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Preliminary study on the impact of 900 MHz radiation on human sperm: An in vitro molecular approach

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ABSTRACT

The use of technologies that produce and emit electromagnetic fields (EMF) is growing exponentially worldwide. The biological effects of EMF-emitting equipment, such as mobile phones and other wireless devices, have been studied in the last decade using *in vitro* and *in vivo* methods. Infertility is a growing health problem, and nearly half of cases are because of male-factor. This study investigated the direct *in vitro* effects of 900 MHz radiation exposure on sperm parameters, genetic status, apoptotic markers, and the PI3K/AKT signaling pathway in healthy normozoospermic men. Semen samples were divided into four groups, two control (30 min and 1 h) and two EMF exposure (30 min and 1 h). Sperm parameters (motility, progressive motility, acrosomal index, morphology), genetic status (DNA fragmentation and chromatin integrity), apoptotic markers (cytokine-c and caspase-3 expression) and the PI3K/AKT signaling pathway (phosphoinoitide 3-kinase-PI3K- and phosphorylated AKT- p-AKT-) were analysed. Sperm motility were significantly reduced in 30 min EMF exposure while a significant increase in the expression of p-AKT were observed in 1 h EMF exposure group. An increased vacuolisation, acrosomal defect, extension of subacrosomal space, uncondensed chromatin structure, apoptotic signs and disrupted axoneme were observed in both EMF groups which were not observed in the control group. Other sperm parameters (morphology and acrosomal index), genetic status, apoptotic markers and the PI3K expression rates had no significant change.

1. Introduction

Infertility affects approximately 15 % of couples hoping to conceive, and nearly half of cases result from deficiencies in male reproductive systems [3]. Male infertility is a growing public health problem which may be affected from a wide variety of factors can affect male reproductive health, including genetic, hormonal, anatomic and environmental factors [17,27].

Among all radiation, electromagnetic is one of the factors that can be harmful to reproductive health. Concerns about the harmful effects of radiofrequency electromagnetic radiation [4] emitted from mobile phones and towers, an inseparable feature of daily life, are growing

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[58]. The rapid growth of mobile phone use has in turn increased exposure to electromagnetic fields (EMF) [44]. A number of studies have reported that short- or long-term exposure to EMF can have deleterious effects on fertility in humans and animals, including decreased sperm count and motility [16,2,24,61,76], pregnancy loss [53] and impaired foetal development [14]. However, others have found no significant effects on reproductive outcomes [19,36,37,68,69,71].

Studies investigating the impacts of cell phones on male reproductive organs basically focused on sperm parameters [16,24,40,42,43,57]. However, an overall biologic analyses on sperm cells exposed to EMF are not consistently apparent. Semen analysis is the cornerstone used in the determination of male fertility and is the first step in diagnosing individuals with infertility (Pozzi et al. [73]). Semen analysis is performed by measuring sperm parameters which include semen volume, pH, motility, morphology, leukocyte count, detection of immature germ cells, and agglutination assessment [29,66]. Semen assessment using WHO reference values has a sensitivity of 89.6 % when results obtained with semen parameters performed using only the methodology established by the WHO for testing [32]. Semen parameters are used for determination of reproductive health, diagnosis and/or prognosis of reproductive disorders, evaluation of treatment effects on sperm production (e.g. toxicology and nutritional studies) because of defects in sperm parameters are associated with disorders in the male reproductive system [12].

Among the biological effects, apoptosis is of great importance since the apoptotic pathway is known to be stimulated as a response to various stimuli [45] and has been shown to cause subfertility [5]. Apoptosis is a complex event which is detected by observing commonly accepted markers including DNA fragmentation, and caspase activation. Among the pathways accompanying apoptosis in sperm cells, PI3K/AKT signaling pathway is one of which has a major role, basically in preventing the initiation of apoptotic cascades [45].

The aim of the recent study was to investigate the 'direct' *in vitro* effects of 900 MHz EMF exposure, with a waveform identical to that of a mobile phone, on sperm parameters (motility, morphology and acrosomal index), genetic status (DNA fragmentation and chromatin integrity), apoptotic markers (cyt-c and caspase-3 expression) and the PI3K/AKT signaling pathway.

2. Material and methods

Fifteen normozoospermic men who applied for infertility investigation to VM Medical Park Hospital, IVF Center, Samsun, Turkey were included in the study. Men with azoospermia, cryptozoospermia, any known chronic disease, genetic anomaly or testicular dysfunction were excluded. The volunteers were 21–50 years old and had no previous history of testicular surgery. All semen samples were processed and evaluated by infertility specialists in the center. EMF exposure was performed at Ondokuz Mayıs University Faculty of Medicine Department of Histology and Embryology. Validated methods were used to ensure levels of exposure consistent with those currently emitted by mobile phones [25,26,70]. All other processes for analysing sperm quality and quantities were conducted at the Istanbul Medipol University Histology Laboratory and Regenerative and Restorative Research Centre. All the analyses were performed in a blinded manner.

2.1. Semen analysis and preparation

Semen samples were obtained by masturbation into sterile containers after 2–5 days of sexual abstinence. After liquefaction, semen analysis was performed according to World Health Organization (WHO) criteria [74]. Shortly, after EMF exposure on semen samples, the forwardly progressive sperm motility (A motility) (total progressive motility %), total motility rates (total motility %) were assessed manually with using Makler chamber [74]. 40X objective was used to evaluate the motility. Motility percentages were calculated with counting progressive moving sperm (Motility A) and all motile sperm (progressive and nonprogressive) [74]. After EMF exposure, 10-µl semen samples were spread onto positive charged slides and sperm smears were prepared which were used for assessment of sperm morphology, chromatin integrity and protein profiling. A least 100 spermatozoa were evaluated and scored. According to WHO guideline, morphologically normal spermatozoa have an acrosome that covers 40 %-70 % of the head and an oval head. Sperms with no anomalies in the neck, midpiece, or tail were considered to have normal morphology and a percentage of normal morphology were calculated.

2.2. EMF exposure system

Each semen sample was divided into four 1 ml aliquots and transferred to a culture dish (35x10mm) (Corning/ThermoFisher, United Kingdom). Two aliquots were exposed to 900 MHz EMF for 30 minutes or one hour. The other aliquots constituted the respective control group with no EMF exposure. The EMF was created using a signal generator operating at 900 MHz (Microwave Test Transmitter, Set Electronic Ltd, Isparta, Turkey) and a monopole antenna that capable of emitting electromagnetic waves. Sperm samples were placed into a 16-chamber round polycarbonate cage, specifically designed for uniform EMF exposure (shown in Fig. 1). An EMF meter was used to measure the electric field density (Holaday Sanayi A.Ş., Adapazarı, Türkiye) (Bilgici et al. Altun et al.).

2.3. Protein expressions

Immunohistochemistry analyses were performed to evaluate protein expression. Antibodies used were as follows: p-Akt (1:250, Santa Cruz, sc-7985), PKI3 (1:250, Santa Cruz, sc-1637), caspase-3 (1:300, Santa Cruz, sc-56046), cytochrome complex (cyt-c) (1:300, Santa Cruz, sc-13156). Air-dried smears were fixed with 4 % paraformaldehyde (Sigma, SZBF1870V) and then hydrolysed with 3 % H_2O_2 (Sigma-Aldrich) in methanol (JT Baker, 80452500). Antigen retrieval was performed with citrate buffer (Bio-Optica) for 20 min at 200 watts in a microwave. All washing procedures were performed in triplicate with phosphate buffer solution (PBS). An immunohistochemical (IHC) staining kit (Abcam, ab93677) and DAB chromogen kit (Abcam, ab64238) were used for the detection of target proteins according to the manufacturer's instructions. Counterstaining was performed with Mayer's hematoxylin. At least 100 randomly selected sperm cells for each participant were analysed in terms of the head, midpiece, and tail at 100X magnification under a light microscope; the proportions of each feature (head, midpiece and tail) were calculated as percentage values. Average positive staining of the head, midpiece and tail was calculated to obtain an Immune Reactive Scoring (IRS) value. All scoring procedures were performed blind.

2.4. Morphology and acrosomal index

DIFF-quick staining was used to examine the morphology and acrosomal index of sperm cells. Sperm smears were prepared following EMF exposure, and the Diff-Quick protocol was then applied. The smears were fixed for 15 seconds within the fixation solution. Stain 1 (xanthene solution) was applied for 15 minutes, and stain 2 (thiazine solution) for 10 seconds. The samples were then washed with distilled water. Analysis was carried out according to Kruger's strict criteria [46].

2.5. DNA Fragmentation analysis

Sperm chromatin dispersion (SCD) test (Halotech, Spain) was performed for DNA fragmentation analysis according to the manufacturer's protocol. Sperm cell evaluation was carried out on at least 100 sperm cells under 100X magnification in brightfield microscopy for each sample according to [39]. Briefly, spermatozoa with large halos whom

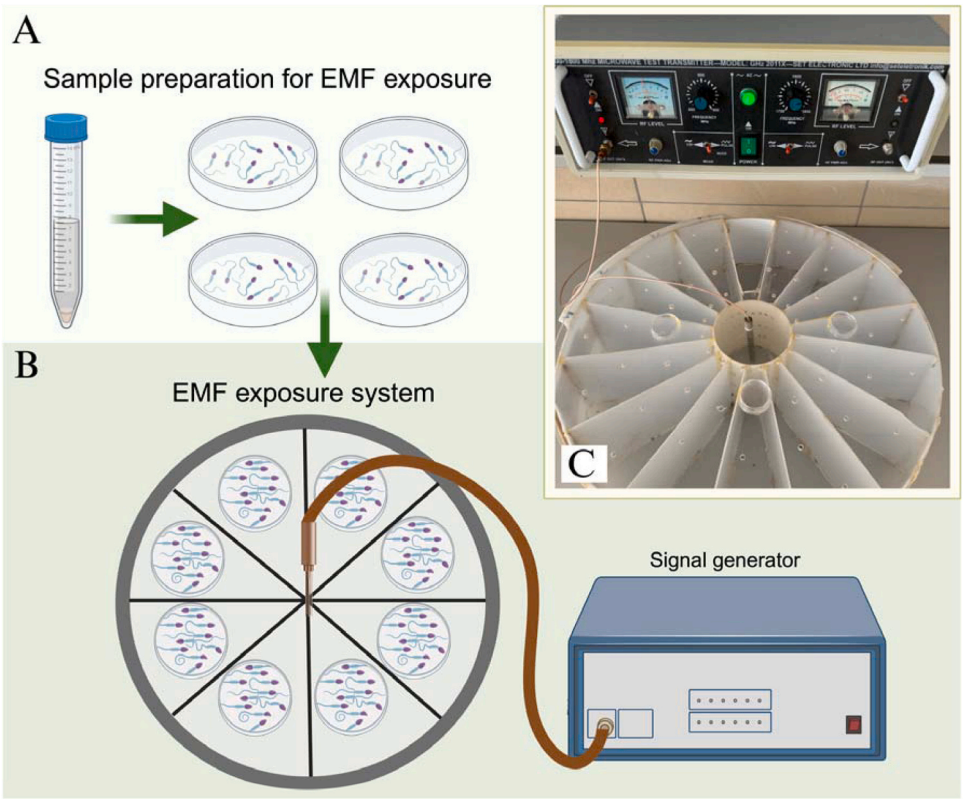

Fig. 1. A demonstration of the EMF exposure system (A and B). Sperm samples were placed in cell culture dishes (A), which were then positioned inside a circular polycarbonate cage. The electromagnetic field (EMF) was generated by a signal generator operating at a frequency of 900 MHz (B). Fig. C shows a real image of the experimental setup, providing a visual confirmation of the actual arrangement used in the study. (Created in BioRender. Kaplan, A. (2024) BioRender. com/m93a669).

thickness is equal or longer than the diameter of the core and medium halos whom thickness is between longer than 1/3 of and shorter than the diameter of the core defined as 'sperm with intact DNA'. If the sperms have small or no halos, they have DNA fragmentation. Sperm cell with a small halo whom thickness was equal or shorter than 1/3 of the diameter of the core and sperm cells with no halo were defined as 'sperm with fragmented DNA' [39].

2.6. Chromatin integrity

Toluidine blue (TB) staining was used to analyse sperm chromatin structure and condensation (Kim et al.). Air-dried sperm smears were fixed with 96 % ethanol-acetone (1:1) (Merck, Germany) at 4°C for 1 h. The sperm cells were then hydrolysed in 0.1 N HCl (Merck, Germany) at 4°C for five minutes. Staining was carried out with 0.05 % TB (Merck, Germany) for five minutes. Sperm cell heads with good chromatin integrity were stained light blue, and these were defined as normal, TB (+). Sperms with diminished integrity appeared deep violet (purple), and were defined as abnormal, TB (-). One hundred randomly selected sperm cells were analysed for each sample, after which the TB (+) and TB (-) ratios were evaluated.

2.7. Electron microscopic analysis

Semen samples were fixed with 2.5 % glutaraldehyde (EMS, USA) for electron microscopic analysis. Routine electron microscopic tissue-processing procedures were performed, involving fixation in 1 % osmium tetroxide (EMS, USA) and dehydration in acetone series (Merck, Germany) and propylene oxide (EMS, USA). The sperm cells were then embedded in resin (AGR1030, Agar Scientific, UK), and 70 nm ultra-thin sections were taken using an ultramicrotome (Leica Ultracut UCT, Germany) and placed onto copper grids. The sections contrasted lead citrate (Leica Ultrastain II, Germany) and 1 % uranyl acetate (EMS, USA). Images were captured at differing magnifications using scanning transmission electron microscopy (STEM) (JEOL-JSM-7001F). The area of sperm vacuole area was calculated by using ImageJ Software (NIH) on 70 nm-thick sections, the vacuole was more than 25 % of the head's cross-sectional area defined as vacuolisation and compared with groups [10].

2.8. Statistical analysis

Statistical Package for Social Sciences (SPSS, Version 21 for Windows; SPSS, Inc., Chicago, IL, USA) was used for all statistical analysis. Kolmogorov-Smirnov test was used to check normality of the data. Mann Whitney-*U* test were used for group comparisons. p values $<.05$ were considered statistically significant.

3. Results

3.1. Sperm parameters

After 30 min EMF exposure, Total sperm motility decreased significantly, while sperm progressive motility parameters did not change; resp p=0.028; p=0.755; p=0.089 (Fig. 2). No significant change was observed in none of the parameters after 1 h exposure; p=0.316; p=0.833; p=0.487, resp. Sperm morphology and acrosomal index revealed no significant changes after exposure to both 30 min and 1 h EMF; p=0.59, p=1, p=0.93, p=0.83, resp.

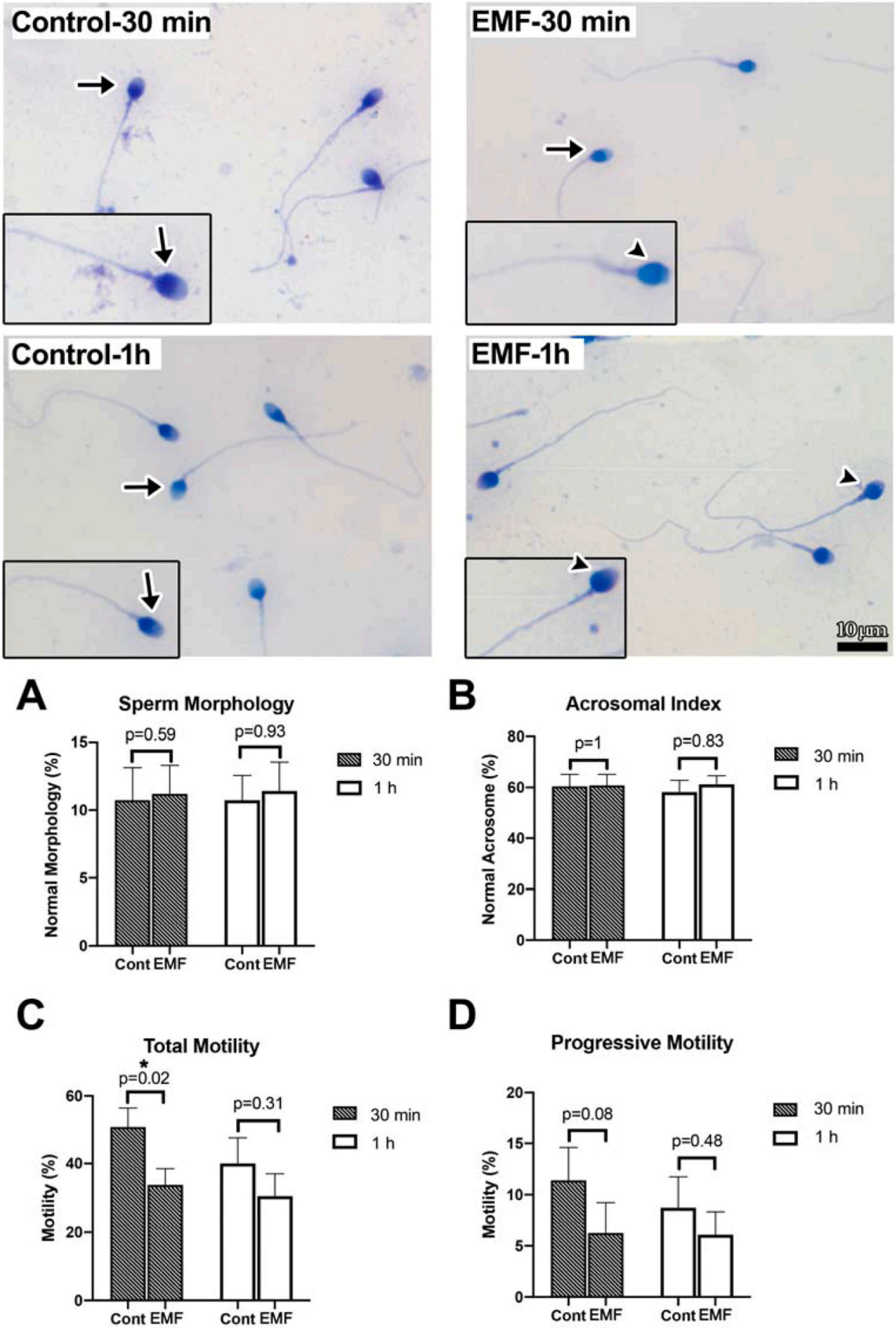

Fig. 2. Sperm parameters analyses. Sperm morphology (A), acrosomal index (B), Total sperm motility (C), and progressive sperm motility (D) *$p \leq 0.05$. Signal on the graph what was statistically significant (*). Sperm normal acrosomal morphology is shown by arrows (→) and abnormal acrosomal morphology by arrowheads (▸).

3.2. Cell cycle signaling pathway proteins

PI3K expression were not changed after 30 min and 1 h EMF exposure, there is no significant differences between groups; p=0.89, p=0.69, resp. 1 h of EMF exposure caused a highly significant increase in p-Akt expression in sperm cells while there is no significant difference 30 min EMF exposure; p=0.006, p=0.37, resp (Fig. 3).

3.3. Apoptotic proteins

Caspase-3 and Cyt-c expression levels were not significantly changed after 30 min EMF exposure (p=0.51, p=0.32, resp). Additionally, the level of caspase-3 and cyt-c were not significantly changed (p=0.08, p=0.09, resp). There were no significant differences between the groups (Fig. 4).

3.4. DNA fragmentation and chromatin integrity

No statistically significant differences were found between the control and EMF exposure groups for DNA fragmentation and chromatin integrity; p=0.35, p=0.25, p=0.35, p=0.23, resp (Fig. 5).

3.5. Ultrastructural analysis

We examined the ultrastructure of the sperm head, acrosome, plasma membrane and the axoneme. An increased vacuolisation were observed in the head region of both EMF groups when compared with the control group. Vacuoles in the nucleus had a vacuolisation area of > 25 % of the head (Fig. 6a, c) and were surrounded with membrane whorls (MWs) (Fig. 6a, d) and some without a membrane (Fig. 6a, b).

In addition, in EMF groups, loss of the acrosomes, swollen and waving in acrosome membranes, extension of subacrosomal space (Fig. 7e, g, h), uncondensed chromatin structure (Fig. 7g, h) were observed in the sperm cell head. Apoptotic cells (Fig. 7h) and disrupted axoneme (Fig. 7f, g) were also observed in EMF exposed samples.

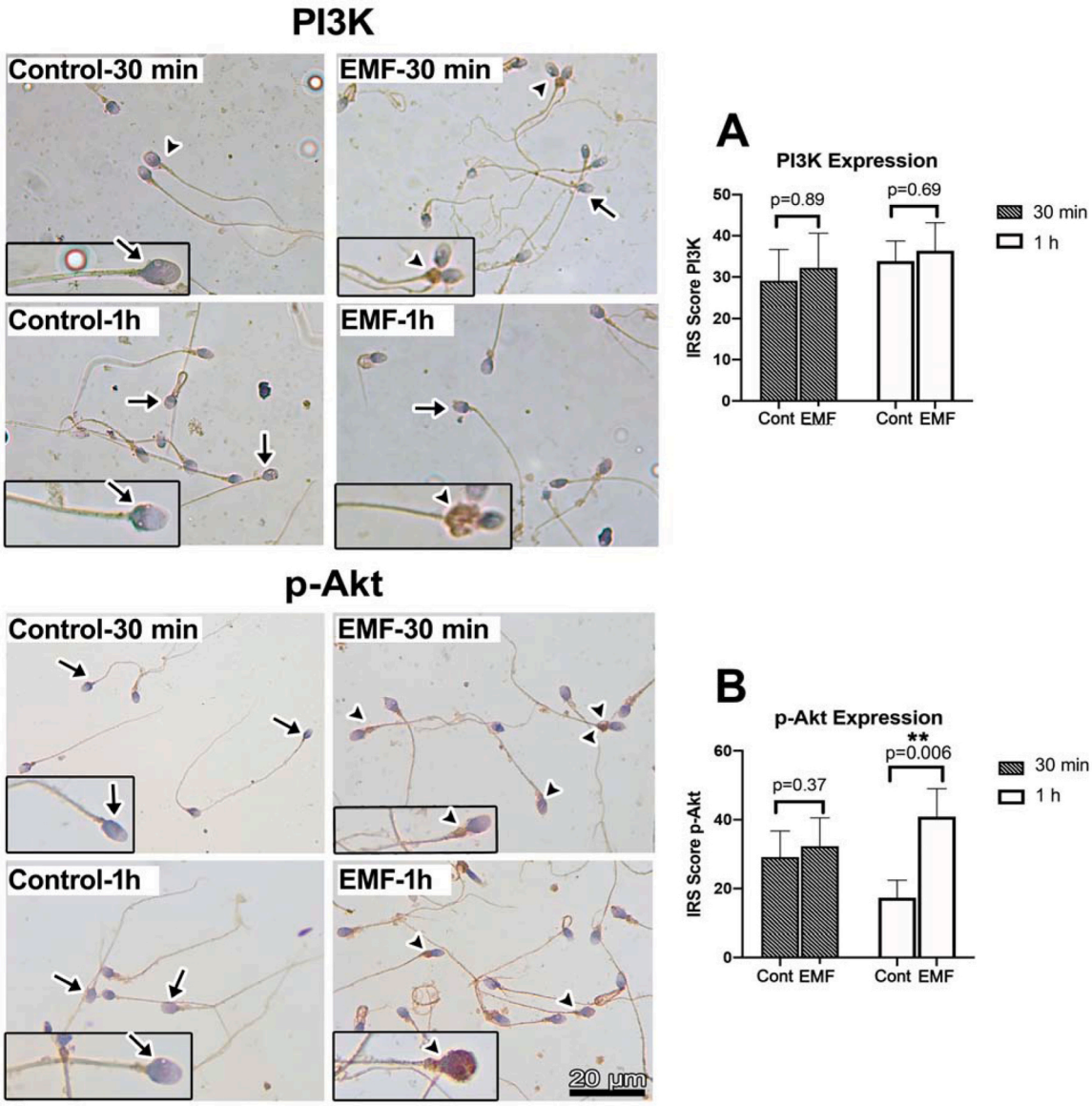

Fig. 3. Immunohistochemistry of sperm cells using anti-PI3K and anti-p-Akt antibodies. Positive sperms are shown by arrowheads (▸) and negative sperms by arrows (→). (A, B) The graphs represent groups' mean values (± SD) with/without EMF exposure. (A) IRS score of PI3K expression in sperm cells. (B) IRS score of p-Akt expression in sperm cells. p-Akt expression after 1 h exposure was significantly increased from that in the control groups; p=0.006, resp. (100X magnification).

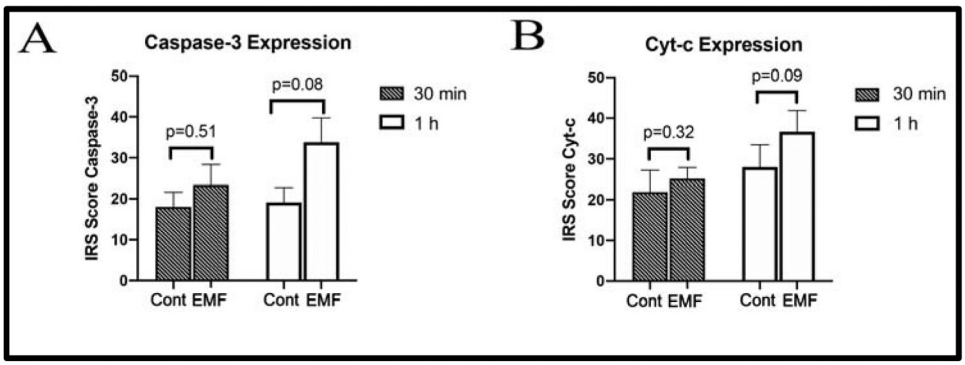

Fig. 4. The effect of EMF exposure on apoptosis proteins. (A, B) The graphs represent the groups' mean values (±SD) with/without EMF exposure. (A) IRS score for caspase-3 expression in sperm cells. (B) IRS score for cyt-c expression in sperm cells. There were no significant differences between the groups.

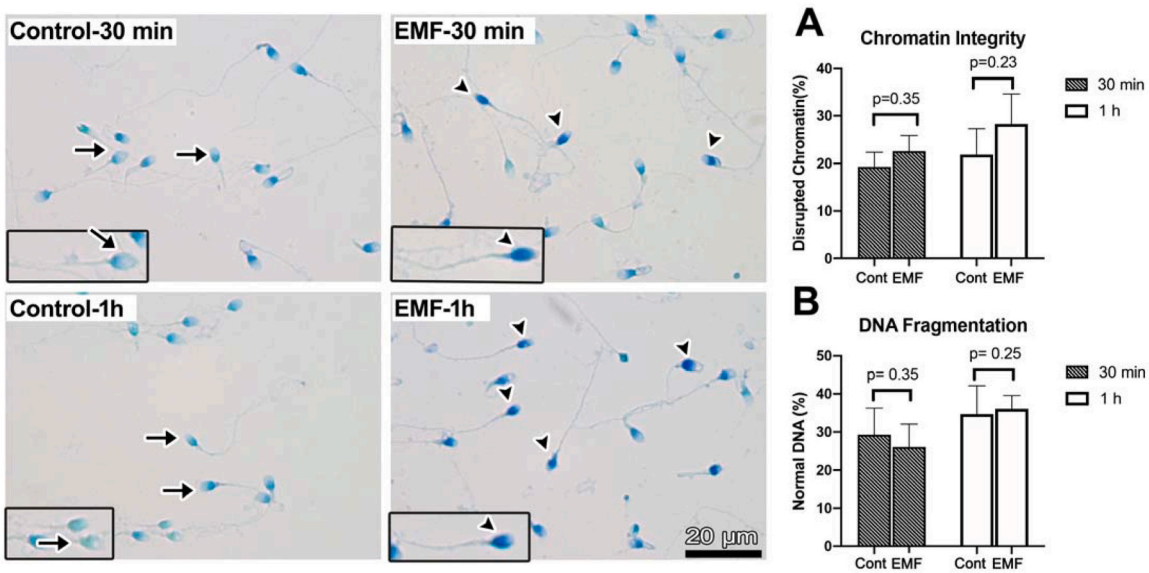

Fig. 5. Sperm cells with intact chromatin structures in the control groups are marked with arrows. In contrast, sperm cells in the EMF-exposed group, which exhibit disrupted chromatin integrity, are marked with arrowheads. (A) Chromatin integrity (n=14) and (B) DNA fragmentation (n=14) analysis revealed no significant differences between the groups.

4. Discussion

Debate is continuing on the effects of environmental factors on sperm quality and fertility, including smoking, alcohol, preservatives, toxic metabolites, pollution, increased testicular temperature, and EMF, the subject of the current study [38]. Increasing industrial development has led to growing exposure to several kinds of EMF, including the type emitted by mobile phones. 900 MHz EMF exposure was studied because of its relevance to commonly used mobile phone frequencies. Although some studies have reported that exposure to EMF may adversely affect fertility in humans and animals [14,21,53], other research has reported limited effects on fertility and reproduction [19,37,68,69,71]. The fertilization capacity of spermatozoa has also been reported to be adversely affected by EMF emitted by mobile phones [2,20,28,42,43]. The mechanisms suggested as contributing to the development of adverse effects include the triggering of apoptosis [47], DNA fragmentation of the mitochondrial and nuclear genome [20,63], protein synthesis [54] and free radical formation [2,22,43,6,60,7] which can induce structural and functional defects in sperm [2,22,35], modifications in cellular signalling pathways including the extracellular signal-regulated kinase (ERK) pathway, apoptosis and impaired cell cycle regulation [13,51].

This study analysed the *in vitro* effect of EMF on semen parameters and its molecular effects in sperm samples from the exposure and control groups. The results showed that total motility is adversely affected by 30 minute exposure with an overall decrease of 17 %, although the prolonged exposure time (1 h) caused no additional effect on the results. However, other sperm parameters were not affected by either 30 min or 1 h. Our results are partially in accordance (in terms of motility) with other studies which reported an association between semen parameters and EMF exposure including motility, viability and morphology [19,23,36], as well as elevated oxidative stress levels ([18]; S.-K. [49,50,55,67]). Although no significant morphological alterations were observed under light microscopy, evident morphological changes were detected at the ultrastructural level through electron microscopic evaluation, which will be discussed in the final section. It may be suggested that the morphologic perturbations raised by the EMF exposure are only evident ultrastructural level by electron microscopy, which cannot be detected by the magnification power of light microscopy. More EMF exposure times and levels may change this outcome and may have more evident effects on light microscopic images as well.

Another important mechanism that can be affected by EMF is apoptosis, which plays a crucial role in eliminating damaged or non-functional sperm cells [67]. The mechanism is also essential for regulating the numbers of proliferating germ cells. Conflicting results have been reported concerning the association between EMF and apoptosis.

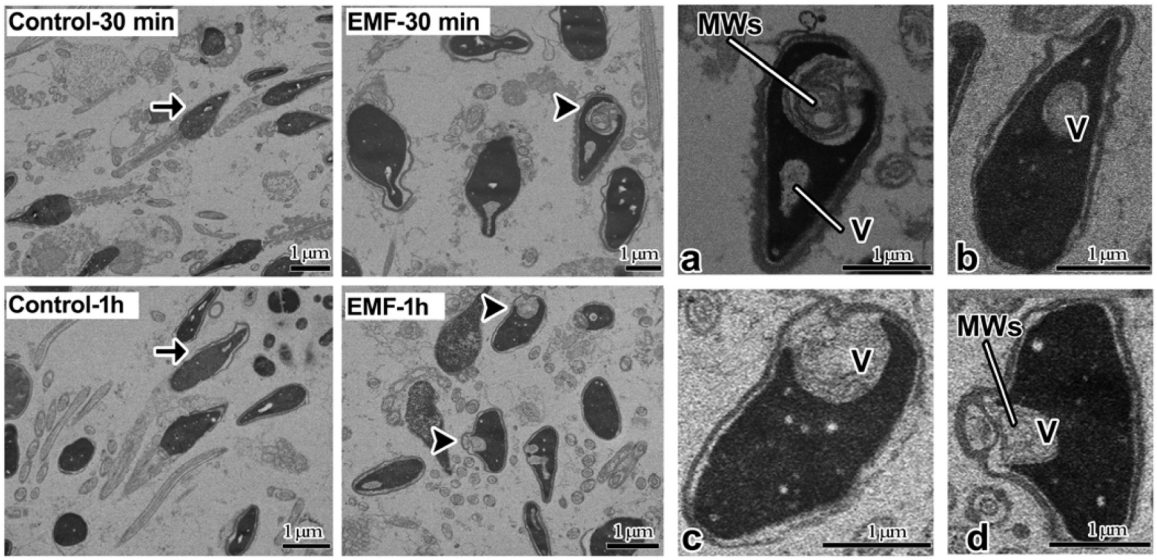

Fig. 6. Electron microscopic images of the sperm samples. Sperm without vacuoles can be seen in the control groups (arrows). In the EMF exposure groups, the arrowheads point sperms with vacuoles. Membrane-surrounded vacuoles in the sperm nuclei in the 30 min EMF exposure group (a) and vacuoles without membranes (V) (a, b) were demonstrated. Membrane surrounded vacuole in the sperm nuclei in the 1 h EMF exposure group (d) and vacuoles without membranes (V) (c) were demonstrated.

Tumkaya et al. reported that 45-day exposure to EMF radiation did not induce apoptosis in the rat testis [71]. Similarly, Lee et al. [48] reported no significant effect on rat testicular levels of Bcl-2, p21, p53 and caspase-3 proteins exposed to EMF for 12 weeks (H.-J. [48]). In contrast, however, Kesari and Behari [41] reported that exposure to 900 MHz radiofrequency radiation caused apoptosis as measured by an increase in caspase-3 activity in sperm cells as well as sperm maturation [41]. Yilmaz et al. [75] demonstrated that mobile phones did not alter anti-apoptotic protein levels examined by means of Bcl-2 levels in the rat testis exposed to EMF for one month [75]. Agarwal et al. reported that radiation exposure may increase apoptosis rates [4,47].

We evaluated the DNA fragmentation, cyt-c, and caspase-3 expression patterns as apoptosis markers in EMF-exposed sperm. Caspase-3 is one of the proteins of the caspase cascade, activated during apoptosis to damage the cell and organelles by cutting targets including proteins, nucleic acid and lipids in the cell. It is used to detect the last phase of the apoptotic process. Caspase-3 is detected in the perinuclear field as well as in the cytoplasm of sperm cells. Another protein that plays a central role in triggering the intrinsic pathway of apoptosis is the cyt-c located in mitochondria. We found no difference in the expression levels of these proteins regardless of the exposure times. Different markers capable of indicating the early apoptotic process should be tested in order to visualize any possible effect. The reason that we couldn't observe any apoptotic marker may be because the apoptotic process mainly occurs during spermatogenesis and spermiogenesis when the sperm are more susceptible to environmental impacts [47], which may not be observed evidently in ejaculated sperm due to the in vitro nature of the study.

Mature sperm cells are known to be more resistant since mitotic activity and differentiation are completed, and the cell is transcriptionally in an inactive state [15]. DNA fragmentation rates remained altered in one recent study. Several studies have suggested that one of the environmental factors that may be involved in the aetiology of DNA damage in human sperm is increased exposure to electromagnetic radiation emitted from mobile phones [76]. Research has also suggested that the energy transmitted by EMF is not sufficiently high to damage the DNA directly [1]. However, other studies have reported alterations in DNA integrity after exposure to 50 Hz extremely low frequency (ELF) magnetic fields (MFs) [31,52,65], and have proposed several hypotheses concerning how ELF might affect the structure of DNA [30,33,56,64] including protein synthesis, which dramatically affects cell functions [54]. We found no significant difference in all the apoptotic markers observed confirming the results of Adair et al. The different results obtained is probably due to the different patient populations and techniques used. In our study we used normozoospermic patients, in order to minimise the effects of confounders which may affect the outcome.

Among the pathways accompanying apoptosis in sperm cells, PI3K/AKT signaling pathway is one of which has a major role, basicly in preventing the initiation of apoptotic cascades [45]. The pathway is reported to effect motility, survival and hyper-activation ([45]; Parte et al. Sagare-Patil et al. Gallardo Bolaños et al.). Pharmacological inhibition of PI3K is reported to attenuate sperm motility, hyperactivation, inhibit the acrosome reaction (Aparicio et al., 2005; Breitbart et al., 2010) and prevent spontaneous acrosome reaction (Tsirulnikov et al., 2019). Although we found no significant difference in PI3K expression in this study, p-Akt expression -the activated form of Akt- increased significantly after 1 h of EMF exposure which has an overall increase rate of 23.58 %. The results confirm the findings that Akt activation (increase in Akt expression) suppresses capacitation and apoptotic process in sperm (Pujianto et al.). Based on the data obtained, it may be concluded that EMF-induced stress increase Akt phosphorylation, (p-Akt expression) in sperm cells which further affects motility and inhibit apoptosis.

Ultrastructural analysis revealed the presence of vacuoles most commonly at the base of the sperm head in both EMF exposure groups. This finding was suggested to be a marker of DNA damage [8]. We used a detailed quantitative scoring for the evaluations of sperm morphology [10] in which a vacuolisation rate of > 25 % of head area is defined as abnormal while the remaining are classified as normal. In the recent study, we observed an abnormal vacuolisation rate (> 25 % of head) in both EMF groups which was not observed in the control group. Additionally, vacuolisation types were different in EMF groups, some of which had membrane whorls (MWs) and some were covered with a membrane with no connection to the sub acrosomal space. In addition, in EMF groups, loss of the acrosomes, swollen and waving in acrosome membranes, extension of subacrosomal space, uncondensed chromatin structure, apoptotic cells and disrupted axoneme were observed which are all signs of abnormal structure. These abnormalities were all related with several functional parameters of the sperm as reported by previous studies which indicated spermatozoa with large vacuoles are related with decreased blastocysts formation rates [11,72], swollen acrosomal

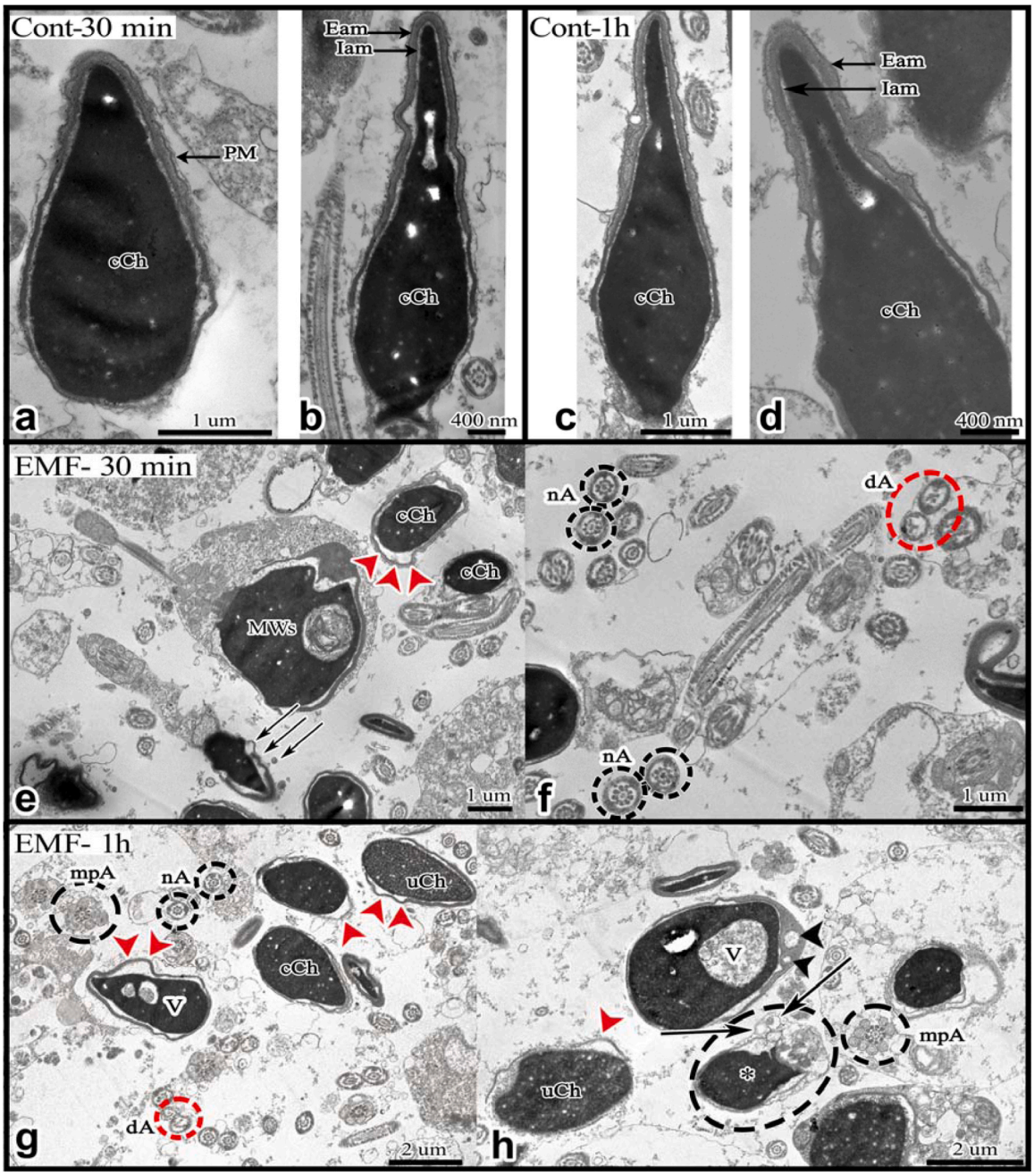

Fig. 7. Transmission electron micrographs of human sperm. (a,b) Condensed chromatin (cCh), plasma membrane (PM), external acrosome membrane (Eam), internal acrosome membrane (Iam) can be seen clearly in control groups. (e, g, h) Condensed chromatin (cCh) and uncondensed chromatin (uCh) sperms can be seen in the EMF 30 min and 1 h groups. (e, g, h) In the EMF exposure groups, the red arrowheads point to great extension of the subacrosomal space. (e, g) Membrane-surrounded vacuoles (MWs) in the sperm nuclei in the 30 min EMF exposure group and vacuoles without membranes (V) in 1 h exposure group can be seen. (h) Acrosome vacuoles shown with black arrowhead in 1 h EMF exposure group. Note that apoptotic sperm (*) with blebbing membrane (arrow). (f, g) Normal axoneme (nA) and disrupted axoneme (dA) can be seen in the EMF exposure groups. (e) Note that in 30 min EMF exposure group, plasma membrane blebbing shown with black arrow can be examined. (mid piece axonem: mpA).

membrane was related with acrosomal exocytosis in which is a sign of early acrosomal reaction [59,62,9], and plasma membrane blebbing, impaired mitochondrial integrity, defects of nuclear envelope, and nuclear fragmentation were associated with apoptosis of sperm [34].

Our study provides valuable data concerning the direct cellular effects of EMF on several functional parameters of sperm. Although the cells' *in vivo* conditions and thus their natural environment, are shielded and protected by ensheating tissues and body parts, several other concerns arise regarding the potential impacts of EMF on the highly susceptible period of spermatogenesis since there is a high possibility of errors being incorporated where cells are proliferating. Additionally, the recent study involved a relatively small sample, which reduces the statistical power in terms of determining a true effect for several of the parameters studied.

The basic limitation of our study was its relatively small sample size. The main reason is the exclusion criteria and all the patients do not have enough semen volume to use it for the ICSI procedure as well as our study. Another limitation was to use 900 MHz only, which is because our study basicly aimed to test the effects of mobile phone radiation which may be more effective at higher frequencies. For these reasons, new

studies are being planned with different dosages and durations in order to add to the present knowledge about the topic.

According to the results of this study, it may be concluded that sperm motility is affected adversely from EMF exposure although apoptotic process is not affected. It may be suggested that the exposure time and/or frequency at this level (900 MHz, up to 1 h) may not be sufficient enough to trigger the apoptotic process. Activated Akt (p- Akt) levels increased significantly indicating a possible role of the PI3K/AKT signaling pathway after EMF exposure. This study investigated *in vitro* effects of EMF exposure on sperm cells to observe the direct effects of it on sperm cells ignoring the protective effect of testicular tissues and environment. Hovewer, results of both *in vivo* and *in vitro* studies should be considered together to obtain reliable data about the effects of EMF on sperm cells. Additionally, further studies are needed in which different frequencies, exposure times, and durations were conducted in order to evaluate any possible cut-off levels and also to observe to what extent does EMF exposure effects the cells. Due to the continuing unexplained growth in fertility problems, and the exponential growth in EMF exposure and other potential sperm-damaging environmental factors, the effort to evaluate the impact of EMF on sperm merits the most serious attention.

Statement of ethics

The research commenced following receipt of approval from the Istanbul Medipol University Ethical Committee (date: 12/06/2018, numbers 10840098–604.01.01-E.52380 and 10840098–604.01.01-E.52378) and of informed consent from all patients.

Author contribution

IK, S Karabulut, AAK, S Kaplan designed the study. S Karabulut, AAK, KKT, M Alagoz, M Akdeniz collect the data, performed the experiment, and analyzed the data under DLD, S Kaplan, S Karabulut and IK's supervision. All authors contributed to the interpretation of the findings from this study, were involved in drafting or critically reviewing the article for its intellectual content and gave their final approval for publication of this version of the article.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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