Oxidative Stress in Applied Basic Research and Clinical Practice

Ashok Agarwal
R. John Aitken
Juan G. Alvarez *Editors*

Studies on Men's Health and Fertility

Humana Press

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We dedicate this book to the late
Professor Thaddeus Mann FRS,
University of Cambridge, and
Professor Bayard Storey,
Emeritus Professor of Obstetrics and
Gynecology, University of Pennsylvania,
who pioneered our understanding
of reactive oxygen species and
oxidative stress in the control
of mammalian sperm function.

Foreword

> Oxidative stress is a universal phenomenon of aerobic life, you cannot escape it, nor should you wish to [1].

In the early days of research in the field, oxygen radicals and other "reactive oxygen/nitrogen species" (RONS) were universally thought of as deleterious molecules that must be eliminated at all costs by high levels of endogenous or exogenous antioxidants. Indeed, at high levels they *are* deleterious, e.g. to spermatozoa, other parts of the reproductive system and indeed to all cells and tissues. Sperm must be protected by their own antioxidants and by those in the bodily secretions surrounding them. Yet we now realise that RONS play key physiological roles, helping us to adapt to stress, defending us against infection and regulating physiological/pathological processes such as signal transduction and the intensity of inflammation [2–5].

The reproductive system is a beautiful example of all these principles. RONS at the correct level help to modulate uterine function, ovulation, the progress (or failure) of pregnancy and the behaviour of sperm, e.g. in response to inflammation in the surrounding tissues or even to electromagnetic radiation. Sperm generate reactive oxygen species (ROS) in mitochondria and by NADPH oxidase enzymes and these ROS may regulate sperm function (e.g. capacitation). Yet ROS can also damage sperm, e.g. during storage or handling procedures for in vitro fertilisation or during thermal stress. The highly polyunsaturated sperm lipids are a particular target.

This book "Studies on Men's Health and Fertility" is therefore extremely timely. Edited by three experts who have contributed enormously to the field, Ashok Agarwal, Juan Alvarez and Robert John Aitken, it examines all aspects of the roles of RONS in male fertility/infertility and semen quality; as well as their role in other conditions such as testicular torsion, variococele and erectile dysfunction. Each chapter is well written, carefully edited and appropriately referenced. I learned a great deal from reading this book, and I am sure that you the reader will do so as well.

I recommend it strongly.

Singapore — Barry Halliwell

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Part I
Basic Research

Chapter 1
Electromagnetic Radiation and Oxidative Stress in the Male Germ Line

Geoffry N. De Iuliis, Bruce V. King, and R. John Aitken

Abstract The beneficial impacts of mobile-based communications on society are considerable. Health concerns over the broadcast of radio frequency electromagnetic waves, which carry the information for this medium, are now gaining momentum but are not without its controversies. Studies in the past that aim to determine whether concerns are warranted are sometimes lacking in impact because of poor understanding of radiation science. Nevertheless, the studies completed to date are important in developing the field toward the goal of confirming or disproving claims that radio frequency electromagnetic radiation (RF-EMR) is a serious health issue. We focus on what has been achieved to date, toward determining the effects of RF-EMR on the male reproductive system and information presented which may underpin the potential mechanisms at play. We suggest that oxidative stress may have a key role in the detrimental effects observed in the human spermatozoon and that this cell type may be a unique model to determine the potential mechanism of action given its sensitivities to such stressors.

Keywords Electromagnetic radiation • Oxidative stress • Male germ line • Sperm oxidative stress • DNA damage

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1.1 Introduction

This chapter will aim to outline the basic principles of electromagnetic radiation (EMR) and what is known about their interactions with biological systems, and then will discuss some early and the most recent findings of EMR and mobile phone exposure and male fertility. The main intention will be to work towards shedding light on the potential mechanisms of action of this radiation on spermatozoa focusing on oxidative stress as the mediator.

In recent times, there has been some controversy over the impact of the physical factor, radio frequency electromagnetic radiation (RF-EMR) broadly on human health. Several studies have found an association between human health and exposure to RF-EMR, with emphasis on a range of clinical conditions including childhood leukaemia, brain tumours, genotoxicity and neurodegenerative disease [1, 2]. One such controversial area surrounds studies that indicate elevated risk of brain tumours after 10 years of mobile phone use [3]. However, for every one of these studies, there seems to be another refuting the claims [4]. Nevertheless, these studies are important in developing the field toward the goal of confirming or disproving claims that RF-EMR is a serious health issue. Although work which focuses on the rest of the body aids our understanding of the purported phenomena, this chapter will only focus on work relating to reproduction. To date, the "real" clinical effects of RF-EMR on human health and reproduction are not proven and still controversial; however, if we obtain a basic understanding of the physics of EMR and experimental design together with our knowledge of male reproduction and sperm cell biology, we are well placed to take this field forward markedly.

1.2 EMR Defined

EMR is a form of radiation that ranges from extremely high-energy cosmic and gamma rays at frequencies above 10^{18} Hz down through the visible spectrum (frequencies near 10^{15} Hz) to the relatively low-energy microwave (10^{10} Hz or 10 GHz) and radio frequencies (10^{8} Hz or 100 MHz) (Fig. 1.1). The part of the spectrum used for mobile phone communications is in the frequency range from 800 MHz to 2.5 GHz, labelled Global System for Mobile Communications (GSM) in Fig. 1.1. EMR may be considered to comprise alternating electric, *E*, and magnetic, *B*, fields. The *E* and *B* fields both generate forces on charged particles in materials, but the forces due to the electric fields are normally much larger, except in magnetic materials. However, in the context of mobile phone exposure, the magnetic component of the radiation may be more significant due to its considerable penetrative ability inside not only in human body, but also in buildings.

As will be seen in the discussion below on mobile phone communications, the EM wave may contain components oscillating at a range of different frequencies. This is the case with modulated EM waves where the amplitude or frequency of

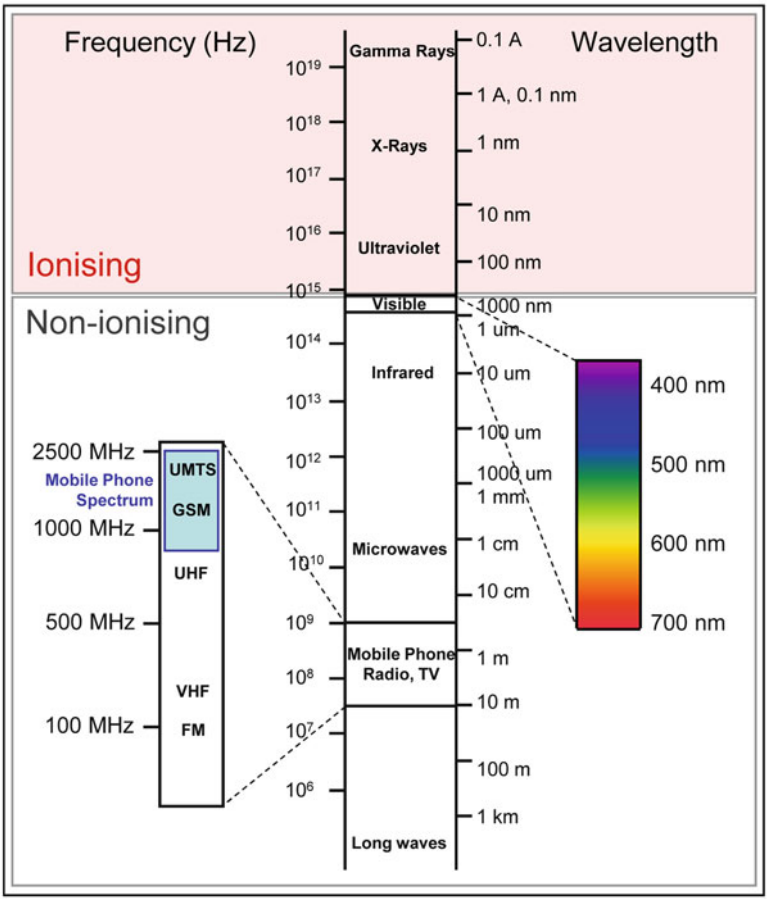

Fig. 1.1 Electromagnetic spectrum. The *blue box* indicates the mobile phone frequency spectrum which begins to enter the microwave spectrum. The energy of mobile phone frequency radiation is far lower than that of ionising radiation (adapted from Physics Central (http://www.physicscentral.com/experiment/askaphysicist/physics-answer.cfm?uid=20110119110703))

the so-called carrier wave is varied in time, in order to carry information. The modulated EM wave contains a component oscillating with the carrier frequency f as well as sideband frequencies $f \pm \Delta f$. The challenge for communications engineers is to maximise the information carried while minimising the frequency spectrum, $2\Delta f$, used.

Most of the radiation studies related to mobile phone communications are done at frequencies used by the GSM phone network. GSM is a digital standard first offered commercially in 1991 and is currently the world's most popular standard for mobile telephony systems, with over 80% of the global mobile market using the standard. GSM networks operate in a number of different carrier frequency ranges

with most 2G GSM networks operating in the 900 or 1,800 MHz bands. Where these bands were already allocated, the 850 and 1,900 MHz bands were used instead (e.g. in Canada and the United States). The more recent 3G networks operate in the 2,100 MHz frequency band in Europe. Although the technology is rapidly evolving, with the incorporation of data transmission and the progression to 4G protocols, the basics of EM radiation emission by GSM mobile phones are relatively unchanged. During a GSM call, speech is converted from analogue sound waves to digital data by the phone itself and transmitted through the mobile phone network by digital means. The EM wave emitted by the phone comprises a range of frequencies. As an example, for a GSM-900 phone, the frequency band 890–915 MHz is used for transmission from the mobile phone to the base station and the band 935–960 MHz from the base station to the mobile phone. In each band, there are 124 separate carrier frequencies spaced 200 kHz apart, starting in the above example at 890.2 MHz. Each 200 kHz frequency is segmented in time, so eight separate channels of information can be sent on each carrier. The digitally encoded information from the codec for all channels together is then used to modulate the frequency of the carrier at a digital rate of 270 kbit s^{-1}. An individual GSM-900 mobile phone will then generate an EM wave with a time-varying frequency within a 200 kHz band on a carrier frequency between 890 and 915 MHz. The intensity of the EMR will also vary in time, since the encoding of the eight separate channels occurs within a 4.615 ms period. So, to properly measure the effect of all mobile phone irradiation on biological systems, experiments should be conducted using pulsed radiation for a range of frequencies within the 850–2,100 MHz band.

The alternating electric, E, and magnetic, B, fields in the EMR interact with materials by exerting forces on charged particles, changing charge distributions in the material. In nonmagnetic materials, the E field causes polarisation (or separation) of bound charges, orientation of permanent dipoles (pairs of opposite charges) and movement of electrons and ions. The first two effects are taken into account by the permittivity, ε, which is a measure of how easily the polarisation of the material changes due to an electric field. Materials primarily affected by the first two processes are called dielectrics. The third effect, the movement of both electrons and positively and negatively charged ions, is accounted for by the conductivity, σ, and materials affected by the third process are known as conductors. The permittivity is typically expressed as a complex quantity

$$\varepsilon = \varepsilon_0(\varepsilon' - j\varepsilon'') = \varepsilon_0\varepsilon' - j(\sigma / \omega) \tag{1.1}$$

where ε_0 is the permittivity of free space (8.85×10^{12} F m^{-1}), $\varepsilon_0\varepsilon'$ is the real part of the complex permittivity (termed the dielectric constant), $j = \sqrt{-1}$ and $\omega = 2\pi f$ is the angular frequency in radians per second. Both ε' and σ increase with increasing water content in the tissue being low for fat and high for blood. The variation of these parameters over the communication frequency range is not large. For testes, ε' is 58 and conductivity is 1.34 S m^{-1} at 900 MHz, whereas at 2.5 GHz, ε' and σ are 57.5 and 2.21 S m^{-1}, respectively [5]. Since ε' is high, tissue such as testicles may

then be considered to be lossy dielectrics. With a lossy dielectric, the transmitted wave is attenuated as it travels into the material. Energy is transferred from the wave to the dielectric as kinetic energy of the charged particles in the dielectric. The loss is related to the average permittivity for biological tissue and depends on frequency, generally decreasing as the frequency increases since the charges cannot respond to rapid changes in high frequency fields. ε represents the conduction of ions as well as friction associated with the alignment of dipoles and vibrational and rotational motion in molecules. The depth of penetration of EM radiation, defined as the distance at which power absorption is approximately 14% of the surface value, is about 4 cm at 1 GHz and 2.5 cm at 2 GHz in tissue. Real world irradiations are, however, more complicated because scattering and refraction of EM waves at interfaces means that energy is deposited in a non-uniform manner into tissue. The energy absorbed from the wave is directly related to the internal E field at the point of absorption. But the incident and internal fields can be quite different depending on the size and shape of the body, its electrical properties, its orientation with respect to the field and its frequency of the EM radiation.

The power absorbed by the sample is related to the specific absorption rate (SAR) where "specific" indicates that the parameter is normalised with respect to mass. The SAR (in W kg^{-1}) is then the power absorbed per unit mass or

$$\mathrm{SAR} = \int_{\mathrm{sample}} \frac{\sigma(r)\,|E(r)|^2}{\rho(r)}\,\mathrm{d}r \tag{1.2}$$

where σ is the sample electrical conductivity (in S m^{-1}), ρ is the sample density (kg m^{-3}) and $|E(r)|^2$ is the square of the magnitude of the electric field, $E(r)$, at point r in the sample. The actual SAR delivered to a region of the body will depend heavily on the depth of the region below the skin, the electrical characteristics of the tissue between the skin and irradiated region and on the exact location and geometry of the RF source.

The US standard is that phones have a SAR level at or below 1.6 W kg^{-1} taken over a volume containing 1 g of tissue, whereas European standards require a SAR maximum of 2 W kg^{-1} averaged over 10 g of tissue. For tissue of density 10^3 kg m^{-3} and 1 Ωm, a SAR of 10 W kg^{-1} corresponds to a field of 100 V m^{-1} and B field of 0.3 μT. However, the actual SAR absorbed by tissue depends on its depth below the surface, the electrical characteristics of the tissue between the source and the target and the presence of external factors which may influence the EMR delivered to the skin. For example, most men put a mobile phone in a front trouser pocket [6]. A 1 W phone placed in the position of the front trouser pocket [7] generates SAR levels of 2 W kg^{-1} in the testes over the frequency range 0.9–4 GHz. This SAR rose to 4 W kg^{-1}, if the effect of metal objects, such as keys, in the pocket was included. Given that GSM-850–900 handsets can have a peak power level of 2 W, then peak SARs in the testes could reach to more than 10 W kg^{-1} under worst case scenarios. However, at typical phone power levels of 0.5 W, SARs would be a more realistic 2 W kg^{-1}.

EMR energy absorption increases the average energy level of random molecular excitation, resulting in a temperature rise. The power absorbed into a region of tissue will cause an initial increase in temperature ΔT in the time interval Δt given by the bioheat equation [8]

$$k\nabla^2 T - \rho^2 C m_b T + \rho \mathrm{SAR} = c\rho \frac{\partial T}{\partial t}$$

where T is the temperature above the mean arterial temperature, k is the thermal conductivity of the tissue (typically 0.5 $\mathrm{W\,m^{-1}\,K^{-1}}$), C is the heat capacity of the tissue (typically 3,700 $\mathrm{J\,K^{-1}\,kg^{-1}}$), ρ is the density of tissue and blood (typically 1.06 kg $\mathrm{m^{-3}}$) and m_b is the volumetric perfusion rate of blood (typically 0.5–10×10^{-6} $\mathrm{m^3\,kg^{-1}\,s^{-1}}$). The first two terms in the above equation represent heat loss from the irradiated area by conduction through the tissue and by blood flow, respectively. The third term is the heat gain due to the irradiation and the fourth term is the temperature rise of the irradiated region with time. On commencement of an irradiation, there is minimal heat loss so the temperature increases linearly with time. For typical tissue, a SAR of 1 $\mathrm{W\,kg^{-1}}$ will cause a 1°C temperature increase per second. As the temperature of the irradiated region rises above the surroundings, heat energy will be transferred away from the irradiated region by thermal conduction through tissue and convective heat flow through the blood, reducing the rate of temperature increase. The sum effect of these channels for heat loss results in an effective thermal conductivity of approximately 10 $\mathrm{W\,m^{-2}\,K^{-1}}$. Finally, the system will come to thermal equilibrium with the EM energy delivered to the irradiated region balanced by the heat energy leaving the region in any time interval. An irradiated spherical region of tissue of mass 10 g would then typically show an equilibrium temperature rise of about 1°C at a SAR of 2 $\mathrm{W\,kg^{-1}}$. It is very well known that the heating of tissue will induce a stress response that is invariably damaging for the tissue involved. To study non-thermal effects of RF irradiation, the subject of this chapter, the equilibrium temperature increase should be kept below 0.1°C, requiring typical SARs of less than 0.2 $\mathrm{W\,kg^{-1}}$.

1.3 Physical Models of the Interaction of Mobile Phone Radiation with Cells

At low power levels where thermal effects are unimportant, EMR still maintains the ability to affect cells. Radiation in the very high-energy gamma-ray frequency range, for example, can directly induce ionisation and lead to radical formation. While this has clear implications for biology, the energy associated with the visible region and down to the radio frequency is not sufficient to remove electrons from atomic or molecular orbitals, i.e. they are not ionising radiations. For example, radiofrequency EMR, at the gigahertz frequencies used in mobile phone communications, can be considered to be a stream of particles, or photons, with energies one million times less than the energies required to directly alter the chemistry of molecules.

Several hypotheses exist which may explain the interaction of RF-EMR with biology, including the male reproductive system and spermatozoa. The difficulty comes in identifying a physical mechanism by which RF radiation at low levels, 10^6 times lower than required to directly ionise molecules, can cause biochemical effects. Indeed, as summarised by Phillips et al. [9] "we are at the stage of having inconsistent results and no proven mechanism to explain RF-induced effects on DNA damage". This is in spite of the fact that there are structures in the body that are extremely sensitive to EMR. For example, a single photon of visible light can cause a change in rhodopsin causing a polarisation in a rod cell in the retina [1]. Different authors have surveyed the range of physical mechanisms which may result in RF-induced biochemical effects. Sheppard et al. [2] argue that possible mechanisms including coherence, resonance, signal averaging, field non-uniformity in inhomogeneous dielectric structures and nonlinear effects all produce effects far below the levels of the electric fields associated with normal bodily processes of wound healing and excitation of muscles and the nervous system. Challis [10] identified one possible non-thermal process—free radical generation in biomolecules with large hyperfine splittings and fast relaxation. In this process, if the EM frequency is resonant with the difference between energy levels in a molecule, then the energy from the external signal can be concentrated, leading to an amplified response at the driving frequency. However, the degree of amplification decreases with the losses in the system. Since most ions are associated with water, the energy dissipation by collisions of water molecules increases the loss of the system at RF frequencies and limits the degree of amplification which can be achieved in resonant systems.

Two other processes commonly used to justify RF effects on cells are the development of additional potentials across membranes which lead to a change in ion transport [11–15] and alteration of normal vibrations of molecular bonds, perhaps affecting proteins and the activities and interactions [16, 17] through alterations in protein conformation. An alteration of ion transport across cell membranes is possible, but only for fields of several hundreds of millivolts, much higher than the resting voltages across membranes, even of organelles such as the mitochondria. Modelling of transient voltages across organelle structures show that if the organelle membrane is thicker than the cell membrane and the organelle contains a high ion concentration, it is possible at RF frequencies for the voltage across the organelle membrane to be larger than that across the cell membrane [15]. Indeed, the change in voltage is of the order of ER [18], where E is external field and R is cell radius in the direction parallel to the field—changes may give rise to 100 mV changes in membrane potential. However, if a voltage is applied across a tissue, most of the voltage drop appears across the membrane at low frequencies. At gigahertz frequencies, the capacitance of the membrane effectively shorts out the membrane resistance at gigahertz frequencies, so the above effects are unlikely in that frequency range.

Cotgreave [19] argues that cellular proteins have different structures and would be expected to behave differently when exposed to RF. In addition, many proteins are in electrostatic contact, so RF-EMR may affect organisation of proteins within the cell. Studies have shown denaturation, aggregation and stability of proteins are

affected by RF exposure [20, 21]. Indeed, the efficiency of a protein as an enzyme depends on its conformation. Several side chains of amino acids in proteins are polar and so will behave differently in EM fields. Experiments at high irradiation levels (3 h of 1.95 GHz irradiation at 51 $W kg^{-1}$) have been shown to affect protein folding [22]. So a possible physical model suggests that resonances with charges and dipoles in each protein conformation change the barrier heights for intermediate processes in the refolding pathways [22]. However, dissipative effects discussed previously would reduce the effect of resonant excitation of dynamic excitations, making it difficult to imagine a mechanism at gigahertz frequencies.

In summary, even though several hypotheses exist for effects of non-ionising, mobile phone range EMR on biology, there is no clear proven mechanism. This is a key point to which much of the debate about the reported detrimental effects of EMR are based. Nonetheless, because there is no known mechanism at this point does not prove there is no effect. With this unknown and the many conflicting reports in the literature over the past decade, this field remains controversial.

1.4 Sperm Oxidative Stress and DNA Damage

From what is currently known about sperm cell damage and dysfunction, there is certainly scope for RF-EMR to contribute to this affliction. The three main types of cellular damage which can account for the adverse observations made are membrane, protein and DNA damage. The factors responsible for contributing to this damage in the male germ line can be grouped into several categories. Quality of spermatogenesis defines the susceptibility of cells to damage [23]. Biological factors including diet and stress as well as chemical and physical factors may all have direct and/or indirect effects on the "health" of spermatozoa. These categories are not necessarily mutually exclusive and combination between them is most probable. The complex, multifactorial nature of male infertility makes it a challenging area of research; however, cellular damage originating from environmental factors including the impacts of EMR on the male germ line must be understood if this issue is to be managed. The continued effort to determine the clinical significance of environmental-born EMR exposure on reproduction (as well as human health) and to gain an understanding of the mechanisms involved is urgently required as the exposure of RF-EMR to humans will only escalate.

There is growing evidence that environmental factors may be a key factor in male infertility [24]. Sperm concentration or microscopic analysis of sperm quality has dominated focus in the past, when studying xenobiotic or other environmental exposures; however, recently, more attention has been centred on the effects of sperm DNA integrity. There is a wealth of reports that link environmental exposures to sperm DNA damage and reduced fertility [25–27], with RF-EMR recently included. To put the risks in some perspective, evidence suggests the ability of DNA damaged cells to initiate fertilisation is somewhat compromised; however, it is not necessarily precluded from fertilising an oocyte. Indeed, DNA damage in the male germ line

has been linked with a range of adverse clinical outcomes, including poor fertility rates in vitro, but also subsequent disruptions of embryonic development, increased rates of miscarriage and poor childhood health [27–29] including cancer. Therefore, it is crucial that we understand the clinical implications and mechanisms of RF-EMR if indeed it plays any part in elevating DNA damage in the male germ line.

Regardless of the recent attention sperm DNA damage has enjoyed, the aetiology of this damage remains unknown. While the cellular mechanisms underpinning these effects have not been completely resolved, it has been suggested that oxidative stress derived from numerous possible pathways could be a key factor [17, 30]. There are several reports that also strongly link this mode of action to the adverse effects observed after EMR exposure [31–34], strengthening the potential role of this environmental factor in this affliction. Oxidative stress has also been implicated in a range of other infertility pathologies, including loss of sperm motility and vitality, which is also a common observation after RF-EMR exposure. Failure of sperm–oocyte fusion is also another result of oxidative stress [35]. We now know that human spermatozoa are capable of generating significant amounts of ROS [36, 37], both spontaneously and when exposed to xenobiotic or physical environmental factors [38]. Furthermore, these highly specialised cells are intrinsically sensitive to ROS and may enter a state of oxidative stress [39] with little hindrance. Therefore, the induction of ROS by environmental factors may account for the majority of cellular damage and dysfunction observed in human spermatozoa. Mobile phone radiation has the potential to elevate ROS leading to a state of oxidative stress which in turn impacts sperm motility vitality and DNA integrity; however, the fundamental mechanism by which ROS is generated is unknown. Two main ideas that lead to a state of oxidative stress in spermatozoa by RF-EMR are the disruption of the sperm mitochondria [40] and the activation of plasma membrane NADH oxidases [41].

Many studies have reported the presence of reactive oxygen species within human spermatozoa and its consequences for the gamete [42–44]. From our understanding of the cell biology of human spermatozoa, it is perhaps no surprise that these cells are then susceptible to oxidative stress and DNA damage. The minute volume of cytoplasm in these highly specialised cells limits the antioxidant capacity usually afforded to other cell types. Once oxidative stress is initiated, fertilisation is compromised through the loss of motility and ability to fuse to the oocyte. These outcomes arise due to lipid peroxidation of the abundant redox-sensitive polyunsaturated fatty acids in the plasma membrane. These peroxides also have the capacity to further propagate oxidative stress by a lipid peroxidation cascade [45]. Oxidative stress also leads to a range of protein damage, including alkylation (by lipid peroxides products) and oxidation [46]. The sperm chromatin does not escape the negative effects of this stress where DNA damage, ranging from oxidation, adduct formation and strand breaks result [26, 39, 47]. Further strengthening the central role of oxidative stress in sperm damage, several studies have shown that supplementary antioxidants have had some protective role against damage induced by EMR [48–50]. The area of antioxidant treatment for male infertility is a rapidly growing one; nevertheless, it is largely driven by empirical data. The work in this area confirms the major importance of ROS and oxidative stress in spermatozoa exposed to RF-EMR;

however, in a broader sense more understanding of oxidant–antioxidant interactions are needed here before rational clinical applications can be established. Similarly, care must be taken when aligning the adverse observations made after RF-EMR or mobile phone exposure to clinical relevance. The biophysics of EMR is exceedingly complex, evident by some examples of poor experimental design and lack of insight offered to date. There have been few experiments done on spermatozoa and the evidence for low-level genotoxic effects is weak [51] at this current time. More appropriately designed studies need to be conducted if any headway is to be made in this field.

1.5 Studies on EMR and Male Infertility

Within the last decade, there have been several well-designed and executed studies on the effects of mobile phone radiation on spermatozoa, covering three main areas, clinical/epidemiological, human (in vitro) and in vivo animal models. The association between RF-EMR and male infertility was initially suggested by an epidemiological study in 2001 where it was speculated that differences in fertility parameters from Chinese males in various professions may be due to EMR exposures [52]. More pertinent epidemiological data from a study in 2005 found negative correlations between mobile phone usage and various attributes of semen quality, particularly motility [53]. Studies on male reproduction in mouse or rat models showing that mobile phone radiation affected testicular histology, including decreased seminiferous tubule diameter, appeared in 1999 [54], but wasn't investigated further until 2003 [55] and 2004 [56] with the effects of low frequency EMR on murine models. There were some conflicting data within these two latter studies; however, both indicated that testis histology was altered in the exposed group. The former study also showed a decrease in testis size, while the latter did not. The latter study did, however, show one of the first instances of DNA damage (fragmentation within spermatogonia only) in the male germ line after mobile phone exposure.

This work was immediately followed by an experimental study involving exposure of male mice to RF-EMR via a wave guide. Exposures were at a frequency of 900 MHz at 90 mW kg^{-1} for 12 h day^{-1} for 7 days. This study revealed a significant impact on the integrity of the sperm mitochondrial genome, but no effect on the nuclear DNA or microscopic parameters [57], somewhat confirming the detrimental effects of RF-EMR on DNA integrity. Then further negative impacts of mobile phone usage on semen quality in human males were observed in a study that found significant reductions in sperm motility after exposure to a mobile phone after only 5 min exposure (talk mode) at a 10 cm range in vitro [58]; shortly after, a study also reported losses of motility and vitality after mobile phone exposure for 6 h day^{-1} for 18 weeks on rats held 1 cm from mobile phones measured at an SAR ranging from 0.9 to 1.8 W kg^{-1} in standby and talk modes, respectively. The spermatozoa of animals exposed to mobile phones also exhibited an up-regulation of CAD-1 and ICAM-1 RNA levels [59] (proteins associated with cell adhesion).

In three elegant studies, some concerning links with mobile phone use and male infertility were presented. Fejes et al. [53] found over 371 men that the duration of possession and use of mobile phones negatively correlated with semen quality. Wdowiak et al. [60] similarly showed that lower motility, vitality and poor morphology correlated with the frequency of mobile phone use over the 304 men studied. Agarwal et al. [61] confirmed these findings showing that sperm cell motility, vitality, normal morphology as well as sperm counts were defective in men who used mobile phones more frequently [61]. Also within this earlier time frame of research, various researchers proposed that the RF-EMR also exerts a range of negative genotoxic effects on different cell types including mature sperm cells [57, 62, 63]. These effects include chromatid exchange, aneuploidy and defective chromosome recombination. The "real world" clinical significance of this body of work was not confirmed; however, these studies were greatly important in providing a platform for continuing high-quality research and focusing effort into the potential harmful effects of mobile phone use. The work in this field up until 2007 is reviewed by Deepinder et al. [64].

From 2008 to the present (2011), several additional studies have shown adverse effects; in a clinical setting, in model systems and studies on human spermatozoa in vitro. Importantly, some studies have begun to shed light on how RF-EMR may drive the adverse effects observed by some researchers in the past. Nevertheless, this latest period of research has also generated several studies showing no detrimental effects of RF-EMR or mobile phone use.

Despite several groups reporting no effects of RF-EMR on male reproduction, other groups with very similar experimental design have reported a common finding of lower motility [65]. Some groups also showed that signs of oxidative stress in the exposed cohort, where increases in markers such as 8-OH-dG [31] and lipid peroxidation and the reduction of antioxidant levels [65], were present in human sperm in vitro, as well as in murine models. One study reported an increase in testicular sperm count in the rat after 1.95 GHz cellular phone radiation with a SAR or 0.08–0.4 $W\,kg^{-1}$ after 5 $h\,day^{-1}$ for 5 weeks [66]. A further study in the rat using exposures of 90 min day^{-1}, 5 days $week^{-1}$ for 12 weeks and using 848.5 MHz frequency at an SAR of 2.0 $W\,kg^{-1}$ found no changes compared to controls in testis histology or several other markers including lipid peroxidation, expression of p53, bcl2 or caspase [67], again exemplifying the range of conflicting data.

A detailed human (in vitro) study was completed by our research group in 2009 [31]. Our aims were to uncover a chain of cause and effect from RF-EMR radiation to the resulting motility and vitality loss and increased levels of DNA damage. We completed this by exposing purified human spermatozoa in a waveguide in a power-dependant fashion (0.4–27.5 $W\,kg^{-1}$ at 1.8 GHz). In step with increasing SAR, motility and vitality were significantly reduced, while the mitochondrial generation of reactive oxygen species and DNA fragmentation were significantly elevated ($P<0.001$). Furthermore, we also observed highly significant relationships between SAR, mitochondrial ROS levels, the oxidative DNA damage bio-marker, 8-OH-dG, and DNA fragmentation after RF-EMR exposure. This study has identified that RF-EMR may interact with the mitochondria in the sperm mid-piece, which then

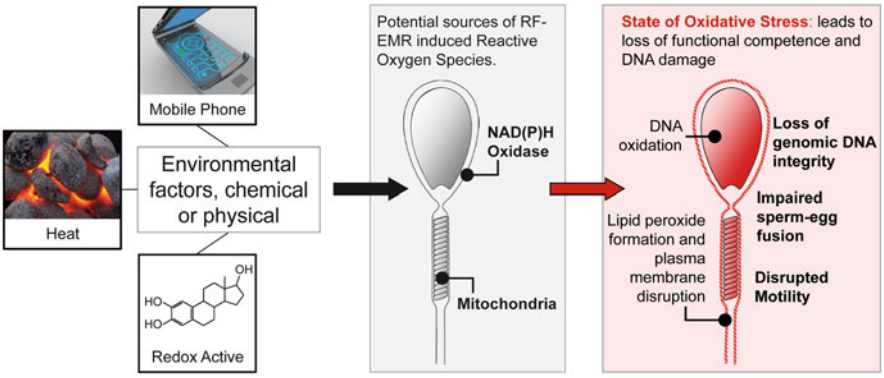

Fig. 1.2 Proposed oxidative stress model of the effects of mobile phone frequency radiation on the human spermatozoon. Evidence suggests that, like several other factors, radio frequency electromagnetic radiation (RF-EMR) can induce a non-thermal oxidative stress response in the gamete, possibly through interactions with NAD(P)H oxidases in the plasma membrane or by perturbation of mitochondria. This stress then leads to the range of adverse effects commonly observed under experimental conditions

leads to ROS generation and a state of oxidative stress. This stress manifests in a loss of motility and vitality (through lipid peroxidation) and the presence of oxidative DNA damage and DNA strand breaks in the nucleus. Our conclusion from this study was that RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and, ultimately DNA fragmentation. This study shed light on a potential mechanism by which "real-life" mobile phone radiation may affect biology. These findings confirm other published data that RF-EMR can indeed impact the male germ line and further that extensive mobile phone use may have negative impacts on males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring. This work was then supported by Agarwal et al. [68], showing that human sperm in vitro suffered the same oxidative stress by and increase in ROS levels and a decrease in the total antioxidant capacity of the cells after exposure to a mobile phone for only 1 h. Whereas we hypothesis that the source of ROS is the sperm mitochondria, Agarwal et al. suggest that NADH oxidase on the plasma membrane is responsible. Nonetheless, there is growing confidence that ROS has a key role to play in the potential mechanism of adverse effects of RF-EMR on the male germ line (Fig. 1.2). The relationship of oxidative stress to the detrimental effects of mobile phone use is also reviewed in this recent article [69].

Certainly in the last 6 years, several papers have implicated ROS as the mediator for RF-EMR-based cellular damage [65]. This hypothesis is not confined to the germ line, where similar studies in other cell types also seem to conform to this proposed pathway [70, 71]. Several studies have demonstrated that antioxidant