Section 10

Mobile Phones, Radiofrequency Waves, and Male Infertility

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Summary

- The purpose of this section is to synthesize pertinent research concerning the relationship between exposure to radiofrequency (RF) and effects on semen parameters and male infertility.

- Relevant publications on the epidemiology of reproductive effects from RF on human male in vivo and in vitro sperm studies, as well as selected animal research studies, were assessed. The literature was exclusively on exposure from mobile phones.

- Unlike the mixed findings found in occupational health studies of radar EMF exposures, the epidemiological studies of men assessed for infertility were consistent in demonstrating decreased sperm motility associated with increased use of mobile phones.

- In vitro laboratory studies, which involved exposing semen samples to controlled mobile phone RF exposure, generally noted a decrease in sperm motility, among other adverse effects. An exception was one study using purified, rather than unprocessed sperm, which lacks leukocytes and other factors important for sperm motility.

- While animal studies allow more control of the laboratory environment, the applicability of findings to humans is questionable. Studies of one type of rat (Wistar) tended to show adverse effects on semen parameters and implications of infertility associated with RF exposure, unlike those of Sprague-Dawley rats.

- Apart from the known thermal effects of RF, oxidative stress due to increased Reactive Oxygen Species (ROS) or decreased antioxidants is a plausible explanation for non-thermal effects of RF on sperm cells.

- Many of the epidemiological, in vitro and animal studies that were reviewed demonstrated biological effects on sperm motility related to RF exposure. Whether male fertility is impacted by RF is not yet clear. The positive findings highlighted here are unique among research endeavours examining possible health effects attributed to RF exposure and deserve more extensive research.

10.1 Introduction

Over the last 30 to 40 years, public concern over health effects related to RF has grown.[1,2] A specific concern is the possible effects of exposure to RF on fertility and viability of offspring.

Infertility affects about 15–20% of heterosexual couples of reproductive age, with half attributed to male factor infertility.[3-6] Often the amount of sperm produced is adequate, but the spermatozoa are functionally defective.[7] The quality of DNA carried within the sperm has been recognized as an additional factor in infertility.[8,9]

This section of the toolkit attempts to inform public health practitioners in their dialogue with decision makers and the public by providing a synthesis of pertinent research of the health effects of RF which may affect male infertility, and a summary of possible mechanisms for such effects. The majority of the literature on reproductive function describes the possible effects of RF on male sperm. The full spectrum of reproduction and development, including male sexual function and pregnancy outcomes such as spontaneous abortion and congenital malformations, as well as child development, will not be covered in the toolkit.

The purpose of this toolkit section is to assess current human and animal research into RF effects on sperm and male factor infertility.

10.2 Methods

Peer-reviewed papers from PubMed, Scopus, Ovid and Medline databases were searched from 2005 to 2011. Grey literature, including government documents, were also searched. The studies were limited to English. MeSH terms for radiofrequency radiation, male, fertility and infertility were among the keywords used and combined. Two recent reviews specific to male infertility and mobile phone RF exposure were used as a starting point in evaluating studies to include in this toolkit.[10,11] From the reference lists, abstracts were obtained where the titles were relevant to the subject of potential male infertility effects due to exposures to typical population levels of RF. Very few papers from the abstracts were excluded.

10.3 Results and Discussion

10.3.1 Human studies

Sperm cells are useful for the study of the cellular effects of RF as their characteristics are well known and the cells are easy to obtain. Human studies have been either retrospective observational studies, mainly on the extent of mobile phone use among men with infertility problems, or in vitro analyses of RF effects on human semen. Brief descriptions of the epidemiological and in vitro studies are given in Table 1 below.

Table 1. Human studies on the effects of exposure to RF on male semen parameters

Population	Methodology	Exposure	Endpoint Assessed	Results	Considerations
Fejes et al. (2005)[12] Is there a relationship between cell phone use and semen quality?					
371 infertility clinic patients, 30.8 ± 4.4 yrs	Retrospective; interview; self-report mobile phone use	Self-reported past cell phone use; 1. Low transmitter, <15 min/day 2. High, >60 min/day	Sperm concentration, motility	Decreased proportion of rapid progressive sperm motility with increased transmission time; increased slowly progressive sperm with increased transmission time	Self-report; unclear how duration of possession and use were assessed
Wdowiak et al. (2007)[13] Evaluation of the effect of using mobile phones on male fertility					
304 infertility clinic patients	Retrospective; questionnaire; self-report of GSM mobile phone use	Self-report of GSM mobile phone use 1. No use 2. 1–2 yrs sporadically used 3. >2 yrs regularly used	Sperm morphology, motility, concentration	Increased abnormal morphology with increased duration of phone use; decreased progressive motility with frequent phone use	No age range given; no significant differences between 3 study groups in terms of smoking, occupation, age, home region; different results according to "frequency" and "duration" of use but specifics unclear
Agarwal et al. (2008)[14] Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study					
361 infertility clinic patients, 31.81 ± 6.12 yrs	Retrospective observational; 4 groups stratified by self-recalled mobile phone use	Self-report of mobile phone use 1. No use 2. <2 hrs/day 3. 2–4 hrs/day 4. >4 hrs/day	Sperm count, motility, viability	Decreased sperm count, motility, viability, morphology, dependent on duration of daily exposure to mobile phone	Did not validate mobile phone use; did not classify type of phone; did not account for any confounders other than age; did not take other RF exposures into account

Population	Methodology	Exposure	Endpoint Assessed	Results	Considerations
Gutschi et al. (2011)[15] Impact of cell phone use on men's semen parameters					
2110 infertility clinic patients, 21.6 ± 6.6 yrs	Retrospective , recorded mobile phone use	Self-report mobile phone use (n=991) and non-use (n=1119)	Serum hormones, sperm count, motility morphology	Mobile phone users have increased pathological morphology (68.0% vs. 58.18%); lower % rapidly progressive motility (23.98% vs. 25.19%); higher free testosterone and lower luteinising hormone; all p< 0.05	Poor exposure ascertainment (no info on frequency, duration, placement of phone etc.) or other environmental confounders
Kilgallon and Simmons (2005)[16] Image content influences men's semen quality					
52 university students, 18–35 yrs	Experiment involving random allocation of explicit images; retrospective "lifestyle" survey	Self-report mobile phone use; explicit images viewed	Sperm motility, concentration	Lower sperm concentration and percentage motile sperm if mobile phone carried in hip pocket or belt	Only study that controlled for numerous "lifestyle" factors while assessing mobile phone use effect; not primary endpoint (intention of study) so details unclear
Erogul et al. (2006)[17] Effects of electromagnetic radiation from a cellular phone on human sperm motility: an in vitro study					
27 healthy urology patients with normal semen parameters, 27 ± 3.2 yrs	In vitro; experimental split samples; neat ejaculate sample	GSM phone, 900 MHz, 2 W peak power, average power density 0.02 mW/cm^2 for 5 min, 10 cm away	Sperm motility	Decreased rapid motility; increased percentage of non-motile sperm; duration of possession and use negatively correlated with semen quality	Two observers per sample

Population	Methodology	Exposure	Endpoint Assessed	Results	Considerations
Agarwal et al. (2009)[18] Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study					
23 normal and 9 infertile patients	In vitro; neat ejaculate sample; exposed and control aliquots	GSM talk mode Sony Ericsson w3001 with AT&T 850 MHz, SAR 1.46 W/kg, max power <1 W for 1 hr, 2.5 cm away	Sperm motility, viability; reactive oxygen species ROS), total antioxidant capacity (TAC), ROS-TAC score; sperm DNA damage	Decreased sperm motility, viability, ROS-TAC; increased ROS; no difference TAC; no DNA damage	Age range unclear; didn't measure sample temperature; seminal leukocyte counts; may not mimic carrying the phone on belt / in pocket
De Iuliis et al. (2009)[19] Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro					
22 Normospermic 24.1 ± 1.1 yrs	In vitro; purified sperm; exposed and control aliquots	Waveguide function generator 1.8 GHz, SAR range 0.4–27.5 W/kg, 16 hrs	Sperm motility, vitality; ROS, oxidative stress; DNA damage	With increased SAR, there was decreased sperm motility, vitality; increased mitochondrial ROS and DNA fragments	Temperature was controlled; purified sperm samples were used
Falzone et al. (2008)[20] In vitro effect of pulsed 900 MHz GSM radiation on mitochondrial membrane potential and motility of human spermatozoa					
Semen samples from 12 subjects	In vitro; purified sperm; motility assessed by computer-assisted sperm analysis (CASA)	Signal generator; pulsed 900 MHz GSM-like RF at 2 or 5.7 W/kg, 1 hr	Mitochondrial membrane potential; sperm motility, kinematic parameters	Decreased sperm kinematic parameters straight line velocity (VSL) and beat-cross frequency (BCF) at 5.7 W/kg; no effect at lower SAR of 2 W/kg; no effect of mitochondrial membrane potential	Age range not clear
Falzone et al. (2011)[21] The effect of pulsed 900-MHz GSM mobile phone radiation on the acrosome reaction, head morphometry and zona binding of human spermatozoa					
12 samples from subjects aged 23 ± 5 yrs	In vitro; purified sperm; CASA	Signal generator; 900 MHz GSM-like, SAR 2 W/kg, 1 hr	Sperm morphology; acrosome reaction; sperm-oocyte interaction (binding)	Decreased competence to bind zona pellucida; no effect acrosome reaction	Flow cytometry cannot assess acrosome reaction stage, so an effect may not have been detected

10.3.2 Epidemiological studies

There have been a number of occupational health studies conducted on military and police exposed to radar devices, rather than mobile phone use.

Weyandt et al. (1996)[22] assessed exposures to microwave RF and aerosolized lead exposure in US military personnel from the Army Intelligence Corps and found that those with microwave exposures (assessed by duty assignment and questionnaire) had lower sperm counts. The same research group (Schrader, 1998)[23] later found no differences in sperm count or function. It was felt that exposure to intelligence radar in the first study would expose personnel to higher amounts of EMF than communication or missile tracking radar. Danish soldiers in another study on exposure to tracking radar with an estimated low maximal mean exposure of 0.01 mW/cm^2 had a non-significant reduction in sperm concentration.[24]

Fejes et al. (2005)[12] set out to conduct what they described as "the first human population study of the possible relationship between mobile phone use and semen quality." They enrolled 371 men who presented with infertility problems, assessing a number of aspects of mobile phone use including duration of possession, duration in standby mode when closer than 50 cm, and duration of daily use. Semen samples were collected by standard technique after five days of abstinence and analysed after liquefaction, according to standard WHO criteria for analysis and classification of sperm samples.[25]

Motility was assessed by percentage of sperm defined as rapid progressive (capable of penetrating the oocyte membrane), non-progressive (sperm which do not move forward) and immotile (dead); sperm count was done and analysis was repeated three weeks later with each subject providing a second sample under similar conditions. As with most studies investigating the cause of infertility, exclusion criteria for participants comprised behaviours and conditions known to affect sperm and semen quality including smoking, alcohol use, drug abuse, severe systemic illness or trauma within six weeks of the study, detectable organic alteration of reproductive organs or infection, and altered hormone levels of follicle stimulating hormone (FSH) and leutenizing hormone LH or testosterone. Of 611 consecutive men considered for inclusion, only 371 met inclusion criteria; all were Caucasian, ranged between 17–41 years with an average of 30.8 ± 4.4 years, and included a representation of a variety of social classes as assessed by level of education.[12]

As for assessment of RF exposure, low-transmitters were defined as those using a mobile phone less than 15 minutes per day; high transmitters, more than 60 minutes per day; short-standby, those who kept the phone in standby for less than one hour per day; and long-standby, for more than 20 hours per day.

It was found that duration of possession correlated negatively with the proportion of rapidly progressive sperm; the proportion of slowly progressive sperm also increased

with increasing daily transmission.[12] No significant findings were found between the long and short stand-by groups. Fejes et al. (2005)[12] concluded that there seems to be an adverse effect on sperm motility related to mobile phone use. They noted, however, that they did not account for a number of factors which influence potential RF effects from mobile phones, including the technology of the phone (e.g., pulse wave Global System for Mobile Communications (GSM) vs. continuous wave Code Division Multiple Access (CDMA)).[12,26] Further limitations were the inclusion only of men with presumed infertility who were enrolled due to seeking treatment, which may not be representative of the general population, and not considering other factors such as occupation.

Wdowiak et al. (2007)[13] studied the effect of mobile phone use on fertility in Polish men presenting for infertility assessment. They enrolled 304 men using three categories of exposure combining cumulative use and duration of use over time; 99 subjects did not use mobile phones, 157 had used GSM phones sporadically over 1–2 years, and 48 reported regular use for more than two years. Subjects answered a questionnaire and survey regarding phone use, and semen samples taken after 2–7 days of abstinence were evaluated according to WHO parameters. Exclusion criteria included those with varicocele, systemic illness, features of reproductive organ inflammation, BMI below 17 or above 30, and history of hormonal or reproductive development disorders. Questionnaires attempted to classify subjects by rural, town or city location (based on population size of residence,) amount of smoking, occupation, age and phone use. In evaluating the three subject groups, no significant differences in smoking, age, residence or occupation were found.

Concentration of sperm was classified into five groups according to the number of sperm cells in the ejaculate sample: severe; moderate; and light oligospermia (low concentration of sperm); and normospermia (normal sperm count and motility) respectively. Motility was assessed in four groups based on percentage of sperm in type A live forward progressive state. Morphology was assessed in five groups looking at percentage of normal sperm, with less than 3% being normal.

Using the above criteria, Wdowiak et al. found that 65.7% of men who did not use a mobile phone had a normal spermiogram, whereas only 35.4% of those using a phone regularly did.[13] Similarly those with no phone use had a greater percentage of sperm with normal morphology and motility; however, frequency of use according to the three exposure groups did not show a statistically significant association.

The researchers noted their results were congruent with those of other studies and concluded that the percentage of live progressively motile sperm of normal morphology decreased with frequency of GSM mobile phone use.[13] However, they failed to provide specific questionnaire questions or to validate the use of their questionnaire as an instrument to assess mobile phone exposure. Though they attempted to account for some confounding by asking subjects about occupation and smoking and

assessing differences between such groups, they failed to include other potential confounders such as alcohol use and other RF exposures. There was also no specific mention of age range of subjects, though it was stated that age did not make a significant difference in results.

In a prospective study of 13 men who used GSM mobile phones for six hours per day for one month, Agarwal et al. (2008)[14] evaluated sperm parameters in men undergoing investigation for infertility in an observational study. A total of 361 subjects were divided into those with no mobile phone use, those who used the phone for less than two hours per day, those using for two to four hours per day, and those with use more than four hours per day. In analysis using age as a covariate, it was found to be non-significant, which the authors interpreted to mean results were not biased by advanced age. Exclusion criteria were also similar to the previous studies, including smoking, chewing tobacco, alcohol use, male genital problems, tuberculosis, diabetes mellitus and hypertension. Samples were collected in standard fashion after five days of abstinence and analyzed according to WHO criteria.[27] The technicians analyzing the semen samples were blinded to the subjects' use of mobile phones.

Mean sperm motility, viability and normal morphology showed significant adverse effects in the mobile phone user groups, both in men with normal and abnormal sperm counts. A dose-response relationship was found as the assessed semen parameters declined with increasing mobile phone use, independent of the quality of the original sample.

Limitations for this study included data for type of phone and other variables known to influence RF exposure (e.g., occupation and other RF sources) not being collected. Age was the only covariate analyzed. Validation of mobile phone use was also not done, and Agarwal et al. relied only on subjective recall of history of use.[14] However, validation of mobile phone use has been performed for other studies and it has been found that subject recall is often reasonably adequate.[28,29]

The retrospective study by Gutschi et al. (2011)[15] was notable in that a large number of fertility clinic patients were included in the study. However, exposure ascertainment was crude, comparing those who used mobile phones to those who did not. With approximately 1000 subjects per group, even a small difference in sperm motility (23.9% for mobile phone users vs. 25.1% for non-users) was statistically significant (p<0.01), as were differences in morphology and serum-free testosterone and luteinizing hormone levels. Misclassification of the simple exposure variable of mobile phone use or not is likely. The authors admit to limitations in exposure ascertainment including no information on frequency and duration of use, whether the mobile phone was placed in pants pockets, or the influence of other environmental confounders such as occupational exposures. This study appears to be a secondary analysis since information on mobile phone use was "recorded" with no description given of a questionnaire or interview survey component.

A study by Kilgallon and Simmons (2005)[16] looking at the effect of type of image viewed on ejaculate parameters (not a study designed to assess the effects of RF), found that men who carried a mobile phone on a belt or in a hip pocket had lower sperm motility and lower sperm concentration according to WHO parameters (1999)[27] than those who did not carry a phone or those who carried a phone on a different body location.

This study recruited 52 heterosexual men aged 18–35 years old from the University of Western Australia and randomized them to look at sexually explicit images. Detailed questionnaires on lifestyle were filled out by participants, including questions on mobile phone use and the carrying position of mobile phones. While not looking specifically at the effects of mobile phones on semen quality, the authors concluded that even after control of all other lifestyle variables assessed by the study questionnaire, storage of a mobile phone near the testes (in a hip pocket or on a belt) had a significant negative impact on both sperm concentration and the percentage of motile sperm. As the study was not meant to address such associations, no information on mobile phone use (type of phone, duration of talk use, storage in on, off or stand-by mode, etc.) was provided, nor was exposure to other RF sources elicited. However, the study does seem to provide suggestive evidence of a relationship between proximity of mobile phone (worn on the hip pocket or belt) with semen quality.

10.3.3 Limitations of epidemiological studies

Although the studies included large enough numbers of men to have adequate study power, the populations were not broad enough to draw conclusions applicable to those outside the study population. Perhaps most limiting in population applications was the use only of infertile men as subjects, as well as the inclusion of predominately men of Caucasian/European origin. Thus the validity of applying results to the general male population is questionable.

As retrospective studies are, by definition, based on participant recall, assessment of mobile phone usage by study subjects is also uncertain. No study attempted to validate subject recall as a method of assessment of phone use, and so as a proxy of RF exposure. Further, few details were elicited or presented on specifics of exposure: how the phone was held; proximity to base stations (towers); type of phone; frequency; modality, etc. Specific duration (years of use) and accumulation of use (accumulation of minutes) was also vague.

While each retrospective observational study attempted to control for confounders first with exclusion criteria and then with analysis to assess significance between results when adjusting for confounders such as age, they were limited in their ability to do so. Although the data is compelling for an *association* between mobile phone use and altered sperm parameters, there is no evidence implicating mobile phone use as a *causative* factor. While one may be reasonably sure that among the Caucasian/

European men seeking treatment for infertility, self-reported mobile phone use was associated with alterations in semen quality (predominantly sperm motility), there is little clarity about a causative link, or accounting of confounding factors or even about the specifics of exposure relevant to effects on sperm function (type of phone, duration of use, etc.).

10.3.4 In vitro studies

Agarwal et al. (2009)[18] performed a small-scale prospective pilot study on unprocessed semen samples from 23 normal donors and nine infertile donors and assessed semen samples according to WHO parameters.[27] Semen samples were obtained by standard means after a period of abstinence of 48–72 hours, and after liquefaction, the samples were divided in half. One aliquot was exposed for one hour to a 850 MHz RF-pulsed mobile phone 2.5 cm away (having a maximal power <1 W and an estimated SAR of 1.45 W/kg). The phone's frequency was confirmed with an RF spectrum analyzer. The other half (control aliquot) was kept in identical conditions but was not exposed to the mobile phone. For control samples, power density was measured as being between 0.01 and 0.1 microwatt/cm^2 and the experimental samples, 2.5 cm from the phone antenna, were between 1 and 40 microwatt/cm^2.

Sperm motility and viability were negatively affected by exposure to RF. No significant differences in sperm concentration were found, nor was an alteration of DNA integrity observed in the experimental samples. Though room temperature was measured and controlled, sample temperature was not monitored. It is assumed that a mobile phone operating at such a low SAR (<2 W/kg) will negligibly increase temperature;[30,31] however, it is still prudent to measure.

Though this study was reasonably well controlled, blinding was not clearly explained in the paper, so it is unknown if technicians analyzing various semen parameters were aware of the purpose of the study or of which samples were considered experimental vs. control. While the distance from the semen sample to the phone was meant to mimic the distance between a phone carried in a pocket or on a belt, from the testes, it does not account for the clothing and tissue layers surrounding the testes in vivo.

De Iuliis et al. (2009)[19] also investigated the effect of RF exposure on human sperm from 22 normospermic donors, aged 24.1 ± 1.1 years old, a younger average age and more narrow distribution than many other studies. They liquefied the semen, which was then purified by separation of sperm from seminal plasma, with isolated sperms washed, centrifuged and then re-suspended. The sperm fraction of each sample was then analyzed for vitality, motility and cell density after the purification process and after experimental or control conditions.

Exposure was to RF of 1.8 GHz in the SAR range of mobile phones, (0.5–1.5 W/kg). A mobile phone was not used to create RF waves; rather a cylindrical waveguide was constructed that allowed RF at a frequency of 1.8 GHz to be propagated along Petri

dishes containing samples. SAR was measured in saline solution within and outside of the experimental system. Temperature of the same saline was also measured throughout the experiment as a measure of sample temperature, and was kept at 21°C to avoid any thermal effects. Samples were exposed to SAR from 0.4–27.5 W/kg for a period of 16 hours, and all experiments were done at least in triplicate.

The investigators found a dose-dependent response for all tested parameters, including sperm motility and vitality. Decreased motility and increased levels of ROS were found in exposed specimens. The authors noted that in vitro studies are limited to approximately 24 hours of sperm viability due to limitations of culture media, and that sperm in vivo survived much longer during the one week transit time from seminiferous tubules to cauda epididymis, which would result in greater exposure to RF waves. As such, it is likely that a higher percentage of sperm may be adversely affected than indicated by this study, even if the presumably more susceptible ones were damaged first. The authors further noted another limitation of the culture media used, being inferior to epididymal plasma for sperm support, as would be found in vivo.[19] However, as a dose-response effect was found, it would seem there is biological and clinical relevance to their findings.

The study by De Iluiis et al. (2009)[19] is one of the best controlled in vitro studies of the effect of RF waves on sperm quality. Experimental parameters were strictly controlled and explained, and rationale for the frequency and SAR is logical and practical. However convincing though, the results were found on purified semen in vitro. Though the authors acknowledge this and point to previous studies supporting in vivo effects and effects on unprocessed samples, it is still difficult to translate this study to mobile phone effects on semen in "real life," and to link the effects observed with infertility.

Erogul et al. (2006)[17] have also looked at in vitro effects of RF waves on semen, in particular motility and concentration. They used a mobile phone to provide 900 MHz frequency, and assessed effect on semen collected from 27 healthy males.

Subjects averaged 27 ± 3.2 years and were recruited from patients visiting a urology clinic. Abstinence of two to seven days was required. Samples were split in half, one aliquot for control and one for experiment. The two groups of samples were rested at room temperature for 25 minutes and then separated; the experimental group was exposed to a GSM 900 MHz mobile phone, peak power 2 W, and average power density 0.02 mW/cm^2 for five minutes at a distance of 10 cm. Semen was analyzed after the rest period and 30 minutes after the exposure period in both experimental and control groups, at the same time, in order to reduce time-dependent motility variation. Analysis was done by two blinded observers; concentration and motility were evaluated through a counting chamber according to WHO criteria.

Significant differences between control and experimental groups were observed, including decreases in rapidly and slowly progressive sperm and increases in no-

motility sperm. No change was seen between groups in non-progressive motility or in concentration.

The authors assert that all environmental factors except for RF exposure were the same in each group, and so the noted change in motility can be explained only by the RF exposure. While the study does seem to be well controlled and conducted, it is mentioned that, despite blinding, inter-observer variability can occur in assessing motility on a qualitative basis, and that even by having two observers who are well trained, human error cannot be discounted.

Falzone et al. (2008)[20] focused specifically on sperm motility after exposure to pulsed 900 MHz RF. They noted that motility is a prerequisite for fertility, as sperm must journey to the ova and must be able to penetrate the zona pellucida. Due to the inherent inter-operator variability in manual semen sample assessment for WHO criteria, a computer assisted sperm analysis (CASA) was used.

Semen samples were collected from 12 healthy, non-smokers after two to three days of abstinence and kept at 37°C. Samples were allowed to liquefy for 30 minutes and parameters were evaluated and confirmed to be normal. Samples were then purified in three steps and the highly motile 95% layer was centrifuged and re-suspended. RF was produced by a signal generator and modulated with by a pulse duration of 0.577 ms with a repetition rate of 4.615 ms to mimic a GSM mobile phone system and administered using a waveguide. Temperature-controlled water was circulated through a 9 mm waterbed beneath the sample Petri dishes to allow control of a constant temperature.

Samples were exposed within the chamber to the 900 MHz GSM-like RF at either a SAR of 2.0 W/kg or 5.7 W/kg for one hour while controls were left beside the chamber for the same amount of time. Sperm were assessed after exposure, at two hours post-exposure and at 24 hours post-exposure. All tests were run in duplicate, and two samples were exposed and two kept as control. Using CASA, the sperm kinetic parameters evaluated were progressively motile sperm and parameters for velocity and frequency of movement. Progressive motility was not found to change significantly with either exposure, and no change in any of the velocity parameters was found with SAR 2.0 W/kg exposure. However, two parameters of motility, straight line velocity and beat-cross frequency, were significantly impaired after exposure to SAR of 5.7 W/kg.

Much criticism of in vitro studies of RF effects on sperm has focused on the influence of cofounders and the mechanism of observation, in particular lack of dosimetry (accurate measure of RF dose) and lack of automated semen analysis use.[32,33] Falzone et al. (2008)[20] attempted to address these concerns by carefully basing dosimetry on numeric simulations validated by temperature-based SAR measurement and carefully controlling the experimental conditions using a constructed chamber. Temperature effects were therefore not a consideration, as the chamber and temperature-controlled

water provided optimal temperature control. Use of CASA technology to assess sperm velocity and motion parameters negated observer bias.

In studies previously described, effect was found on rapid progressive sperm motility, in contrast to the negative findings by Falzone et al.[20] It is possible that the differing samples used (unprocessed vs. purified) is responsible for this by introducing leukocytes and their effects. It is also possible that manual assessment of motility was not as accurate and unbiased as use of CASA. In short, though Falzone et al. (2008)[20] did not find evidence of impaired sperm movement toward the egg (rapid progressive) as other authors did, they did find possible evidence of impaired sperm movement to penetrate the egg once there (hyperactivity).

Falzone et al. (2011)[21] continued their research on the fertility potential of sperm by examining the acrosome reaction, (release of enzymes from the anterior of the head of sperm when contacting the ovum, allowing for penetration and fertilization) head morphometry and zona pellucida binding ability (to protein membrane surrounding the oocyte plasma membrane) of sperm after exposure to 900 MHz of RF at SAR 2.0 W/kg for one hour, using methods similar to their 2008 experiments.

Acrosomal status was assessed at two and 24 hours post exposure, or control. Sperm-ooctye interaction was assessed using oocytes (immature egg cells) obtained from patients undergoing in vitro fertilization. Oocytes were thawed and bisected and the ooplasm was dislodged and kept at room temperature while experimental sperm were added to one half and control sperm not exposed to RF was added to the other. Binding capacity was determined by the ratio of sperm bound to the two halves, comparing the binding ability of the non-RF-exposed sperm to the RF-exposed sperm.

They found that there was no change in acrosome reaction even though morphometric parameters were altered with a significant reduction in sperm head area and acrosome percentage as well as decreased sperm-zona binding ability.[21]

Zona pellucida binding gives a good indication of fertility, therefore the finding of altered sperm binding to the zona pellucida after RF exposure implies an effect on male fertility. However, they caution that the in vitro effects noted should not be directly applied to in vivo situations and that much more research is needed to replicate the results and to explain the mechanism.

The previous studies addressed the relationship of semen parameters with exposure to RF from the use of mobile phones. An exception is the recent prospective in vitro study by Avendano et al. (2012)[34] involving Wi-Fi use in laptop computers. Semen samples from 29 healthy donors were divided into two aliquots incubated under identical conditions, but with one aliquot exposed in a separate room for four hours to a wireless internet-connected actively working laptop, 3 cm away from the specimen (to mimic the typical distance from a laptop placed on the lap to the testes). Laptop exposure induced a decrease in progressive sperm motility and an increase in the

percentage on non-motile sperm compared to unexposed controls (p<0.05). As well, sperm DNA fragmentation increased in the exposed group, allegedly through non-thermal effects (since the room and incubation temperatures, including laptop exposure, were kept constant at 25°C). The researchers concluded that the wireless use of a laptop computer positioned near the male testis may decrease human sperm quality, and with prolonged use there may be an impact on sperm fertility potential. At question was whether an active laptop without wireless internet connection would result in similar effects, which would imply a role of EMF exposure from the battery source.

10.3.5 Limitations of in vitro human studies

The in vitro studies on human semen attempt to address the limitations of epidemiological research. Most of the studies provided better control of exposure conditions, including specific frequency, SAR and power density exposure and more accurate dosimetry calculations. Varying degrees of blinding were attempted, and control samples were universally used. The effect of confounders, like proximity to an RF source, was adequately addressed. As with the epidemiological studies, it is difficult to compare results of the in vitro studies as the exposures and conditions evaluated were not consistent. Differences in use of unprocessed or purified semen and the practical use of evaluating isolated sperm instead of those in a more physiological state contribute to uncertainty in the effect of semen components on sperm motility. Differences in exposure to RF, in frequency, SAR, source and distance also make it difficult to compare results, as do differences in methods of evaluating effect, such as the use of computer assisted versus manual analysis.

Most, although not all studies attempting to control temperature, convincingly ruled out a thermal effect. Further, while one author acknowledged the effect of time on parameters under study, others did not attempt to consider the known effect of time since ejaculation affecting motility, resulting in a progressive decrease in the percentage of motile sperm over time. The human studies generally focused on sperm motility, which has plausibility as an important precursor to fertility; however, it is unknown what characteristics of exposure to RF may have impact on sperm motility. Although effects on sperm motility were found in the in vitro and epidemiological studies, whether these findings translate into "real world" decrements in fertility has yet to be convincingly demonstrated.

10.3.6 Animal studies

Although animal studies often provide more control of the experimental environment, the applicability of animal data to humans is always questionable. Characteristics and findings of the selection of animal studies are presented in Table 2 below.

Table 2. Animal studies on the effects of exposure to RF on male infertility

Subjects	Methodology	Exposure	Endpoint Assessed	Results	Considerations
Dasdag et al. (2003)[35] Whole body exposure of rats to microwaves emitted from a cell phone does not affect the testes					
16 Sprague-Dawley rats	Comparison of 2 groups, exposure and control	GSM phone Nokia 6110, 890–915 MHz peak power 2 W at 250 mW, SAR 0.52 W/kg, 20 min/day x 1 mo, 0.5 cm below cage	Testicular, epididymal lipid; malondialdehyde concentration; p53 immune reactivity; sperm count, morphology; histological structure of testes; rectal temperature	No effects	Low SAR postulated as the reason for no observed effects
Imai et al. (2011)[36] Effects on rat testis of 1.95–GHz W–CDMA for IMT–2000 cellular phones					
72 Sprague-Dawley rats, 5 wks old	Comparison of 3 groups; control, lower SAR, and higher SAR	CDMA phone, 1.95 GHz, SAR 0.4 or 0.08, 5 hrs/day x 5 wks	Testicular, epididymal, prostate weight; body weight; sperm count, morphology, motility; testicular histology; spermatogenic cycle	No effects	Used 5-wk old rats for 5 wks as period of sexual maturation is 5–10 wks
Yan et al. (2007)[37] Effects of cellular phone emission on sperm motility in rats					
16 Sprague-Dawley rats	Comparison of 2 groups exposure and control	CDMA phone, Nokia 3588i, 1.9 GHz trimode, SAR 1.8 W/kg, two 3-hr periods/day x 18 wks, 1 cm away	Epididymis sperm motility, morphology, count; mRNA for cell surface adhesion proteins; face temperature every 12 min; rectal temperature	Higher sperm cell death, abnormal clumping, decreased motility. Adhesion proteins up-regulated	Up-regulation of adhesion proteins associated with clumping: a possible mechanism for infertility?

Subjects	Methodology	Exposure	Endpoint Assessed	Results	Considerations
Dasdag et al. (1999)[38] Whole-body microwave exposure emitted by cellular phones and testicular function of rats					
18 Wistar rats	Comparison of 3 groups to RF (standby, speech, sham)	GSM phone, 890–915 MHz, 2 W max power; 0.141 W/kg, 1. Standby 2 hrs/day x 1 mo 2. Speech 3 x for 1 min over 2 hr/day x 1 mo 3. Control, 0.5 cm under cage	Left caudal epididymal sperm count; testicular histology; rectal temperature each week	Decreased epididymal sperm count in speech group (not statistically significant); decreased seminiferous tubule diameter in speech and standby group; elevated rectal temperature in speech group	Possible thermal effect as testes exposed in close proximity (0.5 cm) to phone
Kesari et al. (2010)[39] Mobile phone usage and male infertility in Wistar rats					
12 Wistar rats	Comparison of 2 groups, control and exposed	Mobile phone, 900 MHz, SAR 0.9 W/kg, 2 hrs/day x 5 wks	Protein kinase C; sperm count; sperm apoptosis; ROS	Decreased protein kinase C and sperm count; increased apoptosis and ROS	Relationship between ROS, PKC
Kesari et al. (2011)[40] Effects of radiofrequency electromagnetic wave exposure from cellular phones on the reproductive pattern in male Wistar rats					
12 Wistar rats	Comparison of 2 groups, control and exposed	GSM phone, 900 MHz, SAR 0.9 W/kg, 2 hrs/day x 5 wks	Antioxidant enzymes; malondialdehyde; histone kinase; micronuclei; reactive oxygen species; sperm cell cycle	Decreased glutathione peroxidise, superoxide dismutase (antioxidants), histone kinase; increased ROS, catalase, malondialdehyde; altered sperm cell cycle	
Mailankot et al. (2009)[41] Radio frequency electromagnetic radiation (RF-EMR) from GSM (0.9/1.8 GHz) mobile phones induces oxidative stress and reduces sperm motility in rats					
12 Wistar rats	Comparison of 2 groups, exposure and control (phone without battery)	GSM phone, 0.9–1.8 GHz, 1 hr/day x 28 days	Caudal epididymal sperm count, motility; glutathione; lipid peroxidation; facial temperature	Decreased sperm motility; increased lipid peroxidation, decreased glutathione in testis and epididymis; no change in sperm count; no temperature effects	

Subjects	Methodology	Exposure	Endpoint Assessed	Results	Considerations
Meo et al. (2011)[42] Hypospermatogenesis and spermatozoa maturation arrest in rats induced by mobile phone radiation					
40 Wistar rats	Comparison of 3 groups, control, exposure of 30 min or 60 min	GSM phone answer mode, 30 or 60 min/day x 3 mos, inside cage	Morphological changes in testes under light microscope	3 of 16 rats exposed for 60 min/day had hypospermatogenesis; another 3 had arrested maturation in testes; no effect was seen on the 16 rats exposed for 30 min/day	Do not specify RF exposure details
Ribeiro et al. (2007)[43] Effects of subchronic exposure to radio frequency from a conventional cellular telephone on testicular function in adult rats					
16 Wistar rats	Comparison of 2 groups, control and exposure	GSM phone, 1835–1850 MHz, 0.125 mW max average power, 1 W max peak power, 1 hr/day x 11 wks	Testicular and epididymal weight; lipid peroxidation; serum total testosterone; epididymal sperm count; seminiferous tubular diameter; rectal temperature	No effect	
Aitken et al. (2005)[44] Impact of radiofrequency electromagnetic radiation on DNA integrity in the male germline					
26 CD1 Swiss mice	Comparison of 2 groups to RF, exposure inside a waveguide and control (outside the waveguide)	3 GHz generator, 900 MHz, SAR 90 mW/kg, 12 hrs/day x 1 wk, cages inside waveguide	Sperm count, vitality and morphology; DNA strand breakage temperature; animal stress	No effect on sperm number, morphology; no DNA strand breaks; mitochondrial genome and nuclear beta-globin locus damage in epididymal sperm	~10x lower SAR than most other studies; no evidence of impact on germ cell development but possible evidence of genotoxicity

Dasdag et al. (2003)[35] continued their earlier research on mobile phone exposure effects on fertility using an animal model (Dasdag et al., 1999)[38] involving 16 Sprague-Dawley rats. Similar to their 1999 study, they exposed rats confined in plexiglass cages to RF waves. A Nokia 6110 GSM phone operating between 890 and 915 MHz, SAR 0.52 W/kg, average power 250 mW and peak power 2 W was placed 0.5 cm below the cage. Subject rats were exposed for 20 minutes per day to the phone in the "talk" position for one month. Control rats were exposed to a turned off phone.

Components measured included testicular lipid composition, malondialdehyde concentration (an index of sperm plasma membrane lipid peroxidation), and histological structure. The left caudal epididymis (where sperm is stored in the testes) was used to harvest semen to determine sperm count and morphology. Rectal temperature was measured to rule out thermal effects. No significant differences were noted in experimental and control groups; however, as there were only eight animals per group, the power of the study to detect significant differences was low.

The authors felt the low SAR accounted for the negative results, which are in contrast to those of a number of other older studies which showed adverse effects on seminiferous tubule epithelium, sperm count and morphology with exposure to high levels of SAR (30–44 W/kg) which themselves were enough to cause thermal effects.[45-47]

Yan et al. (2007)[37] did find adverse effects on sperm after exposing 16 Sprague-Dawley rats to a CDMA phone placed 1 cm away functioning at 1900 MHz, SAR 1.8 W/kg for two three-hour periods each day for 18 weeks.

They examined sperm from the proximal vas deferens (tube conveying sperm), and found that in the exposure group there was a higher cell death count, decreased motility and abnormal sperm clumping. In this study, the RF exposure time was longer, a different type of phone (CDMA instead of GSM) was used, and sperm from a different portion of the reproductive tract was assessed.

Using similar exposure (1950 MHz CDMA phone, SAR 0.04–0.08 W/kg), Imai et al. (2011) [36] examined the effect of RF on developing, five-week-old Sprague-Dawley rats, exposing them for five hours per day for five weeks. They did not find any difference in growth overall or testicular, epididymal (part of the spermatic duct system), or prostate weight. Further, no changes in sperm motility or morphology were found, and histology was normal

Overall, it does not seem that most studies support an adverse effect of RF on Sprague-Dawley rat semen or fertility potential. However, a number of recent studies on Wistar rats do seem to indicate a detrimental effect on sperm motility and, to a lesser extent, sperm count.

In 2011, Meo et al.[42] exposed 16 Wistar rats to one hour of RF each day for three months and found that a portion of the exposed rats showed hypospermatogenesis

(abnormally decreased production of sperm), and another portion had arrested maturation of sperm. Interestingly, the group of 16 rats the investigators had exposed to 30 minutes of RF per day for the same time course, showed no adverse effects, similar to the group of eight rats in the control group.

When Ribeiro et al. (2007)[43] exposed 16 Wistar rats to 11 weeks of GSM RF for an hour each day, they also observed no effects on the histological testicular parameters including testicular and epididymal weight, epididymal sperm count, seminiferous tubule diameter, and rectal temperature. This exposure time was chosen to include six seminiferous epithelium cycles, and so cover the period for spermatogonia to mature to sperm and reach the epididymis. By covering this time frame, they were confident they would detect change through all stages of development and so detect subtle effects if present.

In the study by Mailankot et al. (2009),[41] sperm motility was affected but not sperm count, after exposure of six Wistar rats to one hour of RF for 28 days from a GSM mobile phone.

The potential fertility effects of RF on other rodents (mice and rabbits) have also been investigated. However, not only is it difficult to compare results within a species, it is even more difficult to compare between species.

Aitken et al. (2005)[44] exposed 26 CD1 Swiss mice to GSM equivalent RF at 900 MHz, SAR 90 mW/kg for 12 hours per day for one week and did not find any effect on sperm morphology or motility.

10.3.7 Limitations of animal studies

There are obvious differences in the structure and physiology of the reproductive organs of animals and humans. The small size of the experimental animal means the effective exposure to RF is often greater. Though SAR and power density measures can be used to approximate what a person would be exposed to, it is difficult to be certain. The reproductive system also differs in size as well as in location and placement. A rat's testicles for example are able to move freely through the inguinal canal, and so can migrate into the abdomen, altering the level of RF exposure during the experiment. The way in which animals are exposed also differs from humans; in many studies the animal is confined in a cage with the reproductive organs especially exposed to RF.

As with the human studies, it is difficult to compare results among animal studies. However there is often much better control of experimental conditions. Age, size and care of the animals must be considered. Most rats are kept in standard conditions, with free access to food and water, and are acclimatized to the experimental conditions (though for varying lengths of time) to minimize stress effects. Exposure parameters also differ, between animal experiments and between human ones. Source of RF

production, distance from animal, duration of exposure and intensity of exposure are not standardized. However, most authors have kept exposure parameters within those expected of a mobile phone, to rule out excessive RF exposure leading to possible heating effects.

10.3.8 Possible mechanisms

10.3.8.1 Thermal

It has long been established that thermal effects of RF waves may adversely affect human health, including male reproductive function. Short-term exposure to RF is known to increase testicular temperature and can alter seminiferous tubular epithelium (lining of cells in the testes area where sperm are produced).[45,46] However, exposure to RF from mobile phones has not been shown to generate enough heat to cause thermal effects. For example, even after six hours of mobile phone exposure, rectal temperatures were identical for the exposed and control rats, and therefore biological effects found were attributed to RF.[37]

10.3.8.2 Non-thermal

Though a number of different mechanisms have been proposed, increased oxidative stress (either from increased ROS or decreased antioxidant capacity) seems most likely to be implicated. It can explain observed effects on sperm directly and also indirectly through other possible mechanisms such as DNA damage.

10.3.8.3 Oxidative stress

Many of the effects noted on sperm after RF exposure seem to be related to increases in ROS which have a deleterious effect on sperm resulting in oxidative stress, which is a known factor in male infertility.[14,40,48-54] In 1992, Grundler et al. (1992)[55] showed that RF waves can induce ROS activity in cells.

RF has been shown to stimulate transmembrane NADH oxidase, an enzyme complex which transfers electrons from NADPH (a reduced form of NAD coenzyme) inside the cell across the membrane to be coupled to oxygen, which results in the production of ROS.[56] It is known that sperm have similar plasma membrane reduction-oxygenation (redox) systems, and so may produce increased ROS on RF exposure in a similar manner.[57-59] Mitochondria have been suggested as a source of ROS,[19] as have leukocytes found in semen outside of the sperm.[20]

Apart from inducing and increasing ROS, RF may also alter antioxidant enzymes, and so cause oxidative stress. Changes have been noted in erythroctyes (red blood cells)[60] as well as other tissues[35,38,43,61,62] when antioxidant enzymes have been assayed. However, it is unclear whether the RF is directly causing an effect on the enzymes or whether they are responding to a stress (even an oxidative stress due to increased ROS) effect. Non-enzyme antioxidants, like melatonin, have also been observed to

decline after RF exposure.[62-65] An additive effect may occur, with alteration not only of sperm cell enzymes but of whole body system antioxidants. Melatonin in particular is known to support antioxidant activity in sperm.[66] A number of recent studies have provided experimental evidence suggestive of an oxidative stress mechanism for the effect of RF on sperm.

Agarwal et al. (2009)[18] performed a small scale prospective pilot study on unprocessed semen samples from 23 normal donors and nine infertile donors assessing ROS, total antioxidant capacity (TAC) of seminal plasma, calculated ROS-TAC score and DNA damage by commercial kit. The TAC is the sum of enzymes and non-enzymes considered antioxidants and includes superoxide dismutase, catalase, glutathione peroxidise, ascorbate, urate, vitamin E, pyruvate, glutathione, taurine and hypotaurine. The score reflects the imbalance between ROS and TAC; a lower score is indicative of oxidative stress and infertility. They found that there was evidence for increased oxidative stress, as ROS increased with mobile phone exposure and ROS-TAC score decreased. Since sperm motility and viability were also decreased, the authors felt that induction of ROS and oxidative stress is a plausible mechanism for the deleterious effects seen on sperm exposed to mobile phone RF.

The authors provide a plausible explanation for the mechanism of action, stating that the increased ROS may be due to sperm plasma-membrane redox system stimulation by mobile phone generated RF.[18] However, they also note that an equally plausible mechanism would be an effect of leukocytes present in the unpurified ejaculate. Leukocytes are known to be involved in ROS production. They also note that some studies have found magnetic effects on ROS, and that magnetic fields in the present study were not examined.

De Iuliis et al. (2009)[19] also investigated the effect for RF exposure on human sperm with the hypothesis that oxidative stress is a common causative mechanism for disruption of sperm fertilizing potential and sperm DNA damage. The researchers exposed purified semen samples from 22 normospermic donors to RF frequency of 1.8 GHz with a SAR ranging from 0.4–27.5 W/kg. They performed standard measures of sperm motility and vitality, as well as ROS measurements and DNA damage assessments and all experiments were done at least in triplicate.

The investigators found a dose-dependent response for all tested parameters.[19] From 1 W/kg to 4.3 W/kg a significant increase in ROS was found and it was determined that the ROS were sourced from the sperms' mitochondria. At a SAR of 2.8 W/kg, the results became statistically significant for mitochondrially produced ROS. They noted specifically that rapid change occurred at low SAR exposures which reached a plateau when about 30% of sperm were affected. The researchers posit that even though they attempted to study only high quality purified sperm, a cohort of susceptible sperm exist which perhaps have abnormal, compromised mitochondria.

To confirm that the observed rise in ROS resulted in oxidative stress, expression of 8-hydroxy-2-deoxyguanosine (8-OH-dG), a marker of sperm DNA oxidative damage, was measured. An increase in expression was noted at lower SAR levels, which rose in a dose-dependent manner. A strong positive correlation between 8-OH-dG and MSP was found, indicating that the more ROS are produced, the higher the expression of 8-OH-dG, and so the higher the oxidative stress. An additional assay showed an increase in DNA-strand breakage from a SAR of 2.8 W/kg that increased in a dose-dependent manner and correlated strongly with ROS production and 8-OH-dG production.

The authors noted that in vitro studies are limited to approximately 24 hours of sperm viability due to limitations of culture media.[19] In vivo, sperm survive much longer during the one-week transit time from seminiferous tubules to be stored in the caudal epididymis, which would result in greater exposure to RF waves. As such, it is likely that a higher percentage of sperm may be adversely affected than indicated by this study, even if the presumably more susceptible ones were damaged first. The authors noted another limitation of the culture media used being inferior to epididymal plasma for sperm support, as would be the natural condition in vivo.[19] However, as a dose-response effect was found it, would seem there is biological and clinical relevance to their findings.

The study by De Iuliis et al. [19] is one of the best controlled in vitro studies of the effect of RF waves on sperm quality. Experimental parameters were strictly controlled and explained, and the rationale for the frequency and SAR is logical and practical. Results are internally consistent, and a plausible mechanism is explained based on ROS and oxidative stress. However, the results were found on purified semen in vitro. As such, it is difficult to translate the findings of this study to mobile phone effects on human semen in "real life," and to link such effects (if proven) with infertility.

Mailankot et al. (2009)[41] also looked at indications of oxidative stress in an animal study consisting of six Wistar rats exposed to RF from a GSM mobile phone. The mechanism to explain reduced sperm motility was suggested to be increased oxidative stress as indicated by increased lipid peroxidation (oxidative degradation of lipids) and decreased glutathione (an antioxidant).

Most recently, Kesari et al. (2011)[40] investigated the effect of RF from a GSM mobile phone (SAR of 0.6–0.9 W/kg) on oxidative stress in 12 Wistar rats exposed for two hours per day for five weeks. The mobile phone was placed on top of the cage instead of beneath, as was done by many other investigators. Experiments were done in duplicate in a blind pattern.

Glutathione peroxidise (GPx), catalase (CAT) and superoxide dismutase (SOD) were evaluated in sperm using an antioxidant kit with positive control, and it was found that both GPx and SOD decreased significantly in exposed animals, whereas CAT increased. Malondialdehyde (MDA), a reactive aldehyde known to cause toxic stress in cells, as well as ROS, and micronuclei formation were measured. Both MDA and ROS were

significantly increased in the exposed group, as were micronuclei. Given the increase in ROS and decrease in SOD and GPx (antioxidant enzymes), as well as the trends in MDA and micronuclei, Kesari et al.[40] concluded that the effect of RF was an enhancement of ROS, which likely led to increased lipid peroxidation and antioxidant enzyme alteration, and so oxidative damage. Given the alteration in the other parameters measured, such as increased micronuclei formation, an indication of DNA damage, an impact on fertility was felt likely.

Lack of support for a role of ROS in sperm effects was shown in the 2010 study by Falzone et al. in which exposure to RF had no effect on induction of DNA strand breaks or generation of ROS in purified sperm.[67]

Differences in studies of purified and unprocessed sperm (which have different compositions of mature and immature sperm) may also make sense in the context of an oxidative stress mechanism, as there is more potential for damage in an immature sperm than a mature one.[57] It would therefore follow that the unprocessed sperm in the study by Agarwal et al.[18] would show more effects than the purified, mature sperm in the study by Falzone et al.[20,67]

Overall, oxidative stress seems one of the more plausible mechanisms of RF-induced sperm damage. It has been found fairly consistently in human and animal studies on sperm specifically and on other cells in general. Mechanisms by which oxidative stress is caused by increased ROS and decreased antioxidant have been shown to exist in neurodegenerative diseases such as Parkinson's and Alzheimer's.

10.3.8.4 DNA damage

Increased production of ROS and increased oxidative stress have themselves, independently, been shown to damage DNA and other molecules, and DNA damage is known to be a factor in infertility.[10] However, studies on human lymphocytes have not shown DNA damage after exposure to mobile phone frequency RF for 24 hours.[68]

Although RF does not appear to have sufficient energy to damage DNA directly (as ionizing radiation may), other mechanisms of damage to DNA may be involved such as through ROS and oxidative stress, as well as up-regulation of gene expression and protein formation, including heat shock and adhesion proteins.[69-71]

Aitken et al. (2005)[44] exposed 26 CD1 Swiss mice to GSM equivalent RF at 900 MHz, SAR 90 mW/kg for 12 hours per day for one week and did not find any effect on sperm morphology or motility. Although they found significant damage to mitochondrial genes and the nuclear beta globin locu, no adverse effects on DNA strand breakage in sperm were noted.

As ROS can impact DNA, it is possible that RF may affect sperm quality through some forms of DNA damage, although the effects have not been as reproducible as ROS and oxidative stress effects.

10.3.8.5 Membrane potential and integrity

It is known that RF can induce currents in a cell membrane, and that this may alter the cation (positive ion) distribution (and so charge) in the normally negative membrane. Some evidence shows pulsed RF can dislodge calcium ions (Ca++) from a membrane, resulting in a weaker barrier and leakage, although there is no direct evidence on sperm membranes.[72] However, studies do seem to point to efflux of Ca++ as a factor in altered sperm motility.[51,65,72]

Studies have shown an effect on protein kinase C (PCK), and its alteration is implicated in altered sperm motility. As Ca acts as a secondary messenger, and PCK is one of its targets, this seems to implicate an efflux of Ca in decreased motility.[73]

For instance, Kesari et al. (2010)[39] found a significant decrease in protein kinase C (PKC) and sperm count in Wistar rats exposed to the same conditions described above. As PKC is known to be present in sperm and play a role in both motility and the acrosome reaction, these results point to a potential mechanism for deleterious effects of mobile phone RF on sperm motility and fertility potential.

10.3.8.6 Hormonal effects

RF effects on hormones and the pituitary gland have been studied to a much lesser degree than has sperm motility and morphology. Leydig cells in the testicle produce testosterone under the influence of LH, a hormone produced by the anterior pituitary. It is therefore plausible that alterations in testicular structures and in hormonal levels may be the causative mechanism for RF effects noted.

It is possible that oxidative stress and direct RF effects causing alteration in PKC, which is present in Leydig cells and seminiferous tubules, may explain altered Leydig histology in response to RF. One study showed that Leydig cells were especially sensitive to RF.[74] Alteration of testosterone receptors due to oxidative stress has also been implicated.[75]

There have also been studies showing no effect on hormone levels, testicular or anterior pituitary histology.[76-78] For example, when Ribeiro et al. (2007)[43] exposed 16 Wistar rats to 11 weeks of GSM RF for an hour each day, they observed no effects on total serum testosterone. An earlier study by De Seze et al. (1998)[79] looked at the hormonal effects in humans after exposure of 21 men to a 900 MHz mobile phone used for two hours per day, five days per week for five weeks. Because no effects on FSH, LH, GH, or PRL were noted,[72,80] the authors concluded that intermittent exposure to RF did not induce a cumulative effect on the hormone secretion rate of the pituitary gland.

A criticism of some of these studies is that the lower levels of RF exposure and shorter duration would be insufficient to assess the chronic effects of RF exposure.[64]

10.3.9 Limitations of studies on male infertility

The greatest limitation in evaluating the evidence on mobile phone RF and male infertility is the wide array of methods used to evaluate an even wider array of exposures.

Looking at studies to date, there is no consistency of RF source (phone or not, type of phone, mode of phone), location of RF source (distance, orientation), frequency, SAR, or power, although most investigators attempt to use a mobile phone or cell phone like RF, and so most studies are within 800–1800 MHz, SAR 0.04–<2 W/kg, and power <2 W.

In human epidemiological studies, reporting of mobile phone use is subject to misclassification of exposure. As well, there is no standardization of measuring duration of use in each instance (number of minutes per day) or of length of time of use (number of years since starting to use a mobile phone), nor of differentiating between talking on the phone, having the phone on and answerable, having the phone in standby or off mode, or of using a hands-free device with the phone. Texting, which has become an increasingly common use of mobile phones, has not been addressed, nor is use of other applications on smart phones. It is also difficult to find an ideal control group for human studies, as most people have been exposed to RF waves, and almost all have done so through personal mobile phone use. To complicate things, most people who are "light" users are older, further confounding the data. Few people are aware of previous RF exposures, and so it is difficult to account for them.

While animal studies have been better controlled and better reproduced than human studies, there are still discrepancies between species and between studies. Extrapolation of results to humans is also indirect and possibly irrelevant for some measured parameters.

While a plausible mechanism for fertility effects of RF exposure relating to oxidative stress and ROS has been postulated, the source and target of ROS remains unclear.

The rapidly changing nature of mobile phone technology also limits conclusions. Most studies were done on second generation pulsed GSM mobile phones which are being increasingly replaced by third and fourth generation continuous wave smart phones.

10.4 Conclusion

There is evidence of adverse effects on sperm with RF exposure, both in vitro and on the basis of epidemiological studies. The balance of evidence shows that human sperm exposed to RF exhibits decreased motility, abnormal morphology and increased oxidative stress. However, the number of caveats to the evidence, including the effects of confounders and unstandardized experimental designs, weakens the association considerably.

Almost all of the recent reviews on mobile phones and male fertility published since 2009 have concluded that sperm motility was the most consistent parameter showing a decline with exposure to RF.[11,18,81,82] The 2012 review by La Vignera et al.[11] further adds that sperm morphology is affected. Desai et al. (2009)[101] suggested a mechanism in which RF can stimulate extracellular superoxide production in semen, which would result in decreased sperm motility and viability. The detrimental effects of oxidative stress on sperm motility as well as semen parameters were emphasized by Hamada et al. (2011).[82] The review by Merhi (2012)[83] concluded that the evidence for RF exposure being associated with male infertility was weak due to diverse and inconsistent study conditions and stressed the need for further well-designed studies, as was recommended by all of the reviews.

To date, animal and human data are contradictory and difficult to evaluate due to heterogeneity of study designs including exposures, endpoints and intervening parameters measured. However, the balance of all evidence, animal and human, is consistent with the assertion that exposure of the testes to mobile phone RF may be associated with decreased sperm count, motility, concentration and altered morphology.

Evidence is less robust for decreased fertility; though it does follow logically, it is unproven that altered sperm parameters will adversely affect fertility, and it is unclear at what threshold of sperm parameters such an alteration of fertility would occur. Though sperm count and motility are accepted as measures of infertility, the rationale appears largely to be due to the simplicity of standardization and sampling.

Given that the balance of evidence is for some adverse effect, even if that effect cannot yet be precisely defined, it seems reasonable to proceed with caution. A recommendation is that short-term personal exposure for males be reduced by keeping mobile phones away from their genital area (i.e., not in pants pockets) and limiting mobile phone use. As industry is already moving to arguably safer use of RF in mobile phones, consumer encouragement may help this trend continue.

10.4.1 Gaps in the literature

Epidemiological studies ideally need to be conducted on larger, more heterogeneous populations, rather than limiting research to infertile groups. Studies should include men of all ages, as well as children and subjects going through puberty (though limitations on semen analysis must be considered). Diverse populations should be sought and compared, including race, geographic location, occupation, education and socioeconomic status. A potential study group would be healthy sperm bank donors. Control and adjustment of known confounders should be clearly documented, and consensus on what to consider as a confounder and how to adjust for it should be reached to facilitate study comparison. In this regard, stress effects are an important effect modifier of interest. The problem of finding a "control" population not exposed to mobile phone use may be addressed by careful comparison of duration of

possession, duration of use per day and type of use (i.e., texting, hand-free calling, storage of the phone and in which mode), as well as type of phone and network technology used.

Prospective studies are costly and time-consuming but with appropriate exposure assessment, limitations of bias and random error associated with retrospective observational studies could be avoided and allow more definitive evidence on the association of RF with male infertility.

There have been no studies on the effect of RF exposure to body organs from text messaging; there is also a lag in the study of newer technologies such as smart phones and fourth generation long-term evolution (LTE) devices.

In vitro studies must likewise strive to be comparable; agreement should be reached on the type of semen sample (unprocessed vs. purified, duration of abstinence) and the type of supporting media used. Conditions such as source of RF, proximity of source to sample, parameters of source (frequency, power, SAR, etc.) should be clearly defined. Endpoints should also be evaluated in a systematic, common fashion. Within individual studies, manual and automated analyses could be used, and samples should be run in duplicate or triplicate, and assessed by two observers.

Standardization of biochemical assays and preparation for testing would be helpful, as would clear justification of endpoints used as proxies to assess apoptosis, oxidative stress and other conditions. Use of the same sample for multiple analyses may be useful, but control for time elapsed and other alterations should be noted.

Animal studies may have more of a role in mechanistic determination and less in adverse effect confirmation due to the differences in reproductive anatomy and size. However, randomized controlled trials and true RF-naive controls will be an advantage of animal models. Again, studies should strive to be explicit with respect to experimental conditions and, if possible, similar to facilitate comparison. Stress effects and other confounding effects should be addressed and adjusted for. Consensus on semen location source (vas deferens vs. epididymis) and other easily altered parameters should be agreed upon or analyzed in each study.

Overall, a concerted effort would likely help in drawing more firm conclusions on the effect of mobile phone use on male infertility. Until then, conclusions should only be made within the limits of available knowledge, and should acknowledge said limitations.

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