

Cell phone radiofrequency electromagnetic radiation exposure alters mouse sperm quality and impairs preimplantation development of embryos derived from exposed sperm

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Abstract

Cell phone radiofrequency electromagnetic radiation (RF-EMR) may adversely affect male reproductive function, but its effects on sperm and embryos derived from exposed sperm remain incompletely understood. This study investigated the effects of 30-min in vitro exposure to RF-EMR (900–1800 MHz, call mode; SAR ~ 0.6–1.8 W/kg; 10 cm distance) on mouse sperm quality and subsequent preimplantation embryo development. Sperm count, motility, viability, morphology, DNA fragmentation, acrosome reaction (AR), malondialdehyde (MDA) levels, and mitochondrial gene expression (*mt-Nd1*, *mt-Nd2*, and *mt-Atp6*) were assessed. Embryos generated by in vitro fertilization (IVF) using exposed sperm were monitored by time-lapse imaging. RF-EMR exposure significantly reduced progressive motility, viability, and normal morphology ($P < 0.001$), while sperm count and AR were unaffected. DNA fragmentation significantly increased following exposure ($P < 0.0001$), accompanied by elevated MDA levels ($P = 0.0491$) and downregulation of all assessed mitochondrial genes ($P < 0.05$). Embryos derived from exposed sperm exhibited delayed cleavage and compaction, reduced embryo quality and survival, and increased fragmentation and developmental arrest compared with controls ($P < 0.05$). These findings suggest that short-duration exposure of mouse sperm to cell phone RF-EMR can impair sperm functional and molecular characteristics and is associated with adverse preimplantation development of embryos derived from exposed sperm.

Introduction

The widespread use of cell phones has become an integral part of daily life, such as making calls, checking social media and using artificial intelligence (Adamczewska-Chmiel et al., 2022). It is estimated that over 80% of the world population owns a smartphone (Union, 2025). Cell phones are a source of radiofrequency electromagnetic radiation (RF-EMR). The frequency range of these non-ionizing waves is in the range of 900 to 1800 MHz and the effect of these waves on various organs, especially sex organs, has been raised as a concern (Maluin et al., 2021). In recent years, studies have shown that exposure to mobile waves may damage the sperm biological characteristics. The extent of the effect of these waves can depend on the specific absorption rate, distance from the wave source and the duration of exposure. EMR has thermal and non-thermal effects on living cells. Thermal EMR can damage cells by increasing temperature, whilst non-thermal EMR can affect living cells through various methods, including altering oxidative stress levels, cell membrane potential and signaling pathways (Kowalczyk et al., 1983). Oxidative stress is one of the main sources of damage to sperm cells. During the process of spermiogenesis, sperm cells lose a large amount of their cytoplasm, which weakens the antioxidant system. Also, sperm cells become more susceptible to damage due to the presence of targets of oxidative damage, including unsaturated fatty acids in the sperm membrane, DNA structure, and the presence of reactive oxygen species (ROS) generators, such as mitochondria (Balci et al., 2007; Koppers et al., 2008). Studies have shown that exposure of sperm to cell phone electromagnetic waves reduced sperm motility, and morphology, damaged genetic material and increased the free radical levels (Agarwal et al., 2008; Gorpinchenko et al., 2014). *In vitro* animal studies have more consistently shown reduced sperm motility and viability after RF-EMR exposure, whereas pooled results from human cross-sectional

studies have been less conclusive, likely reflecting differences in exposure intensity, duration and measurement methods across study designs (Yu et al., 2021). Spermatozoa transmit not only the paternal genome but also epigenetic information that can influence subsequent embryonic development (Yan et al., 2022). Time-lapse imaging studies in mouse and human preimplantation embryos have shown that a high degree of paternal sperm DNA fragmentation can delay both pronuclear formation and early cleavage divisions. Embryo morphokinetic parameters can also be affected, since the oocyte's ability to repair paternal DNA damage is limited (Gawecka et al., 2013). Consistent with this, animal studies have reported that paternal exposure to RF-EMR can alter offspring body-weight trajectory and glucose metabolism even in the absence of overt changes in conventional sperm parameters, pointing to more subtle, transgenerational effects of paternal RF-EMR exposure (16). However, as far as we are aware, no studies have examined the effects of cell phone RF-EMR on the morphokinetics of preimplantation embryos resulting from sperm cells exposed to the waves. During the preparation of sperm for *in vitro* fertilization (IVF) treatment, these cells are exposed to mobile electromagnetic waves. The aim of this study was to assess the effect of 900 to 1800 MHz waves emitted from mobile phones on sperm parameters, DNA integrity, free radical levels and their ability to acrosome react (AR). In addition, the morphokinetics of preimplantation embryos resulting from IVF using sperm exposed to the waves was investigated.

Materials and methods

Ethical approval

This study received code of ethics from the Animal Ethics Committee of Yazd University of Medical Sciences (IR.SSU.AEC.1403.013). All protocols were conducted in accordance with the Guidelines for the Use of Laboratory Animals of international guidelines.

Study design

10–12 week-old male ($30\text{g}\pm 3.2$) and 6–8 week-old female ($25\text{g}\pm 2$) mice were purchased from the animal house of Reproductive Sciences. Mice were maintained under standard conditions, i.e., temperature $\pm 22^{\circ}\text{C}$, humidity $\pm 55\%$, 12-h light/dark cycle, 100 lux light intensity during daylight hours, and free access to food and water. This study had two phases. In the first phase, sperm cells were obtained from 25 mice and then divided to two groups: either exposed to RF-EMR and not. In the next phase, cumulus oocyte complexes (COCs) were obtained from 20 mice and randomly divided into two groups. Half of COCs were fertilized vis IVF with sperm from the exposure group and the other half were fertilized via IVF with sperm from the control. The zygotes were then transferred to a time-lapse microscope (TLM; Thermo Fisher Scientific, USA) for 5 days. The morphokinetics of the resulting preimplantation embryos was then assessed (Fig. 1).

Sperm and oocyte collection

Male mice were anesthetized with ketamine and xylazine (9:1) and then euthanized. The epididymis and the beginning of the vas deferens were dissected under sterile surgery and placed in a dish containing 1 mL of pre-warmed Ham's F10 medium with 10% BSA albumin (37°C, 5% CO₂). The tissue was cut carefully and placed in the incubator for 30 min to allow the spermatozoa to swim into the culture medium (Rostamzadeh et al., 2016). The study included two groups: the control group and the exposure group. Sperm from the control group were incubated for 30min without exposure, then used for analysis and IVF. Sperm from the exposure group were exposed to RF for 30 min at a distance of 10 cm and then used for analysis and IVF. For COC retrieval, 10 units of Pregnant Mare Serum Gonadotropin (PMSG (Gonaser, Hipra, Spain), were injected intraperitoneally into the mice, and 10 units of Human Chorionic Gonadotropin (hCG) (Hemapharmed, Iran) were injected 48 h later (Lin et al., 2020). 12 to 14 h after hCG injection, female mice were anesthetized with ketamine / xylazine (9:1), prior to spinal transection. Dissected oviducts were transferred to pre-warmed HTF medium and COCs were removed from the tubes using insulin needles prior to transferring into the culture medium via sterile micropipettes.

IVF procedure

The COCs samples were transferred into 100 µl droplets of Universal IVF Medium (Origio, USA), covered with oil and pre-warmed for 24 h at 37°C and 5% CO₂. Approximately 10 µl of sperm suspension was added to each droplet and after 6 h, fertilization was assessed. Zygotes were transferred to clean droplets of one-step culture medium (Origio, USA) prior to transfer to a 9-well time-lapse dish (Primovision, Vitrolife, Sweden). Embryo morphokinetics were followed for 4 days until they reached the blastocyst stage, with the time-lapse device recording images every 5 min.

RF-EMR exposure condition

The RF-EMR source was a commercial android mobile phone (LG GT-S6310) operating at a carrier frequency range of 900–1800 MHz with a reported specific absorption rate of 0.6–1.8 W/kg. The device was placed at a fixed distance of 10 cm from the sperm dish placed on the incubator tray for 30 min. All exposures were conducted under standard incubation conditions (37 °C, 5%CO₂). The experimental setup was configured to ensure uniform electromagnetic field distribution across all samples; while, effectively mitigating potential confounders, including temperature variations (Seify et al., 2024).

Sperm parameters assessment

For sperm motility assessment, 200 spermatozoa were examined at × 200 magnification via contrast phase microscope (Nikon. Japan). Motility was reported as a percentage of non-motile, non-progressive, and progressive motility. Sperm viability was assessed via eosin-nigrosine staining and sperm morphology was assessed using Diff-Quick staining (IVF co, Iran) of air-dried smears (1000× magnification, 200 sperm cells, with percentage of normal spermatozoa reported (Wyrobek and Bruce, 1975)).

Sperm DNA fragmentation

Sperm DNA fragmentation was assessed using the Halo sperm kit (SDFA kit, IVF, Iran) where the size of the halo formation around the sperm head correlated negatively with the DNA fragmentation index (DFI). Sperm with a medium or large halos were classed as having non-fragmented DNA, whilst sperm without any halo were classed as having fragmented DNA (Beigi et al., 2022). The percentage of sperm with fragmented DNA (no and small halo) was calculated.

Acrosome reaction assessment

For AR evaluation, slides stained with PSA-FITC solution (Sigma Aldrich, USA) were examined using a fluorescence microscope at 450-495 nm and 400 × magnification. Non-reacted spermatozoa that had not undergone the AR was bright across the entire head. Acrosome-reacted spermatozoa, however, showed a fluorescent band in the middle and the rest of the parts did not have fluorescent color (Beigi et al., 2022).

MDA levels assessment

The MDA level was used to assess oxidative stress, as MDA is a well-established indicator of lipid peroxidation and an indirect marker of excessive reactive oxygen species (ROS) production. The MDA concentration in the samples was measured using a ZellBio kit (GmbH, Germany) according to the manufacturer's instructions. Briefly, 100 µl of the sample was transferred into a microtube and 100 µl of the standard solution was added. Then, 100 µl of reagent (R4) and 200 µl of chromogen were added to the microtube. The microtube was placed in a hot water bath for 1 h, prior to ice exposure for 10 min. The sample was centrifuged at 4°C for 15 min. Finally, 200 µL of the supernatant was transferred into the wells of a microplate, and absorbance was measured at 530–540 nm using a microplate reader (Ren et al., 2015).

Gene expression profile

Expression of the mitochondrial genes (*mt-Nd1*, *mt-Nd2*, and *mt-Atp6*) was evaluated in 25 pooled sperm samples per group to minimize inter-individual variability. Total RNA was extracted using TRIzol (Yekta Tajhiz, Iran) and quantified using a NanoDrop spectrophotometer (DeNovix, USA). RNA purity was assessed based on A260/A280 ratios, with values of 1.8–2.0 considered acceptable. First-strand cDNA was synthesized using a commercial reverse transcription kit (Yekta Tajhiz Azma, Iran). Quantitative real-time PCR (qRT-PCR) was performed using a real-time PCR system (QIAGEN, Germany) with SYBR Green Master Mix (Yekta Tajhiz, Iran). Primer sequences are provided in Table 1. Each reaction was performed in a final volume of 20 µL and contained 10 µL of 2× SYBR Green Master Mix. Each sample was analyzed in technical triplicates. Thermal cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 20 s, and 72°C for 20 s. Product specificity was confirmed by melting curve analysis, and no-template controls (NTCs) were included in all runs. Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method and normalized to the *B2m* reference gene.

Table 1 Primer sequences and Amplicon size used for quantitative real-time PCR analysis of mitochondrial genes.

Gene	Primers sequence (5'-3')	Sequence amplified	Product length(bp)
<i>mt-Nd1</i>	F-CCGCAAGGACAAAGACAAAAT	NC_005089.1	121
	R-TGTTCTCTTCCGCAGTATTGT		
<i>mt-Nd2</i>	F-AACATACCCCAACAACAACC	NC_005089.1	196
	R-GGCAGAGATGTAGTTGAGGGT		
<i>mt-Atp6</i>	F-AACCAATGGCATTAGCAGTCCG	NC_005089.1	195
	R-AAATGTAATGGTAGCTGTTGGTGGG		
<i>B2m</i>	F-CCTTCAGCAAGGACTGGTCT	NM_009735.3	139
	R-GATCACATGTCTCGATCCCAG		

Abbreviations: F: Forward, R: Reverse, *mt-Nd1*: Mitochondrially encoded NADH dehydrogenase 1, *mt-Nd2*: Mitochondrially encoded NADH dehydrogenase 2, *mt-Atp6*: Mitochondrially encoded ATP synthase membrane subunit 6, *B2m*: Beta-2-Microglobulin

Time-lapse embryo monitoring

For morphokinetics, 102 embryos were examined using viewer software (Vitrolife, USA) with the capability of taking images at 7 sections every 5 min. Times are taken after HCG injection. Embryo morphokinetics were examined using the following variables: PN fading time (tPNf) ,1-cell to 8-cell division time (t1-t8), time of starting compaction (tSc), time of finishing compaction (tmf), time of blastocoel cavity formation (tSB), time of blastocyst formation (tByz), time of full blastocyst formation (tEyz), time of first sign of hatching (tHNyz), duration of the second cell cycle (CC2 = t4-t2), duration of the third cell cycle (CC3 = t8-t4), synchronization of cell divisions of second cell cycle (S2 = t4-t3) and synchronization of cell divisions of third cell cycle (S3 = t8-t5), duration of transition from blastocoel cavity formation to blastocyst formation (dB= tEyz-tByz) and duration of transition from full blastocyst formation to first sign of hatching (dexp= tHNyz-tEyz)khali. In addition, using these images, cleavage defects in the embryos were examined: 1) Direct Cleavage (DC): division of zygote in <5 h into 3 blastomeres; 2) Reverse Cleavage (RC): fusion of two daughter blastomeres after complete separation or unsuccessful cytokinesis; 3) uneven blastomeres: the presence of blastomeres with a size difference of more than 33% in 2-cell stage; and 4) multinucleation: blastomere have a more than one nucleus (Anbari et al., 2023).

Embryos were divided into 4 groups (A, B, C and D) based on morphology: Grade A with even blastomeres and no fragmentation; Grade B with few even blastomeres and up to 10% fragmentation; Grade C with uneven blastomeres and 10–50% fragmentation rate; and Grade D with uneven blastomeres and >50% fragmentation. Finally, embryos that stopped growing were classed as arrested

embryos (Anbari et al., 2023). Grades A and B as good and C and D considered as low quality embryos, respectively.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics, version 20 (IBM Corp, NY, USA). The normality of data distribution was assessed using the Kolmogorov–Smirnov test. For quantitative variables, the independent samples t-test was used for normally distributed data, while the Mann–Whitney U test was applied for non-normally distributed data, to compare differences between groups. For qualitative variables, the Chi-square or Fisher's exact test was used, as appropriate. Normally distributed continuous variables are presented as mean ± standard deviation (SD), whereas non-normally distributed variables are presented as median (interquartile range, IQR). A two-sided P-value <0.05 was considered significant.

Results

RF-EMR exposure on Sperm Parameters

In comparison between control and exposure groups, no significant difference was seen in sperm concentration (52.32 ± 7.95 million/mL vs. 52.32 ± 7.56 million/mL; Table 2). Sperm motility was significantly lower in exposure group compared to control. Data showed that in exposure group, sperm morphology and viability were significantly lower than control group.

Table 2 The effect of RF-EMR on sperm parameters (n=25)

Sperm Parameters (%)	Control group (mean $\pm$ SD)	Experimental group (mean $\pm$ SD)	p-value
Count	52.32 $\pm$ 7.95	52.32 $\pm$ 7.56	1.000
Progressive motility	58.20 $\pm$ 5.09	53.80 $\pm$ 5.75	<0.001
Non-progressive motility	17.24 $\pm$ 7.07	19.00 $\pm$ 4.39	<0.001
Non-motile	24.56 $\pm$ 3.94	27.20 $\pm$ 4.50	<0.001
Viability	79.24 $\pm$ 3.77	76.36 $\pm$ 4.38	<0.001
Normal Morphology	76.44 $\pm$ 3.48	73.48 $\pm$ 3.72	<0.001

Statistical comparisons were performed using an independent-samples t-test and the Mann-Whitney U test for normal and non-normal variables with mean $\pm$ SD and median $\pm$ IQR, respectively. *P < 0.05 was regarded as statistically significant

RF-EMR exposure on Sperm chromatin and acrosome reaction

After exposing the sperm sample to RF-EMR, DNA fragmentation increased significantly compared to control group (16.28 $\pm$ 3.84% vs. 19.12 $\pm$ 4.86%). No significant difference was observed after exposure (21.12 $\pm$ 4.09% vs. 21.28 $\pm$ 4.35%; Fig. 2).

RF-EMR exposure on MDA levels

Exposure significantly increased MDA levels in the exposed group compared to the control (P =0.0491). This elevation indicates enhanced lipid peroxidation under the applied exposure condition (Fig. 3).

RF-EMR exposure on gene expression profile

RF-EMR exposure significantly decreased the transcript levels of all assessed mitochondrial genes compared to the control. Each gene showed a significant reduction ($P < 0.05$), with consistent direction of change across all targets (Fig. 4).

Table 3 The effect of RF-EMR on embryo morphokinetic individual variables (n=102)

Individual variables	Control group	Exposure group	P-Value
tPNf	28.68±1.18	29.19±1.33	0.24
t2	30.68±1.21	31.78±1.74	0.002*
t3	51.33[1.37]	52.33[1.95]	<.001*
t4	52.03 [1.34]	53.58[1.53]	<.001*
t5	64.13[1.07]	64.48[1.91]	<.001*
t6	64.38[1.75]	65.23[1.95]	<.001*
t7	65.07±0.99	66.17±1.63	0.003*
t8	65.55±1.03	66.61±1.80	0.009*
tSc	70.43[3.63]	72.18[3.10]	<.001*
tMf	78.21±3.18	80.33±5.89	0.006*
tSB	91.87±4.01	92.12±4.67	0.085
tByz	99.62±3.74	99.49±4.82	0.380
tEyz	106.49±4.15	107.38±5.78	0.402
tHNyz	112.78±5.30	114.80±6.34	0.115

The result of disappearance of PN (tPNf), 1-cell to 8-cell division time (t1-t8), time of starting compaction (tSc), time of finishing compaction (tmf), time of blastocoel cavity formation (tSB), time of blastocyst formation (tByz), time of full blastocyst formation(tEyz), time of first sign of hatching(tHNyz). The data were expressed with an independent *t*-test and Mann-Whitney *U* test for normal and abnormal variables with mean ± SD and median ± IQR, respectively

Table 4 The effect of RF-EMR on embryo calculated morphokinetic variables (n= 102)

Individual variables	Control group	Exposure group	P-Value
CC2a	20.62±0.92	20.67±1.24	0.44
CC2b	21.11±1.06	21.35±1.74	0.43
S2	0.30[0.72]	0.70[0.75]	0.034*
CC3a	12.13. ±0.87	11.78±1.02	0.054
CC3b	12.26±0.89	12.23±1.16	0.23
CC3c	13.26±0.87	13.03±1.29	0.28
CC3d	13.74±0.87	13.47±1.38	0.71
S3	1.50 [1.15]	1.25[1.19]	0.480
dB	7.74±3.06	7.37±2.58	0.098
Dexp	6.28±3.56	7.42±3.97	0.264

A significant difference ($P < 0.05$) was observed specifically in s2 time interval. duration of the second cell cycle (CC2 = t_4-t_2), duration of the third cell cycle (CC3 = t_8-t_4), synchronization of cell divisions of second cell cycle (S2 = t_4-t_3) and synchronization of cell divisions of third cell cycle (S3 = t_8-t_5), duration of transition from blastocoel cavity to blastocyst formation (dB= $t_{Eyz}-t_{Byz}$) and duration of transition from full blastocyst formation to first sign of hatching (dexp= $t_{HNyz}-t_{Eyz}$). The data were analyzed with an independent *t*-test and Mann-Whitney *U* test for normal and non-normal variables with mean $\pm$ SD and median $\pm$ IQR, respectively

Table 5 The effect of RF-EMR on embryo cleavage abnormalities (n=102)

Cleavage abnormalities	Control (%)	Exposure (%)	OR (95%CI)	P-value
Fragmentation	6.9 (7)	19(18.6)	3.732(1.402-9.934)	0.006
Reverse cleavage	2(2)	4(3.9)	2.085(0.365-11.925)	0.40
Direct cleavage	3(2.9)	7(6.9)	2.545(0.620-10.428)	0.18
Uneven blastomere	3(2.9)	8(7.8)	2.977(0.742-11.942)	0.11
Multinucleation	0	0	-	-
Arrest	5(4.9)	13(12.7)	3.14(1.030-9.620)	0.038

Data were analyzed using Pearson's chi-square test for normally distributed variables and Fisher's exact test for non-normally distributed variables, respectively OR, odds ratio; CI, confidence interval

RF-EMR exposure on embryo development

The average duration of cell divisions from the 1-cell to the 8-cell stage was significantly delayed in exposure compared to the control group ($P \leq 0.05$). Furthermore, the time to start compaction and the time for the embryo to reach full compaction were significantly longer in exposure compared to the control group ($P \leq 0.05$). In addition, duration of transition from 2 cell to 4 cell was significantly longer in exposure compared to control group ($P=0.034$). In the other morphokinetic variables and time interval, there was no significant difference between two groups (tables 3 and 4). Good quality embryos (A and B) and low quality embryos (C and D) were significantly reduced and increased in embryos formed with sperm exposed to RF-EMR compared to control group, respectively ($P = 0.04$).

Also, the percentage of fragmented and arrested embryos in exposure group were significantly higher than control group. Changes in other qualitative variables, such as multinucleation, DC and RC were similar between the two groups (Table 5).

Discussion

This study evaluated the effect of a short exposure to RF-EMR from a mobile phone on a wide range of sperm functional parameters, simultaneously assessing both cellular and molecular endpoints, as well as the morphokinetics of embryos derived from sperm exposed to waves. Exposure to RF-EMR significantly increased MDA in cells and reduced sperm motility, morphology, viability and mitochondrial gene expression. Furthermore, RF-EMR exposure resulted in a significant increased DNA fragmentation.

Recently, a meta-analysis by Jamaludin et al investigated the time-dependent impacts of Wi-Fi radiation on male rats and clearly showed a significant increase in MDA level (Jamaludin et al., 2025). Similarly, exposed epididymal sperm to 1.8 GHz radiation enhanced mitochondrial ROS production (Houston et al., 2018). Our findings are consistent with these studies, as they measured ROS directly or used MDA as a biomarker. RF-EMR damages the mitochondrial electron transport chain, causing electron leakage and excessive production of superoxide anions and hydrogen peroxide. The ROS damage polyunsaturated fatty acids in the sperm plasma membrane, leading to lipid peroxidation and MDA formation. Therefore, elevated MDA confirms that oxidative stress is a primary mechanistic event in our model (De Iuliis et al., 2009). Importantly, our study indicated that while significant functional defects were observed, sperm concentration remained unchanged in the exposed group. This finding is in agreement with a significant body of literature concluding that mobile phone exposure was associated with reduced motility and viability, with no apparent effect on sperm concentration (Adams et al., 2014; Agarwal et al., 2009). Likewise, Veerachari and colleagues reported that sperm counts were not significantly altered, while mobile phone emissions induced oxidative stress (Veerachari and Srini, 2012). The observed lack of change in sperm concentration despite increase in MDA, suggests that the primary effect of the 30-min RF-EMR exposure was functional and structural damage to sperm, rather than the direct physical elimination or cell lysis. In addition, Baldini et al reported that an exposure to mobile phone and Wi-Fi radiation significantly decreased progressive sperm motility in the ART laboratory (Baldini et al., 2025). A large-scale meta-analysis by Cordelli et al demonstrated that experimental RF-EMF exposure in both animal models and human sperm significantly reduced *in vitro* sperm motility and vitality (Cordelli et al.,

2024). Similarly, Hassanzadeh et al showed that exposure of normozoospermic human sperm to cell phone radiation caused lower viability and motility (Hassanzadeh-Taheri et al., 2022). The reduction in normal morphology observed in our study is also supported by Falzone and co-workers. Collectively, these studies support the concept that RF-EMR induced oxidative stress impairs the structural and functional integrity of spermatozoa, leading to diminished sperm functional role (Darvish et al., 2021). The elevated DNA fragmentation observed in our exposed group is consistent with previous reports. Butković et al observed that *in vitro* exposure of boar semen to 5G RF-EMR significantly increased spermatozoa DNA fragmentation (Butković et al., 2024). Others have also shown that DNA fragmentation was increased significantly in the exposed sperm cells (Gorpinchenko et al., 2014; Zalata et al., 2015). This may happen in a two-step pathway: RF-EMR induces mitochondrial electron transport chain dysfunction, leading to excessive ROS production. Subsequently, ROS damage the sperm DNA, causing single and double-strand breaks (Houston et al., 2016). Notably, our study demonstrated that the AR was not significantly altered after 30 min of RF-EMR exposure. Falzone et al. exposed human spermatozoa to 900 MHz radiation for 1 h and observed that the AR remained unchanged (Falzone et al., 2011). One possible explanation is that the AR is mainly mediated by voltage-gated calcium channels, which may be less dependent on mitochondrial ATP production compared to motility and viability. Additionally, the short exposure duration (30 min) is likely insufficient to deplete acrosomal enzyme stores or disrupt acrosomal membrane integrity (Nolan and Hammerstedt, 1997). Nevertheless, others reported conflicting results regarding the effects of RF-EMR on sperm quality. Cordelli et al assigned a "very low certainty" to the evidence regarding DNA/chromatin alterations and morphology following RF-EMF exposure in animal studies. Trosic et al also stated that exposure of mobile phone waves for 1 hour a day for two weeks had no effect on sperm morphology and motility in rats. These contradictions may be due to methodological differences, such as variations in exposure duration, longer distance, frequency range, SAR values, species-specific sensitivity and experimental design (Trošić et al., 2013; Weller et al., 2025). Our study was purposefully chosen with a short, high-intensity exposure model (30 min, distance 10 cm, call mode) to simulate a plausible ART condition, where a cell phone is held close to the sperm samples. Several studies showed a decrease in an expression level of mitochondrial genes after exposure with RF-EMW. Zahmatkesh et al. observed that RF-EMR exposure significantly reduced the expression of multiple spermatogenesis-related genes in rats (Zahmatkesh et al., 2024). Although, their gene targets differ from our mitochondrial genes, the pattern of downregulation agrees with ours for *mt-Nd1*, *mt-Nd2*, and *mt-Atp6* expressions. Our results are consistent with these findings, as we observed a lower relative expression of *mt-Nd1*, *mt-Nd2*, and *mt-Atp6* genes in exposed sperm compared to the control group. This decrease in expression confirms the increased production of ROS in the exposed group and decrease in motility of sperm. Reduced expression of these genes related to the electron transport chain, which results in reduced ATP production and, consequently, reduced sperm motility (Al Smadi et al., 2021). However, it is noteworthy that Wang et al. reported no significant reduction in the relative expression of the *Sod* gene in *Drosophila melanogaster* following exposure to 3.5 GHz EMR (Wang et al., 2022). This discrepancy may be explained by the lack of substantial ROS generation in their experimental conditions, which likely preserved the integrity of the antioxidant defense system. Furthermore, the non-thermal biological effects of RF-EMR are highly contingent upon a

range of interacting biological and physical parameters, including frequency, exposure duration, and organism-specific sensitivity (Fatehi et al., 2018). Our results showed that there was a significant delay in cleavage divisions from time point t2-t8 in the exposed group compared to the control. In addition, the time of compaction start and completion were longer in the exposed group. Recently, Sedo et al. reported that there is an inverse relationship between the sperm DNA damage and the rate of embryo development (Sedó et al., 2017). Also, Koohestanidehaghi et al showed that exposure of mouse embryos to RF-EMR for 30 min caused a delay in cleavage development and an increase in the time to reach the blastocyst(Koohestanidehaghi et al., 2023) . The delay in embryo development is possibly related to sperm DNA damage, caused by the ROS increase due to exposure to RF-EMR. The damaged paternal genome causes defects in the embryo genome and delayed development (Nguyen et al., 2023). On the other hand, Suzuki et al reported no significant difference in embryogenesis and blastocyst formation (Suzuki et al., 2017). This difference is not contradictory but may be a consequence of different approaches. The type of signal modulation plays a crucial role; the pulsed GSM call mode signal) 900–1800 MHz (, which was used in our study, induces more oxidative stress and has greater biological effects on cells compared to the more continuous W-CDMA (3G data mode) signal used by Suzuki et al (Höytö et al., 2008). These findings suggest that not all cell phone radiation is equal and therefore may have different effects on cells.

Also, we observed a decrease in the quality of embryos derived from exposed sperm. This finding is in line with previous reports showing that the quality of embryos derived from mice exposed to RF-EMR waves was lower than the control (Fatehi et al., 2018). Moreover, we observed fragmentation and embryo arrest was significantly higher in exposed group. Our results corroborate the earlier work of Seify et al, who concluded that fragmentation and embryo arrest was increased after 30 min exposure of zygotes. One possible explanation is that increase in ROS production induced by EMR can lead to damage to mitochondrial function (Seify et al., 2024). Reduced mitochondrial function leads to diminished ATP production, which can cause problems with embryo division. ROS can also cause DNA damage, which can lead to altered gene expression and apoptotic events (Soto-Heras and Paramio, 2020). Taken together, we observed that exposure to RF-EMR can adversely affect the morphology and embryo development. The high fragmentation rate in the exposed group delays development and decreases embryo quality. These results highlight the potential detrimental effects of RF-EMR on embryo growth. This study has several strengths. By simultaneously evaluating sperm functional parameters, oxidative stress, mitochondrial gene expression, and the subsequent morphokinetics of embryos generated by IVF, we provided a mechanistic to functional link between paternal RF-EMR exposure and embryo outcomes for first time. The use of continuous time-lapse monitoring allowed us to capture subtle, stage-specific kinetic deviations that would be missed by conventional static observation. Nonetheless, the limitations were exposure of spermatozoa to RF-EMR *in vitro*, away from the protective physiological environment of the male reproductive tract. Finally, this study used a single exposure duration in a mouse model; species-specific differences in gamete biology, that warrants caution when generalizing these findings to human fertility outcomes.

Conclusion

The exposure of sperm to the 900-1800 MHz mobile phone has detrimental consequences on both sperm and embryos derived from exposed sperm cells in animal model. sperm parameters and mitochondrial gene expression decreased after exposure. Also, DNA damage and MDA significantly increased in exposed group. Furthermore, there were significant morphokinetic variables between exposed and control group, with delays to cleavage and compaction, and higher rates of fragmentation and embryo arrest in the exposure group. In particular, the potential effects on sperm and the possible synergistic impact on embryo development are two arguments that need to be explored.

Declarations

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Authors contribution

Seyed Abbas Datli Beigi: Investigation, Writing – Original Draft, Data Curation, Writing – review & editing, Formal analyze Mohammad Ali Khalili: Supervision, Project administration, Writing – review & editing, Validation Fatemeh Anbari: Validation, Formal analysis, Methodology Brayan J. Woodward: Writing – Review & Editing, Visualization Fatemeh Zare: Methodology, Writing – review & editing Behrooz Aflatoonian: Resources, Project administration, conceptualization.

Data availability statement

Data will be made available on reasonable request.

Ethical approval

This study received approval from the Animal Ethics Committee of Yazd University of Medical Sciences (Approval No. IR.SSU.AEC.1403.013). All experimental procedures involving mice were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of international guidelines, and the study was performed in line with the ARRIVE guidelines for reporting animal research.

Consent to Participate

This is not applicable.

Consent to Publish

This is not applicable.

Competing interests

The authors declare no competing interests.

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Figures

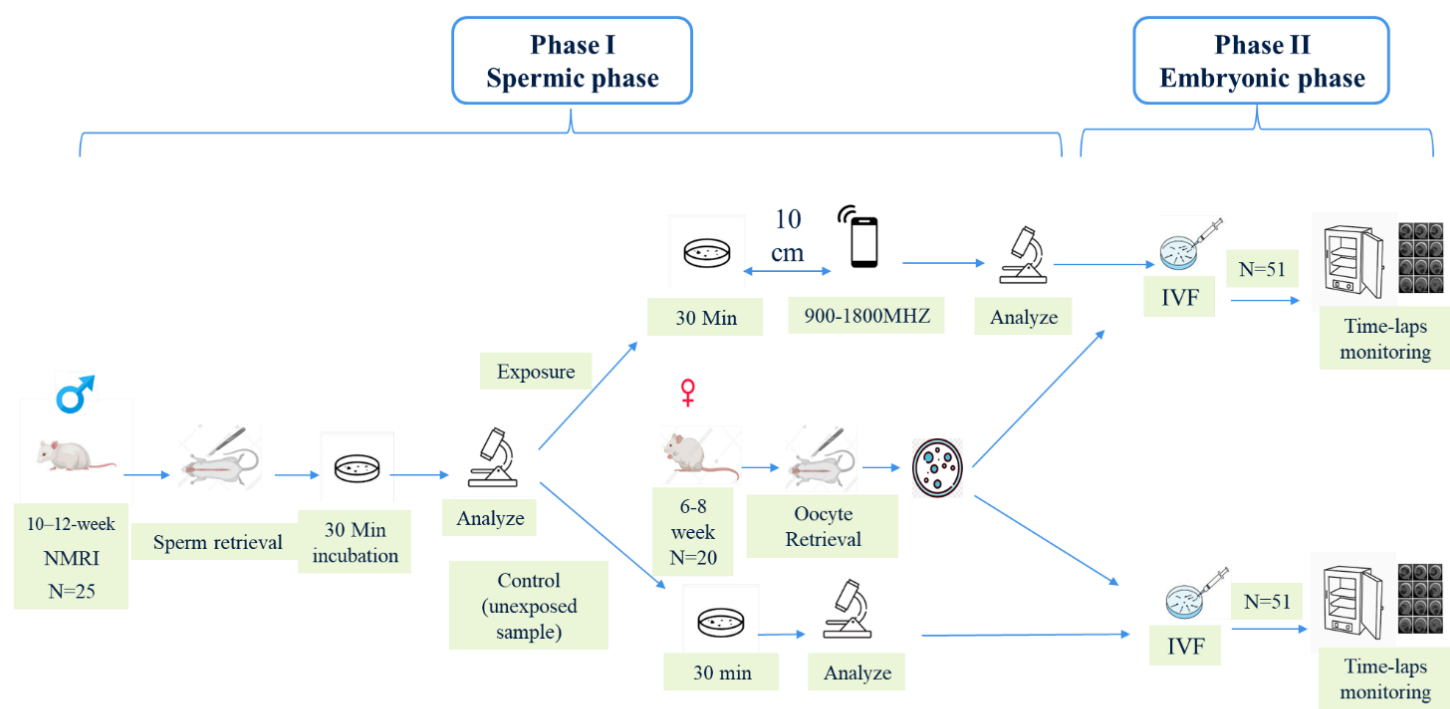


Figure 1

Experiment design chart. Sperm samples divided to two groups: exposure and control. In the exposure group, the sperm sample were exposed to mobile phone RF-EMR for 30 min. Oocytes were then exposed to the sperm samples to be fertilized by IVF. Sperm motility, viability, morphology, DNA fragmentation,

and AR were assessed. MDA levels were measured as a biomarker of lipid peroxidation. The transcript levels of mitochondrial genes (*mt-Nd1*, *mt-Nd2*, and *mt-Atp6*) were quantified by qRT-PCR. The development of preimplantation embryos was assessed using time-lapse monitoring

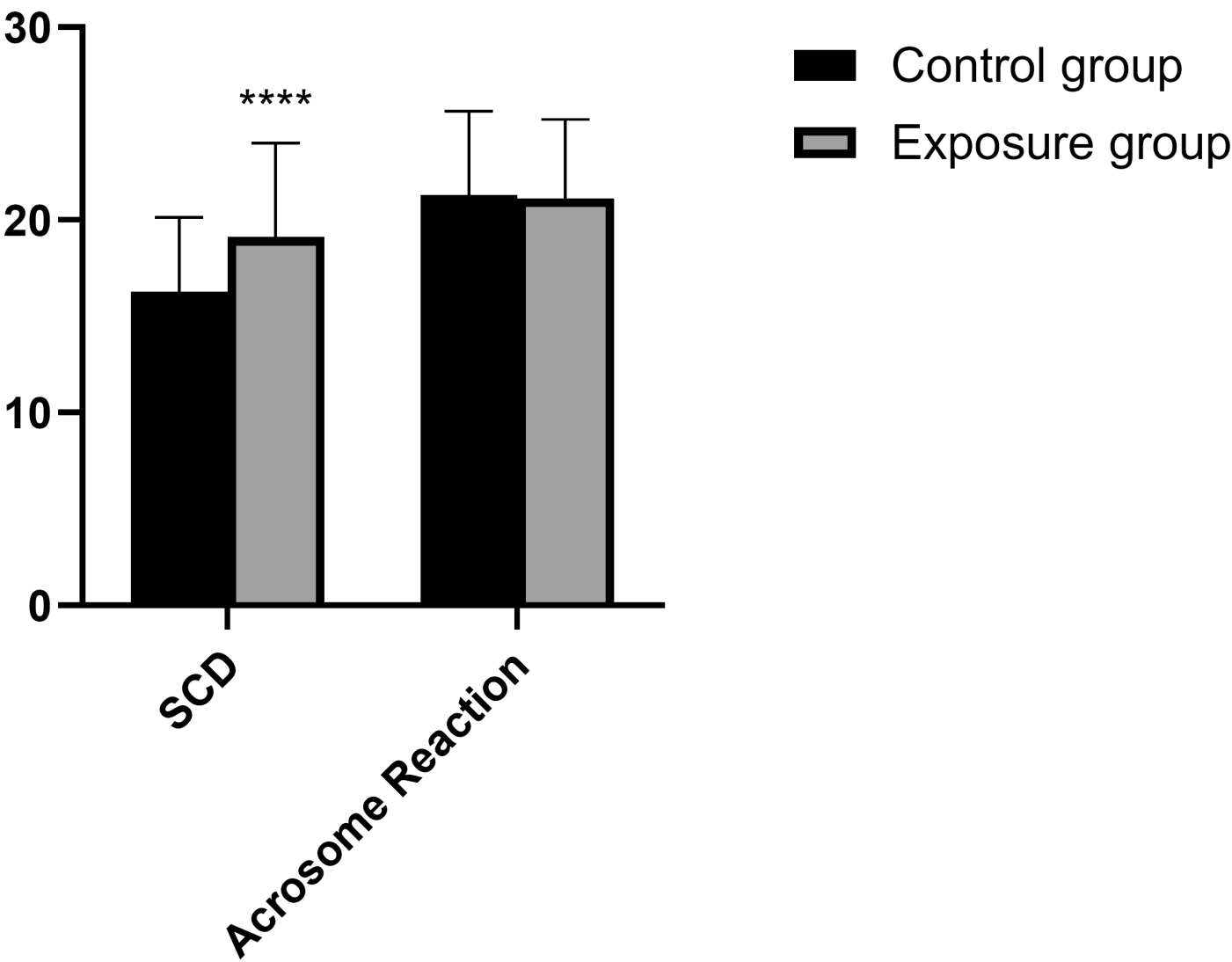


Figure 2

The effect of RF-EMR on sperm DNA integrity and acrosome reaction. There was a significant change between control and exposed group in DNA fragmentation ($P<0.0001$). However, AR, was similar in both groups ($P=0.77$)

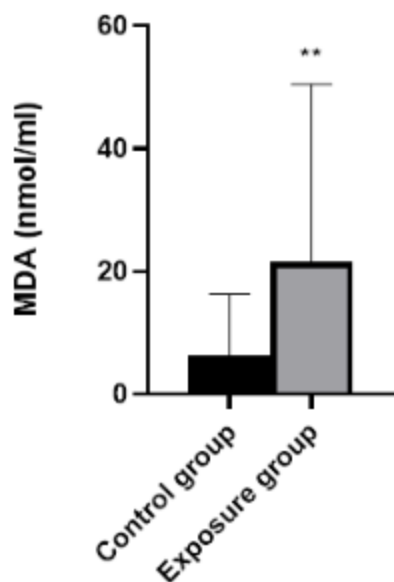


Figure 3

The effect of RF-EMR on MDA levels. Significant change was observed in exposure group ($P=0.0491$)

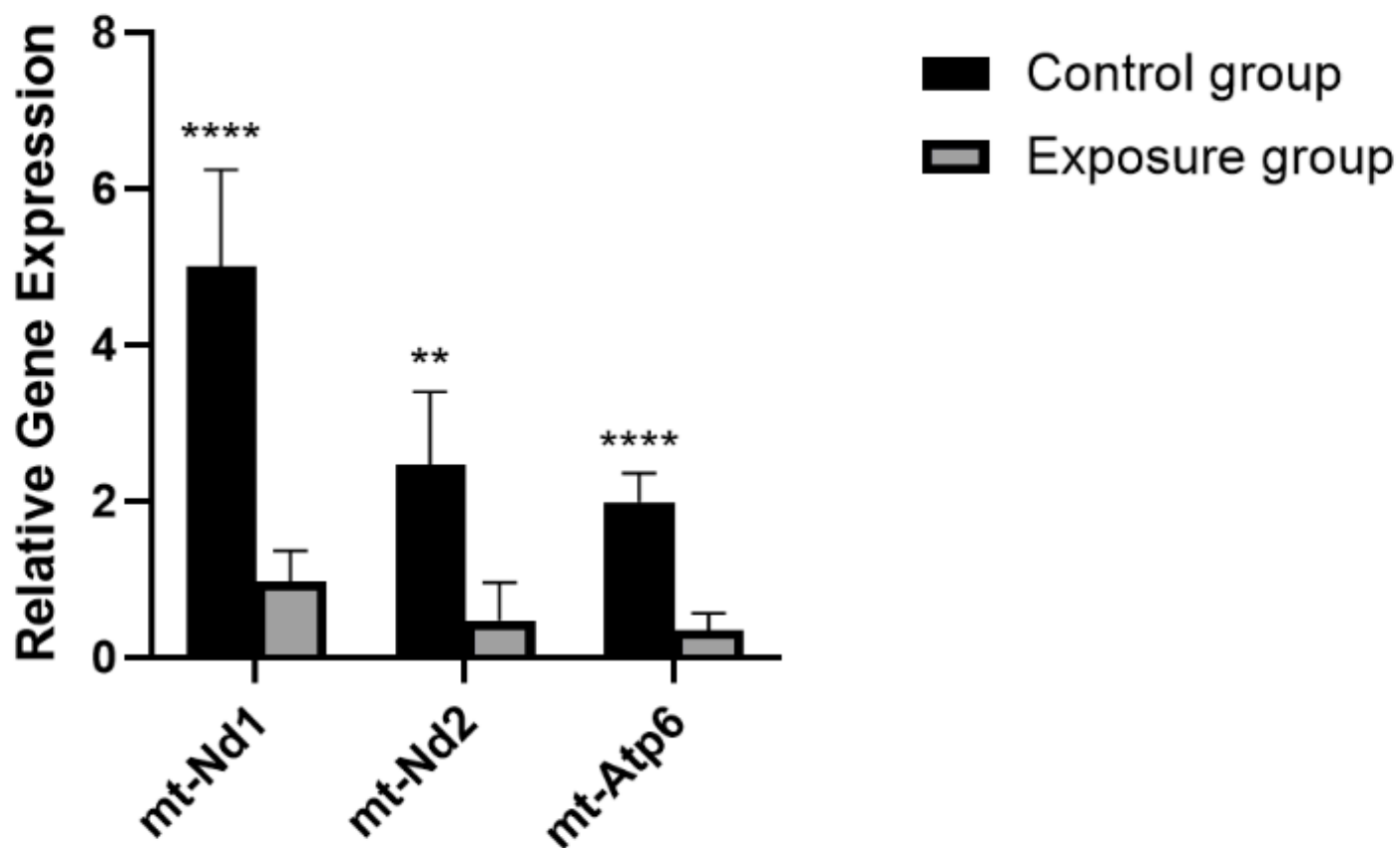


Figure 4

The effect of RF-EMR on the expression of mitochondrial target genes. The data were analyzed with an independent *t*-test and Mann-Whitney *U* test for normal and non-normal variables with mean $\pm$ SD and median $\pm$ IQR/2, respectively. $P < 0.05$ was considered significant (**** $P = 0.0001$ and ** $P=0.01$).