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Mobile Phones and Male Fertility: A Mini Review of Sperm DNA Fragmentation and Its Relationship with Radiofrequency Electromagnetic Radiation

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ABSTRACT

Introduction: Concerns over male infertility have grown, with over fifty percent of infertility cases linked to male causes. The impact of radiofrequency electromagnetic radiation (RF-EMR) of cellphones on the fragmentation of sperm has been investigated. This mini-review evaluates previous investigations on the relation between RF-EMR and sperm DNA fragmentation (SDF).
Methods: A systematic review was carried out using Google Scholar and PubMed databases up to July 2020. MeSH terms related to DNA fragmentation, sperm, mobile phones, radiofrequency, and related synonyms identified relevant studies. Nine studies were selected, and their methodologies examined.
Results: The studies reviewed presented diverse findings on the correlation between RF-EMR from cellphones and SDF. Out of nine investigations—five in vitro and four in vivo—all in vivo research works found significant DNA fragmentation in men who used their phones extensively, especially when carried in pants pockets. Three of the five in vitro tests showed a substantial effect, while the other two found no significant change between exposed and unexposed samples.
Conclusion: Although more studies reported decreased sperm quality with prolonged and intense RF-EMR exposure, the evidence regarding DNA fragmentation remains inconclusive. Given how often cellphones are used, it is critical to further investigate their potential impact on male fertility and reproductive health. Existing evidence emphasizes the necessity of more studies in this field.

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Introduction

Concern about the rise in male infertility, which explains over fifty percent of all infertility cases, has been noticed around the world, which has prompted targeted investigations to address its probable causes (1). Globally, between eight and twelve percent of couples struggle with infertility, and male factors account for 50% of these occurrences (2). Additionally, the fertility rate of men under the age of 30 has dropped by 15% globally (3). Evidence suggests that the amount and human sperm quality have decreased over the last few decades (4–7). Sperm counts among white males have significantly decreased by 50–60%, according to a recent research (8). According to World Health Organization standards, at least one semen parameter was below the suggested threshold limits in 61.1% of cases involving men who appeared healthy (9). The etiology of male infertility should be taken into account

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from all perspectives, and potential processes should be conceived and researched in order to achieve treatment for male infertility. In this context, it is crucial to comprehend the molecular and genetic mechanisms involved in sperm activity. Intact sperm DNA in association with sperm function tests has a significant influence on reproductive outcomes. The functional and physical characteristics of sperm are directly influenced by sperm DNA fragmentation (SDF), a condition that prevents sperm from engaging in reproductive activities and can be produced by a range of external and internal factors (10,11). The molecular mechanisms of DNA fragmentation are still largely unclear from a scientific perspective. However, it has been suggested that aging, abnormal lipid metabolism, genetics, physical and environmental factors, including heat and electromagnetic radiation exposure, are implicated (4,12,13). Due to the extensive availability of substrates for free radical assault, oxidative stress is recognized to be the primary cause of DNA damage along with scrotal hyperthermia. Conditions that can produce oxidative stress include infections, radiofrequency radiation, and leucocyte production of reactive oxygen species (ROS) (14,15).

Exposure to the radiofrequency electromagnetic radiation (RF-EMR) is an increasingly important area of concern in human health along with the advance of technology. Extended exposure RF-EMR, which is emitted by cellphones, is one of the environmental factors that may be linked to SDF (14,16). There has been controversy over whether mobile phones and other EMR-emitting devices have an impact on male fertility and sperm quality (17). Mobile phones operate at frequencies between 800 and 2500 MHz (rarely exceeding 3 GHz in LTE and 5G) which can be absorbed by the human body (16,18).

Several studies have reported a correlation between prolonged cellphone use and sperm quality deterioration and DNA fragmentation. This association was found by Fejes et al. (19) who investigated 371 cases and discovered a correlation between using a cellphone frequently and having less sperm motility in men. Aitken et al. (20) further supported this idea by exposing mice to RF-EMR. While sperm count, morphology, and vitality were not impaired by RF-EMR exposure in mice, significant damage to the nuclear β-globin gene and the mitochondrial genome was observed by a DNA integrity analysis (20). According to Agarwal et al. (16), exposure to RF-EMW had a significant impact on sperm motility, viability, and increased ROS levels. However, there were not noteworthy variations in DNA damage among the exposed and unexposed groups (16). Similarly, Iuliis et al. (13) reported a significant increase in DNA fragmentation and ROS formation in mitochondria, along with a notable decrease in the motility and viability of human spermatozoa after exposure to RF-EMR. In a recent research work, Al-Bayyari (21) noted that sperm counts, motility, morphology, and viability all decreased with phone use, potentially leading to male infertility. It is noteworthy that the research by Rago et al. (22) is one of the few studies that, despite demonstrating noticeably higher DNA fragmentation in frequent phone users, found no significant difference in other sperm characteristics based on the duration of mobile phone usage.

However, several studies have not found a connection between DNA fragmentation and sperm quality reduction with cellphone usage. Falzone et al. (23) discovered that mobile phone radiation had no discernible influence on spermatozoa apoptosis in their investigation of the effects of radiation from mobile phones on human spermatozoa..

Overall, most research shows how mobile phone radiation affects sperm qualities such as motility, viability, normal morphology, and count. The impact of RF-EMR on sperm DNA fragmentation is far less understood. This suggests a need to understand the range of opinions about this impact. The work presented here is one of the first reviews to examine the effects of RF-EMR on SDF.

Materials and Method

The current study uses data published on Google Scholar and PubMed databases up to 5 July 2020 in order to gain insights into SDF. Older studies published before 2005 were excluded due to the rapid advancement of technology. For the purpose of analysis, the MeSH terms for DNA fragmentation, sperm, mobile/cell phones, radiofrequency and other related terms were combined, along with additional synonyms. This investigation was conducted using the following query: "((break) OR (fragment) OR (damage) OR (SDF (Sperm DNA Fragmentation)) AND ((radiofrequency) OR (radio-frequency) OR (mobile) OR (ELF (Extremely Low Frequency)) OR (EMF (Electromagnetic Fields)) AND ((sperm) OR (fertile) OR (semen) OR (reproductive)) AND (DNA)". Independent searches were conducted by two contributing authors. Any discrepancy or conflict between the two researchers' search findings was settled through discussion and reanalysis.

Following the initial search, a few articles were disqualified according to the abstract and title. Duplicate studies were removed. After reading the entire text of the selected articles, the two authors decided regarding their relevance. Each disagreement was reconsidered and settled. Finally, nine publications were selected for the systematic review. The database search and study selection procedure based on a PRISMA flow diagram is shown in Figure 1. When accessible, the following data was taken from each article: Number of cases, age range, and health status of the study groups, radiation exposure conditions (frequency, strength (SAR (Specific absorption rate)), duration, and distance), outcomes of laboratory tests, and significant observations. This study endeavor was authorized on September 8, 2020, by the Ethics Committee of Mashhad University of Medical Sciences. Since this is a systematic review of previously published work, informed consent was not necessary.

Results

In the initial search, more than 2,864 sources were identified from Google Scholar and PubMed. Following screening and discussions, nine full-text publications were included in the investigation. Table 1 provides the summary statistics of the included studies. It can be seen that four studies analyzed instances in vivo based on individuals' mobile phone usage habits (22,24–26), while the remaining five investigated exposing sperm to radiation in a laboratory setting under controlled conditions (13,16,23,27,28). Most studies included healthy males or infertile men with normal sperm conditions, while excluding those who smoked, frequently used laptops or other radiation-emitting devices, or lived in close proximity to high-voltage power lines. The research by Radwan et al. (25) that included smokers was the exception. It should be highlighted that these two criteria are not included in Table 1 since the phone operating frequency was between 850 and 1900 MHz and the sperm samples had been collected after at least three days of abstinence in all included trials.

Table 2 provides a summary of the listed studies' findings. The P-value, if available, and the DNA fragmentation index (DFI) have been determined. Although seven research works found a significant difference, at least for heavy users or highly-radiated samples, two studies (16,23) found that there was no discernible distinction among the radiation-exposed and non-exposed groups.

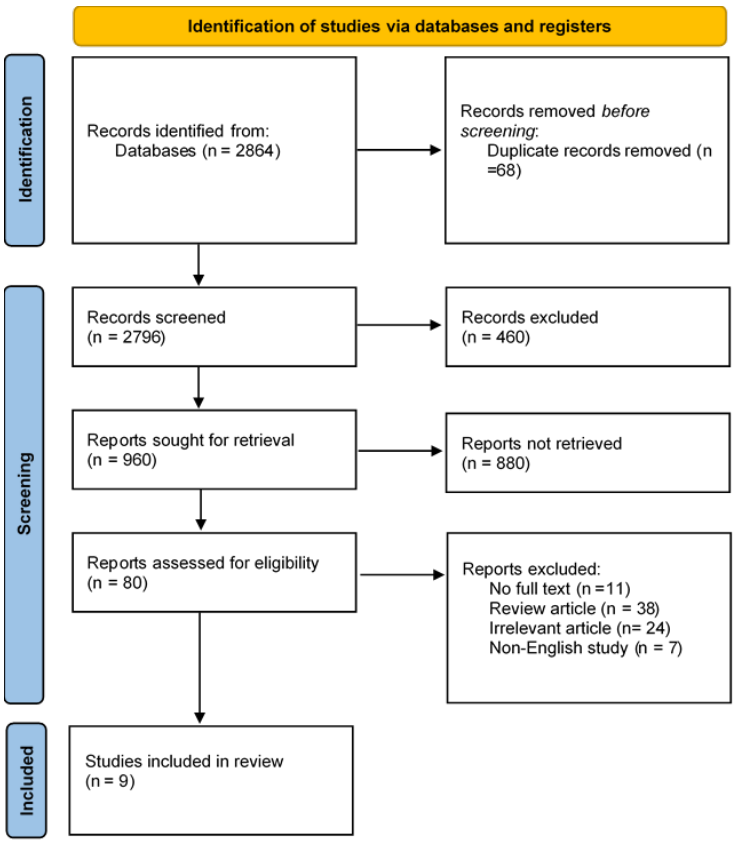

Figure 1. PRISMA flow chart for the process of choosing studies

Table 1. Summary of characteristics of the included studies.

Study	Year	N (Total)	In *Vitro*/ *Vivo*	Features (Inclusion)	Age (Y) range/Avg	BMI (kg/ m2)	Country	Exposure Duration	Distance	SAR (W/ Kg)	Method
Rago et al., (2013)	2013	63	*in vivo*	Healthy, fertile, non-smoking	18-35	19-24.5	Italy	-	-	-	TUNEL assay
De Iuliis, Newey et al., (2009)	2009	22	*in vitro*	No known prior male reproductive pathologies including varicocele and infection	24.16 ± 1.1		Australia		~cm (waveguide)	0.4-27.5	TUNEL assay
Mostafa et al., (2012)	2012	100	*in vivo*	Infertile men, but non-smokers, residing or working away from base station, no direct exposure to another source of EMW, no time to watch television.	25-60		Egypt	-	-	-	Comet assay
Gorpinchenko et al., (2014)	2014	32	*in vitro*	Healthy (normozoospermia), childless couples	27.5 ± 3.5		Ukraine	5h, a call every 10min	5 cm (thermostat)		SCD
Zalata et al., (2015)	2015	124	*in vitro*				Egypt	1h	10 cm		Flowcytometry
Falzone et al., (2010)	2010	12	*in vitro*	Healthy, non-smoking			South Africa	1h	~cm (chamber)	2, 5.7	TUNEL assay
Agarwal et al., (2009)	2009	32	*in vitro*	23 healthy and 9 infertile			USA	1h	2.5 cm	Power <1 W, SAR 1.46	TUNEL assay
Ding et al., (2018)	2018	270	*in vivo*	Healthy, active reproductive age, non-smoker, not exposed to other radiations, no chronic disease	28 ± 5 27 ± 6 28 ± 5	22.85 ± 2.68 22.51 ± 2.59 22.81 ± 2.22	China	-	-	-	Comet assay
Radwan et al., (2016)	2016	236	*in vivo*	Infertile but normal semen concentration	22.7–44.8 (32.2)	25–29.9 47.6% 30–40 21%	Poland	-	-	-	SCSA Flowcytometry

BMI: body mass index, SAR: specific absorption rate, SCD: sperm chromatin dispersion test (SCD), TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling

Discussion

Our daily exposure to RF-EMRs has significantly increased due to the widespread use of mobile phones (29,30). Concerns have been raised regarding potential adverse effects on health, particularly concerning the human reproductive system (31–34). Numerous human and animal studies have indicated that RF-EMR exposure, depending on power, duration, frequency, and wave type, can alter sperm parameters and impact reproductive health (35–39). There is more controversy surrounding damage to sperm DNA integrity compared to findings related to adverse effects on sperm count, viability, normal morphology, and motility (40).

This systematic review focused on the DNA fragmentation of human sperm resulting from RF-EWR from mobile phones, while other sources of RF-EMR such as mobile base stations and Wi-Fi networks were not considered. In a study by Avendano et al. (41), sperm was exposed for four hours to radiation from a laptop connected to the internet via Wi-Fi. Reports indicate that there was a significant decrease in sperm motility and an increase in DNA fragmentation (from around 4% to approximately 8%). In addition to sperm, the detrimental effects of RF-EMR on the DNA fragmentation of other cells have also been investigated. In their study, Durdik et al. (42) found that hematopoietic stem/progenitor cells exposed to RF-EMR from mobile phones had higher levels of ROS but no significant difference in terms of DNA fragmentation (42). This research looked over the majority of studies on DNA fragmentation of human sperm. In terms of studies conducted on non-human subjects, Aitken et al. (20) examined male mice following a 7-day exposure and discovered substantial DNA damage in the mice spermatozoa's nuclear and mitochondrial genomes.

The purpose of this study was to evaluate the relationship among exposure to RF-EMR from mobile phones and DNA fragmentation. Nine relevant studies were found for this review.

Laboratory Methods

The most popular laboratory techniques for measuring DNA fragmentation include comet assay, sperm chromatin dispersion (SCD) test, sperm chromatin structure assay (SCSA), and terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) (43). Of the nine reviewed publications, four took reference to TUNEL (13,16,22,23), Mostafa et al. and Ding et al. (26) employed comet assays (15,26), Zalata et al. (28) and Radwan et al. (25) utilized flow cytometry (SCSA), and Gorpinchenko et al. (27) used the SCD technique.

Given the established adverse impacts of heat on the integrity and quality of sperm, the thermal effect of radiation is regulated in almost all of in vitro experiments to ensure that any damage or deterioration in quality is not the result of heat. Additionally, sperm samples were incubated for two hours at temperatures between 21 and 50 degrees Celsius in the study carried out by De

Table 2. Outcomes of the included studies in terms of DNA fragmentation analysis.

Study	N (Total)	Groups (n =)	DNA Frag. Index (%) (Ex vs Unexposed)*	P-Value	Conclusion
Rago et al., (2013)	63	A: no use (10) B: <2 h/day (16) C: 2–4 h/day (17) D: >4 h/day (20) D1: Trousers (12) D2: Shirt (8)	3.0 ± 1.2 % 3.2 ± 1.6 % 3.1 ± 2.2 % 6.6 ± 2.2 % 6.7 ± 1.8 % 5.1 ± 1.3 %	< .05 < .05	No anomaly for A, B, C High DNA fragmentation in group D High DNA fragmentation in trousers users
De Iuliis, Newey et al., (2009)	22	Case (exposed) Control (unexposed) (2 samples: Exp, Unexp)	3%-29% for SARs of up to 27.5 W/kg		Reduced motility and vitality after exposure Significantly elevated ROS and DNA fragmentation (P: 0.001) Highly significant relationships between SAR, 8-OH-dG, and DNA fragmentation after exposure
Mostafa et al., (2012)	100	A: no use (15) B: <2 h/day (30) C: 2–4 h/day (25) D: >4 h/day (30)	18.1 ± 8.1 % 20.3 ± 9.6 % 19.7 ± 7.5 % 24.9 ± 8.9 % (DNA damage using comet tail moment)	04.	Highly significant difference in DNA .damage using comet tail moment Group D showed an increased ROS level and altered DNA strands Extended use of cell phones could have adverse effects on semen quality
Gorpinchenko et al., (2014)	32	Case (exposed) Control (unexposed) (2 samples: Exp, Unexp)	8.8 ± 2.2 % 4.2 ± 1.8 %	05. >	Increased sperm DNA fragmentation and significant decrease in the number of sperm with progressive movement with prolonged exposure
Zalata et al., (2015)	124	normozoospermia (N, 26) asthenozoospermia ((A, 32 asthenoteratozoospermia AT, n = 31) oligoasthenoteratozoospermia) .(OAT, n = 35) (samples: Exp, Unexp 2)		05. >	Significant increase in sperm DNA fragmentation after exposure such that OAT>AT>A>N Poor sperms have higher DNA frag- .mentation index
Falzone et al., (2010)	12	SAR 2.0 (12) SAR 5.7 (12) (2 samples: Exp, Unexp)	30% ≈ 37% ≈ (Read from figure)		Slightly increased DNA fragmentation but no significant difference in any .sperm param Impairment of fertility was not caused by the induction of apoptosis in spermatozoa
Agarwal et al., (2009)	32	Case (exposed) Control (unexposed) (2 samples: Exp, Unexp)	5.77% ± 8.44 6.62% ± 7.80	for) 62. (n=20	Increase in ROS level No significant difference in DNA fragmentation Cell phone in trouser may negatively affect spermatozoa and impair male fertility
(Ding et al., (2018	270	G1: < 30 min/day (n = 89) G2: 31-120 min/day (104) G3: > 121 min/day (77)	% [15.7 ,11.4 ,2.8] ≈ % [47.1 ,17.1 ,14.3] ≈ % [57.1 ,53.6 ,50.0] ≈ [1st Qt, Med, 3rd Qt] Comet tail (Read from figure)		Positive correlation of exposure and damaged sperm DNAs of all three groups Increased DNA fragmentation and decreased sperm quality with extended exposure time
Radwan et al., 2016	236	0–5 years (45) 6–10 years (149) 11–25 years (68) (Based on cell phone possession and use)			High and medium level of occupational stress and age increase DNA fragmentation index (P = 0.03, P = 0.004 and P .(= 0.03, respectively Obesity and cell phone use > 10 years positively associated with percentage of immature sperms (high DNA stainability index) (P = 0.02 and P = 0.04, (respectively Stress and lifestyle factor may affect sperm DNA damage

Iuliis et al. (13). They showed that heat had an impact only at temperatures above 40 ºC. Thus, the maximum temperature rise caused by RF-EMR at the temperature where DNA fragmentation evaluation was conducted (21 ºC) was around 0.4 ºC at the highest SAR of 27.5 W/kg. Increased ROS could not be the result of such a spike (13).

In Vivo Research

Four in vivo studies in our research revealed low sperm parameters for males who use phones frequently throughout the day (15,22,25,26).

Considering how often people use their cellphones, males were divided into four groups in Rago et al.'s (22) in vivo experiment (group A=no use; group B= <2 h/day; group C= 2–4 h/day; and group D= >4 h/day). Additionally, two subgroups of people who kept their phones in their shirts (held farther from the testicles) and trousers (kept devices closer to the testicles) were taken into consideration for the group with the highest usage (> 4 hours per day). When their sperm's

conventional and bio functional parameters were examined, no significant variations were found across the groups—with the exception of the "trouser" sub-group of heavy users (> 4 hours per day), which had a significantly higher percentage of DNA fragmentation (≈ 6.7%). SDF may be the only parameter that is substantially impacted by the duration of phone use (22).

These outcomes are comparable to those reported by Mostafa et al. (15); they found that the group with the highest daily phone usage (> 4 hours/day) had significantly higher ROS levels and altered DNA strands in their spermatozoa compared to the other groups.

A different survey conducted by Radwan et al. (25) has shown the impact of daily life factors on sperm DNA damage in adult men. The substantial difference with other articles was the inclusion criteria where they took smokers into account. In addition to age and obesity, they discovered that mobile phone use for longer than ten years was associated with higher levels of DNA damage. Moreover, the percentage of immature sperms (high DNA stainability) was much higher in those individuals (25).

In a recent research by Ding et al. (26), men exposed to 2.4 GHz Wi-Fi and the EMR 4G 1800 MHz 4G smartphone network were studied. Three groups were formed based on the duration of phone use by the participants: fewer than 30 minutes, 31–120 minutes, and more than 121 minutes. Sperm viability, sperm count, and the proportion of progressive sperms all dramatically declined with longer exposure time (when comparing the three groups), while sperm damage and ROS levels significantly increased (26).

In Vitro Research

According to the results of a previous systematic study, in vitro research showed that RF-EMR had the most adverse effects on sperm motility and viability (36). In our review, five out of the nine studies focused on in vitro research.

Previous observations by De Iuliis et al. (13) involved exposing human spermatozoa to RF-EMR using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay to test for SDF. They observed a notable drop in vitality and motility and an approximately 8% increase in DNA fragmentation at 2.8 W/kg SAR. Higher SAR values were associated with higher DNA fragmentation (a linear region), which persisted until the percentage of fragmentation reached a flat zone between 25% and 29% for SAR values between 10 and 27.5 W/kg. It should be noted that about 4% fragmentation was seen in the 1.0–2.0 W/kg (commercial phone) range. They also reported a positive correlation between mitochondrial ROS production and DNA fragmentation, linking oxidative DNA damage to elevated ROS levels (13).

Gorpinchenko et al. (27) measured the SDF percentage using the SCD test method. They observed a significant variation in the percentage of fragmented DNA between exposed and non-exposed samples after 5 hours of exposure to a mobile phone placed 5 cm from the sperm samples, with intermittent active/sleep phases. Sperm motility was found decreasing with exposure time, while DNA fragmentation showed a positive correlation with exposure time (27). This approach mirrors what used by Zalata et al. (28) who exposed semen samples to radiation for one hour at a SAR level of 1.46 W/kg. Using flow cytometry to analyze DNA fragmentation, they found a statistically significant difference between exposed and unexposed samples. One notable finding was that radiation-induced DNA damage was greater in samples with poorer sperm parameters (28).

In contrast to earlier studies, Falzone et al. (23) evaluated the spermatozoa parameters under two different values of SAR (2.0 and 5.7 W/kg). According to the TUNEL test, there was no discernible difference in the amount of DNA fragmentation between the RF-EMF-exposed and control groups (23). These outcomes resemble those that Agarwal et al. (16) described. DNA damage was measured using the TUNEL technique. In that work, although there was a discernible increase in ROS levels and a decrease in sperm motility, the exposed and unexposed samples did not significantly vary in terms of the DNA integrity index.

Therefore, RF-EMR from mobile phones could be a major, if not the sole, factor contributing to reduced sperm quality, even though DNA fragmentation has not consistently been observed in all studies.

Conclusion

The purpose of this study was to examine the connection between human SDF and RF-EMR. When considered collectively, the data show that although longer and more severe radiation exposure has been linked to a decrease in sperm quality, the conclusions involving DNA fragmentation have not yet been validated. The current study is limited by the absence of a meta-analysis due to the nature of the included research and the absence of key parameters in some of the studies. Further work is needed, particularly controlled studies conducted over longer periods of time, to fully understand the effects of mobile phones' RF-EMR on SDF.

Conflict of interest

The authors of this article state that they have no competing interests.

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