of Wyrobeck (1979). Spermatozoa preparations were stained using 1% Eosin Y die/1 h (Fisher Sci. Co., Fair Lawn, NY) and examined under high power magnification (x1000). 100 cells per slide, 10 smears for each animal, for a total of 5000 cells per analyzed group, were evaluated in a blind way, for the presence of bicephalic or biflagellate forms, and abnormalities in head shape such as enlarged and amorphous head were expressed as a percentage of sperm morphology abnormalities.

All reagents were supplied by Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

Statistical Analysis

The statistical differences among groups were estimated using analysis of variance for normal distributions. In the case of the percentage of sperm morphology abnormalities, the data obtained were first transformed by using the arcsine function. After this, an analysis of variance for normal distributions and the correspondent parametric Tukey test for establishing individual differences were performed. The normality of the data was determined by the Kolmogorov-Smirnov test ($p<0.05$). All analyses were done using the SPSS package version 22.0. Differences were considered to be significant when the probability values were lower than 0.05.

3 I RESULTS

The current *in vivo* study was aimed to evaluate the possible cytogenotoxic effects of magnetic field exposure from a patented therapeutic machine in both male and female Sprague-Dawley rats on the micronuclei frequency and PCE percentages, and on male germ cells; sperm counts, and sperm morphology abnormality percentages. In the figure 1, the frequency of micronuclei from bone marrow of exposed rats is showed. No statistically significant differences were found among groups in both male and female animals, suggesting no clastogenic effect induced by PEMF's or SMF's exposure ($p> 0.05$). On the other hand, figure 2 shows the grouped means of percentages of polychromatic erythrocytes (PCE's) from the analyzed groups. These values indicated no statistically significant differences among groups, for both male and female animals ($p>0.05$). These results suggested that the number of PCE's were no altered by magnetic field exposure of both PEMF's or SMF's.

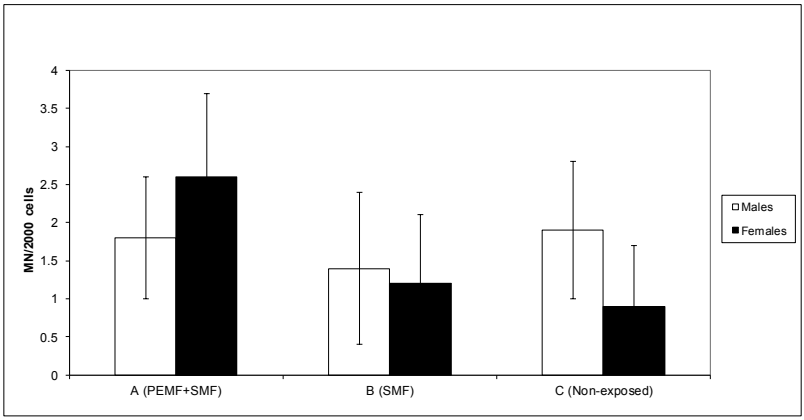

Figure 1. Micronucleus (MN) frequency from bone marrow polychromatic erythrocytes of Sprague Dawley rats. (a) animals exposed to 120 Hz PEMF's combined with SMF's for three consecutive days, two exposures of 50 min each with no-exposure periods of 1 h between exposures, (b) rats exposed to SMFs, without current in the coil, and (c) non-treated animals. No statistically significant differences were found among groups in both male and female animals ($p > 0.05$). Bars represent grouped means ± S.D.

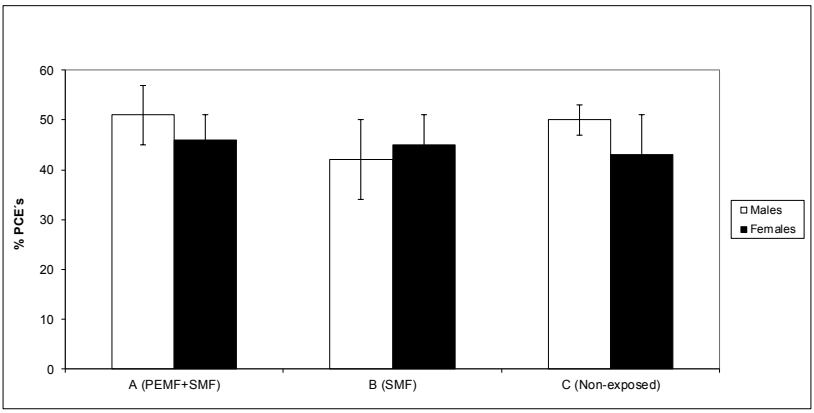

Figure 2. Percentages of polychromatic erythrocytes (PCE's) from bone marrow of Sprague Dawley rats. (*a*) animals exposed to 120 Hz PEMF's combined with SMF's for three consecutive days, two exposures of 50 min each with no-exposure periods of 1 h between exposures, (*b*) rats exposed to SMF's, without current in the coil, and (*c*) non-exposed animals. No statistically significant differences were found among groups in both male and female animals ($p > 0.05$). Bars represent grouped means ± S.D.

With regard to male germ cells analysis, the observed results showed no alterations in either the sperm counts (Figure 3), and morphological characteristics of spermatic cells (Figure 4) when compared the grouped means among groups ($p > 0.05$).

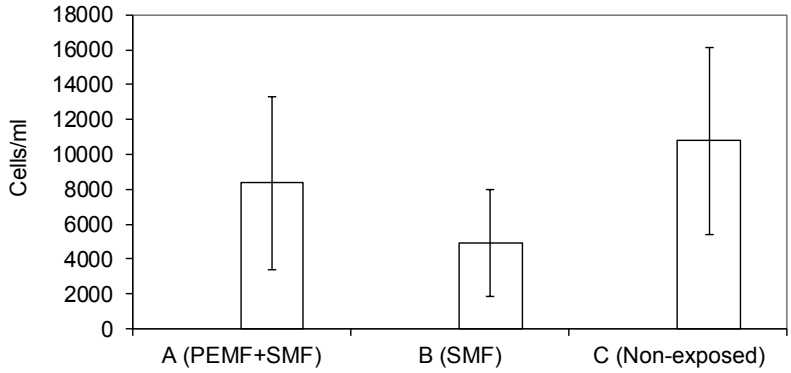

Figure 3. Effect of magnetic field exposure on sperm counts of Sprague Dawley rats. (*a*) animals exposed to 120 Hz PEMF's combined with SMF's for three consecutive days, two exposures of 50 min each with no-exposure periods of 1 h between exposures, (*b*) rats exposed to SMF's, without current in the coil, and (*c*) non-exposed animals. No statistically significant differences were found among groups ($p > 0.05$). Bars represent grouped means ± S.D.

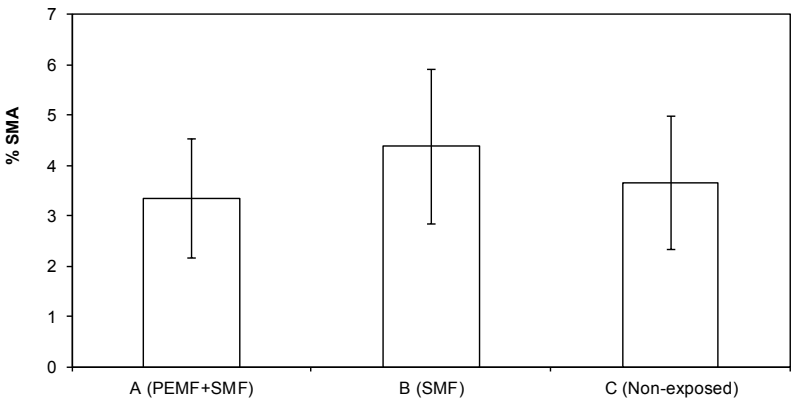

Figure 4. Percentages of sperm morphology abnormalities (SMA) in male germ cells of Sprague Dawley rats. (*a*) animals exposed to 120 Hz PEMF's combined with SMF's for three consecutive days, two exposures of 50 min each with no-exposure periods of 1 h between exposures, (*b*) rats exposed to SMF's, without current in the coil, and (*c*) non-exposed animals. No statistically significant differences were found among groups ($p > 0.05$). Bars represent grouped means ± S.D.

4 | DISCUSSION

Biomedical effects attributed to magnetic fields have been widely discussed during the last years. The question has been raised as to whether exposition to these fields can originate genetic damage or other biological disfunctions. On the other hand, since the early part of the 20 th century, attempts had been undertaken to treat or otherwise cure human diseases using magnetic field techniques. However, many research reports agree that life organisms could be affected in a negative way by exposure to electromagnetic radiation

(Feychting et al., 2005). In the current study, we did not find any measurable genotoxic or cytotoxic effect induced in mature rats by an *in vivo* exposure to a therapeutic machine that combine PEMF's and SMF's exposure conditions.

Results presented here, indicated no alterations in micronuclei frequency and in PCE percentages of treated animals due to magnetic field exposure. These results agreed with previous reports that used the MN assay, indicating no genotoxic or cytotoxic effects induced by magnetic fields (Scarfi et al., 1994; Scarfi et al., 1999; Frahm et al., 2006; Okudan et al., 2010). Furthermore, it was observed that extremely-low frequency EMF's exposure at 1.0 mT and 60 Hz did not increase the MN frequency by ionizing radiation in mouse embryonic fibroblast NIH3T3 cells (Jin et al., 2012). On the contrary, there are several papers with positive results, indicating a genotoxic effect due to magnetic field exposure (Simkó et al., 1998; Celikler et al., 2009). Winker et al. (2005) also claimed for a clastogenic potential of low-frequency EMF's, which may lead to considerable chromosomal damage in dividing human diploid fibroblasts. Moreover, Erdal et al. (2007) found a higher MN frequency in Wistar rat tibial bone marrow cells treated with a long term extremely low-frequency EMF's exposure, in comparison to non-exposed or acutely exposed animals.

The controversial concern regarding EMF's cytogenotoxic effects, is originated from the fact that many scientists believe that EMF's therapeutic devices produce as a little quantity of energy and are therefore too weak to induce any alteration on cells. Moreover, the inconclusive nature of laboratory bioassays and the fact that there are no epidemiological studies of patients exposed to such therapeutic devices turns this concern very complex. In contrast, the International Agency for Research on Cancer (IARC, 2002) included and categorized the extremely low frequency magnetic fields as "possibly carcinogenic to humans"; this was based on pooled analyses of epidemiological research that reported an association between exposure to low-level magnetic fields and several types of cancer.

With regard to the issue that weak fields may have too little energy to induce any genotoxic effect or DNA damage, it has been proposed that because low frequency electromagnetic radiation does not transmit enough energy to affect chemical bonds, then, it is generally accepted that extremely low frequency EMF's are not enable to cause DNA direct damage (Adair, 1998). On the other hand, several hypotheses have been put forward of how EMF's can alter the structure of DNA indirectly. Secondary currents and, hence, a movement of electrons in DNA might be induced (Valberg et al., 1997). This could generate guanine radicals, which, upon reaction with water may be converted to oxidative DNA damage (Giese, 2006). In a recent investigation, Focke et al. (2010) found that exposure of human primary fibroblasts to a 50 Hz EMF's at a flux density of 1.0 mT induced a slight but significant increase of DNA fragmentation as assessed by the Comet Assay. Furthermore, they observed that EMF-induced responses in the Comet Assay were dependent on cell proliferation, suggesting that DNA replication rather than the DNA itself may be altered.

On the other hand, the results obtained in the present research indicated that

exposure to pulsed EMF's or SMF's emitted by the therapeutic device showed no influence on sperm cells of exposed animals compared with the un-exposed controls. The sperm counts were no altered by exposure, suggesting no effect in cell cycle progression. These results coincided with the findings of Lundsberg et al. (1995) who found no association of occupational EMF exposure on sperm concentration among males. By the way, even with higher frequency electromagnetic radiation, Aitken et al. (2005) observed that sperm number, morphology and vitality of male germ cells were not significantly affected when mice were treated with 900 MHz radio-frequency electromagnetic radiation. On the contrary, Furuya et al. (1998) found that the application of EMF's at 50 Hz with intensities between 1.0 mT to 100 mT affected the proliferative and differentiative capacity of mouse spermatogonia. Moreover, anomalous effects on spermatogenesis in mice that were exposed to a 1.5-T static magnetic field were informed by Narra et al. (1996). Ramadan et al. (2002) also reported that exposure of mice to fractionated doses of high oscillating magnetic fields (20 mT) induced a statistically significant decreased sperm count, and daily sperm production. Likewise, a significant decreased sperm count was found in mature Sprague-Dawley rats exposed to a 50 Hz and 25μT for 18 consecutive weeks (Al-Akhras et al., 2006). Furthermore, Hong et al. (2005) reported that a 50 Hz EMF's exposure have the potential to induce DNA strand breaks in testicular cells and sperm chromatin condensation of exposed mice.

Regarding the sperm morphology abnormalities, the outlined results presented here are in good agreement of those of Withers et al. (1985) who did not find alterations in sperm heads, however, in their work they exposed mice to 0.3 T static magnetic fields from a magnetic resonance device. Similarly, the Spanish group of Tablado et al. (1998) demonstrated that morphological characteristics of epididymal sperm of mice were not affected after exposition to 0.7 T therapeutic magnets. We have previously published a lack of alterations on morphological characteristics of male germ cells in mice exposed to a 60 Hz and 2.0 mT magnetic fields (Heredia-Rojas et al., 2004). On the contrary, Roychoudhury et al. (2009) found alterations of spermatozoa and fertilization rates in rabbits exposed to 50 Hz magnetic fields. For human beings, it is generally accepted that there is some evidence that suggests that magnetic field exposure may have deleterious effects on sperm quality (De-Kun et al., 2010).

On the other hand, conflicting findings have been reported by Lorio et al. (2007) who observed significant increased values of kinematic parameters of spermatozoa after an exposure to EMF's of 5 mT and 50 Hz frequency. In contrast, a 5 mT sine wave (50 Hz) and a 2.5 mT square wave (50 Hz) exposure did not produce any significant alteration on sperm motility. These results indicate that EMF's exposure can improve spermatozoa motility, and that this effect depends on the field characteristics.

In conclusion, the current study suggested that an *in vivo* exposure to PEMF's combined with SMF's emitted by a patented therapeutic device, originally designed for treating several types of cancer in humans, did not have any measurable effect on MN frequency and PCE

percentages of exposed rats and on sperm counts and morphological characteristics of male germ cells. These results support, in part, the innocuity of this therapeutical machine in mammals. However, we consider that is necessary to carry out more research using various cell types and cytological endpoints under different experimental conditions to add more evidence of innocuity of the mentioned therapeutic device, and at the same time, help to clarify the controversy concerning the possible cytotoxic and genotoxic risk associated with a therapeutic magnetic field exposure.

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