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YUNIBESITHI YA PRETORIA

THE EFFECT OF NON THERMAL 900 MHZ GSM IRRADIATION ON HUMAN SPERMATOZOA

by

NADIA FALZONE

Submitted in fulfilment of the requirements for the degree

PHILOSOPHIAE DOCTOR
(REPRODUCTIVE BIOLOGY)

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DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY
FACULTY OF HEALTH SCIENCES
UNIVERSITY OF PRETORIA

Supervisor: Dr. C. Huyser
Co-Supervisors: Profs. D. R. Franken and D. Leszczynski

April 2007

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To Paolo

Thank you for your support in reaching this goal.

"I can do everything through Him who gives me strength"

Phil 4: 13.

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DECLARATION BY CANDIDATE

" I hereby declare that the thesis submitted for the degree Philosophiae Doctor, at the University of Pretoria, is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references".

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Name in Block letters Signature

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ABSTRACT

Several studies have highlighted the possibility that radio-frequency electromagnetic fields (RF-EMF) used in mobile phone technology could influence DNA integrity of male germ cells as well as sperm motility. Current knowledge concerning the influence of RF-EMF on male germ cells is extremely limited. In the present study the hypothesis that 900 MHz GSM radiation could induce the activation of stress response in human spermatozoa was investigated.

Ejaculated, density purified, human spermatozoa from donors were exposed to 900 MHz GSM mobile phone radiation at specific absorption rate (SAR) levels of 2.0 and 5.7 W/kg and examined at various time points post exposure. Sperm motility and morphology were evaluated by computer-aided sperm analysis (CASA). The ability of RF-EMF exposed sperm to undergo the acrosome reaction was evaluated by flow cytometry. Sperm binding to the zona pellucida of human oocytes was determined by the hemi-zona (HZA) assay. Apoptotic markers, phosphatidylserine (PS) externalization, change in mitochondrial membrane potential ($\Delta\psi m$), reactive oxygen species (ROS) generation, caspase activation and DNA fragmentation were analysed using flow cytometry. Heat shock protein (Hsp) 27 and 70 expression and activity were analyzed using specific antibodies with flow cytometry and Western blot methods. Stress fibre stabilization (F-actin polymerization) was visualized using fluorescent dye labelled phalloidin.

No effect was seen on kinematic parameters assessed at SAR 2.0 W/kg, however straight line velocity (VSL) and beat cross frequency (BCF) were significantly altered after exposure at SAR 5.7 W/kg. Sperm shrinkage (decrease in surface area) was observed at both exposure levels. RF-EMF did not influence exposed spermatozoa's ability to undergo the acrosome reaction. A significant decrease in sperm-zona binding was observed at both exposure levels. RF radiation did not have an effect on any apoptotic markers. ROS generation increased significantly with an increase in SAR (5.7 W/kg). RF-EMF did not induce a stress response in exposed sperm (no activation of Hsp70 and 27 activity).

These results cannot be ascribed to heating, as the temperature did not increase by more than 0.2 - 0.3ºC during exposure. The decrease in sperm-zona binding is the result of an alternative non-stress inducible pathway. This study should be replicated at lower SAR levels that would simulate the radiation absorption from carrying the cell phone in a pocket close to the testes.

KEY WORDS

human spermatozoa, mobile phone radiation, sperm functionality, stress response, apoptosis.

TABLE OF CONTENTS

Page number

ACKNOWLEDGEMENTS x
STRUCTURE AND SCOPE OF THE THESIS xi
OBJECTIVES OF THE STUDY xiii
SUMMARY xiv
PUBLICATIONS xvi
LIST OF ABBREVIATIONS xviii
LIST OF FIGURES xx
LIST OF TABLES xxxi

SECTION A: LITERATURE SEARCH

CHAPTER 1: BIOLOGICAL EFFECTS OF MOBILE PHONE RADIATION

1.1 INTRODUCTION **2**
1.2 RADIO-FREQUENCY FIELDS FROM MOBILE PHONES – PHYSICS AND DOSIMETRY **3**
1.2.1 Modulation 4
1.2.1.1 FDMA (Frequency Division Multiple Access) 4
1.2.1.2 TDMA (Time Division Multiple Access) 4
1.2.1.3 CDMA (Code Division Multiple Access) 4
1.2.2 Cellular Phone Technologies 5
1.2.3 Output from Mobile Phones 5
1.2.4 RF radiation dose and measurement 6
(i) Micro-antennas: 7
(ii) Miniature thermal probes: 7
(iii) Numerical modelling: 8
1.2.5 Biological basis for limiting exposure to mobile phones 8

1.3 BIOPHYSICAL INTERACTION OF RF-EMF WITH BIOLOGICAL SYSTEMS 10
1.3.1 Biophysical Mechanisms 10
1.4 BIOLOGICAL EFFECTS OF RADIO-FREQUENCY FIELDS FROM MOBILE PHONES 13
1.4.1 Evidence of biological effects of RF-EMF fields 13
1.4.2 Health risks associated with genotoxic effects from RF-EMF exposure 14
1.4.3 Health risks associated with the induction of apoptosis as a result of RF-EMF exposure 15
1.4.4 Health risks associated with Gene/Protein expression as a result of RF-EMF exposure 24
1.4.5 Health risks associated with effects on male germ cells from RF-EMF exposure 31
1.5 STRESS RESPONSE AS A POSSIBLE PATHWAY FOR RF-EMF EXPOSURE 34
1.6 REFERENCES 35

CHAPTER 2: MOLECULAR BASIS FOR CELLULAR STRESS: OCCURRENCE IN HUMAN SPERMATOZOA AND IMPLICATIONS FOR MALE FERTILITY

2.1 INTRODUCTION - GENERAL ASPECTS OF CELLULAR STRESS 50
2.2 BIOCHEMICAL CHARACTERIZATION OF APOPTOSIS 51
2.2.1 The effectors of apoptosis 51
2.2.2 Extrinsic regulation of Apoptosis 52
2.2.3 Intrinsic regulation of Apoptosis 54
2.2.3.1 The role of mitochondria in apoptosis 55
2.2.3.2 The endoplasmic reticulum regulation of apoptosis 56

2.2.4 The role of kinases in the regulation of apoptotic signal transduction 57
2.3 THE HEAT SHOCK RESPONSE: HEAT SHOCK PROTEINS 57
2.3.1 Heat shock protein families 58
2.3.2 Induction and regulation of the heat shock response 58
2.3.3 Hsps as mediators of apoptosis 61
2.3.3.1 Hsp regulation of the intrinsic apoptotic pathway 62
2.3.3.2 Hsp regulation of the extrinsic apoptotic pathway 64
2.3.3.3 Hsp regulation of the MAPK activated apoptotic pathways 64
2.4 CELLULAR STRESS IN HUMAN SPERMATOZOA 66
2.4.1 Heat shock protein expression during spermatogenesis 67
2.4.2 Heat shock proteins: presence and functionality in spermatozoa 68
2.4.2.1 Role of heat shock proteins in fertilization 70
2.4.2.2 Apoptosis in spermatozoa 71
2.4.2.3 The Fas mediated pathway in sperm cell apoptosis 72
2.4.2.4 Apoptosis promotion by caspase activation in spermatozoa 72
2.4.2.5 Association of mitochondrial membrane potential with sperm apoptosis 73
2.4.2.6 Externalisation of phosphatedylserine as an indication of apoptosis in spermatozoa 74
2.4.2.7 DNA fragmentation in spermatozoa as a consequence of apoptosis 75
2.5 REFERENCES 77

SECTION B: THE EFFECT OF NON-THERMAL 900 MHz GSM MOBILE PHONE RADIATION ON HUMAN SPERMATOZOA

CHAPTER 3: CAPACITATION & OOCYTE BINDING

3.1 INTRODUCTION - MOLECULAR BASIS FOR CAPACITATION IN HUMAN SPERMATOZOA 99
3.1.1 Hyperactivated Motility 100
3.1.2 The Human Acrosome Reaction 101
3.1.2.1 Signal transduction between the zona pellucida and the spermatozoon 101
3.2 RF-EMF EXPOSURE SYSTEM AND EXPERIMENTAL PROTOCOL 105
3.2.1 Experimental set-up 105
3.2.2 Collection of semen 106
3.2.3 Density gradient purification and preparation of spermatozoa 107
3.3 CAPACITATION: ASSESSMENT OF THE HUMAN MOTILITY AND THE ACROSOME REACTION 108
3.3.1 Capacitation: Assessment of motility 108
3.3.1.1 Morphometric assessment 109
3.3.2 Capacitation: Assessment of the acrosomal status 110
3.3.2.1 Viability probes used in the acrosome reaction 111
(i) 7-Amino Actinomycin D 112
(ii) Propidium Iodide 112
(iii) Comparison between 7-AAD and PI as viability probes used in the acrosome reaction 113
3.3.2.2 Evaluation of the acrosome reaction by flowcytometry 113
(i) Visual assessment of the acrosome reaction 114
(ii) Induction of the acrosome reaction by calcium ionophore 115
3.4 HEMI-ZONA ASSAY 115
3.4.1 Zona Pellucida Binding - Mechanism: 115

3.4.2 Source and preparation of human zonae pellucidae 116
3.4.3 Sperm-oocyte interaction 117
3.5 STATISTICAL ANALYSIS 118
3.6 RESULTS 119
3.6.1 Motility and Morphology: Computer aided sperm analysis 119
3.6.1.1 Percentage progressive motility 119
3.6.1.2 Velocity parameters 120
3.6.1.3 Motion parameters 123
3.6.1.4 Morphometric analysis 123
3.6.2 Acrosome Reaction 126
3.6.2.1 Comparisson between 7-AAD and PI as viability probes for flow cytometry post fixation and permiabilisation 127
3.6.2.2 Visual assessment of the acrosome reaction 127
3.6.2.3 Assessment of the acrosome reaction by flowcytometry 128
(i) Evaluation of 7-AAD staining 128
(ii) Induction of the acrosome reaction 129
(iii) Evaluation of the acrosome reaction post RF-EMF 132
3.6.3 Sperm-oocyte interaction - Hemizona Assay 132
3.7 DISCUSSION 134
3.8 REFERENCES 139

CHAPTER 4: APOPTOSIS

4.1 INTRODUCTION 151
4.2 EXPERIMENTAL PROTOCOL 152
4.3 ASSESSMENT OF THE APOPTOTIC STATUS IN SPERMATOZOA 154
4.3.1 Phosphatedylserine externalisation determined by the Annexin V assay 154
4.3.1.1 Annexin V-FITC staining protocol 155
4.3.1.2 Annexin V blocking by recombinant Annexin V 155
4.3.1.3 Induction of apoptosis by staurosporine 156

4.3.2 Mitochondrial Membrane Potential 157
4.3.2.1 MitoTracker® Red CMXRos staining procedure: 157
4.3.2.2 Abolishment of $\Delta\psi m$ by Carbamoylcyanide m-chlorophenylhydrazone 157
4.3.3 Detection of superoxide 157
4.3.3.1 Detection of $O_2^{\cdot-}$ with hydroethidine 158
4.3.3.2 Determination of leukocyte contamination in processed spermatozoa 159
4.3.4 Caspase-3 Activation. 159
4.3.4.1 Active caspase-3 PE staining protocol 160
4.3.4.2 Caspase inhibition by CaspACE™ FITC-VAD-FMK 160
4.3.5 DNA Fragmentation 161
4.3.5.1 Fixation protocol for APO-Direct™ samples 161
4.3.5.2 APO-Direct™ staining protocol 162
4.3.5.3 Induction of DNA fragmentation by DNase 162
4.4 STATISTICAL ANALYSIS 163
4.5 RESULTS 163
4.5.1 Phosphatedylserine externalisation determined by the Annexin V assay 163
4.5.1.1 Annexin V blocking by recombinant Annexin V 163
4.5.1.2 Induction of apoptosis by staurosporine 164
4.5.1.3 The effect of 900 MHz GSM irradiation on phosphatedylserine externalisation 165
4.5.2 Mitochondrial Membrane Potential 170
4.5.3 Detection of ROS 172
4.5.3.1 Detection of leukocyte contamination in processed spermatozoa 173
4.5.3.2 Detection of $O_2^{\cdot-}$ with hydroethidine 173
4.5.4 Caspase Activation 175
4.5.4.1 Detection of active caspase-3 175
4.5.4.2 FITC-VAD-FMK detection of activated caspases 175
4.5.5 DNA Fragmentation 177
4.5.6 Correlation between apoptotic markers and ROS 179

4.6 DISCUSSION 182
4.7 REFERENCES 190

CHAPTER 5: HEAT SHOCK PROTEIN & STRESS FIBRE ACTIVATION

5.1 INTRODUCTION 199
5.2 EXPERIMENTAL PROTOCOL 201
5.3 DETERMINATION OF HEAT SHOCK PROTEIN EXPRESSION AND PHOSPHORYLATION AFTER 900MHz GSM RADIATION 203
5.3.1 Flow cytometric analysis of Hsp70 expression and Hsp27 expression and phosphorylation 203
5.3.2 Western Blot Analysis of Hsps 110, 90, 75, 70, 60, 40 and 27 expression 204
5.3.2.1 Protein extraction 204
5.3.2.2 Electrophoresis 205
5.3.2.3 Blotting 205
5.3.2.4 Western blot analysis of Hsp70 and Hsp27 in EA.hy926 cells 205
5.4 PHYSIOLOGICAL EFFECTS OF HSP ACTIVATION 206
5.4.1 Detection of stress fibres in human spermatozoa 206
5.4.2 Detection of stress fibers in MCF-7 cells 207
5.5 STATISTICAL ANALYSIS 207
5.6 RESULTS 207
5.6.1 Flow cytometric analysis and visualisation of Hsp70 and 27 expression and phosphorylation after 900MHz GSM 207
5.6.1.1 Flow cytometric analysis of Hsp27 expression and phosphorylation 207
5.6.1.2 Flow cytometric analysis of Hsp70 expression 208
5.6.2 Western blot analysis of Hsp110, 90, 70, 60, 40 and 27 expression after 900MHz GSM 211

5.6.2.1 Western blot analysis of Hsp27 211
5.6.2.2 Western blot analysis of Hsp70 211
5.6.2.3 Western blot analysis of Hsp110, 90, 75, 60 and 40 214
5.6.3 Detection of stress fibres 217
5.6.3.1 F-actin polymerisation in RF-EMF exposed human spermatozoa 217
5.6.3.2 F-actin polymerisation in RF-EMF exposed MCF-7 cells 219
5.7 DISCUSSION 220
5.8 REFERENCES 224

SECTION C: CONCLUSIONS

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS AND RECOMMENDATIONS 233
6.2 REFERENCES 240

SECTION D: ANNEXURES

ANNEXURE A: SAR SIMULATION RESULTS

A.1 VERTICAL 900MHZ EXPOSURE CHAMBER 244
A.1.1 Temperature control unit 244
A.1.2 Signal generator 246
A.1.3 GSM-Modulator 246
A.1.4 RF-power amplifier 247
A.1.5 Circulator and coaxial termination 247
A.1.6 RF-power meter and power sensor 247
A.2 SAR CALCULATION 247
A.2.1 Field and Temperature measurements 247
A.2.2 Electromagnetic simulations 248

A.2.3 Thermodynamic simulations 249
A.3 MAGNETIC FIELD MEASUREMENTS 252
A.4 REFERENCES 254

ANNEXURE B: MACROSCOPIC AND MICROSCOPIC SPERM PARAMETERS

B.1 EVALUATION OF SPERMATOZOA 255
B.1.1 Semen Parameters – Macroscopic and microscopic evaluation 256
B.1.1.1 Macroscopic analysis 256
(i) Appearance 256
(ii) Coagulation and liquefaction 256
(iii) Colour and odour 257
(iv) Viscosity 257
(v) Volume 258
(vi) pH 258
B.1.1.2 Microscopic analysis 259
(i) Sperm agglutination - SpermMar IgG test 259
(ii) Non-sperm cellular elements 260
(iii) Concentration - Neubauer haemocytometer 260
(iv) Motility - Differential count 261
(v) Morphology (Papanicolaou stain using Tygerberg strict criteria) 262
B.2 REFERENCES 264

ANNEXURE C: DONOR SPERM PARAMETERS - RESULTS

C.1 MACROSCOPIC SEMEN PARAMETERS 266
C.2 MICROSCOPIC SEMEN PARAMETERS 266
C.3 REFERENCES 269

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STRUCTURE AND SCOPE OF THE THESIS

SECTION A: REVIEW OF LITERATURE

Chapter 1 provides an overview of the operating principles of mobile phones, in addition the biological mechanism/s of radio-frequency electromagnetic fields (RF-EMF) are discussed, and scientific evidence is presented of *in-vitro* biological effects. Based on evidence provided that RF-EMF could interact with biological systems by inducing a stress response, a review on current literature elucidating the stress response phenomenon was conducted. **Chapter 2** therefore addresses the molecular basis for cellular stress in somatic cells. The occurrence of a stress pathway in human spermatozoa and the implications thereof for male fertility were considered.

SECTION B: THE EFFECT OF NON-THERMAL 900 MHz GSM MOBILE PHONE RADIATION ON HUMAN SPERMATOZOA

In **Chapters 3**, **4** and **5** the effect of 900 MHz mobile phone radiation on human spermatozoa was evaluated at two specific absorption rates (SAR) of 2.0 and 5.7 W/kg. RF-EMF exposure of spermatozoa was firstly assessed using sperm specific assays (sperm capacitation and sperm-zona binding – **Chapter 3**). Based on these findings the hypothesis that a stress pathway as a result of RF-EMF insult is operational in human spermatozoa was tested. The induction of apoptosis (**Chapter 4**) and activation of heat shock proteins (**Chapter 5**) after RF-EMF were evaluated.

SECTION C: CONCLUSIONS

In **Chapter 6** a summary of the findings of this study are given, conclusions are drawn, and recommendations for future research are provided.

SECTION D: ANNEXURES

Annexure A addresses the characterisation of the vertical RF chamber used in this research. Dosimetric analysis results are also summarised**.** Dosimetric evaluations were conducted at STUK, Radiation and Nuclear Safety Authority, Helsinki, Finland

Annexure B contains a summary of the macroscopic and microscopic sperm parameters used in the evaluation of the sperm donors.

Annexure C summarises the results of the macroscopic and microscopic sperm assessment of each donor.

OBJECTIVES OF THE STUDY

Human semen parameters could serve as valuable indicators of toxic and genotoxic effects of occupational and environmental factors. Furthermore, spermatozoa are terminally differentiated cells that are unable to repair DNA damage, which make sperm an extremely sensitive model to use in the investigation of the effect of environmental stressors such as RF-EMF.

To determine:

1. if and by which mechanism RF radiation from mobile phone emissions affect human spermatozoa and what implication these findings have on male fertility,
2. the effect of RF exposure on sperm capacitation and sperm-zona binding,
3. if a stress response is operational in spermatozoa as a result of RF-EMF, by investigating;
    i. the induction of apoptosis,
    ii. heat shock protein phosphorylation and expression,
4. the suitability of using human spermatozoa as a reproductive model to indicate effects/influences of mobile phone radiation.

SUMMARY

Several studies have highlighted the possibility that radio-frequency electromagnetic fields (RF-EMF) used in mobile phone technology could influence DNA integrity of male germ cells as well as sperm motility. Current knowledge concerning the influence of RF-EMF on male germ cells is extremely limited. The main objective of this research was directed at determining the effect of non-thermal 900 MHz GSM modulated RF-exposure on human sperm fecundity by assessing sperm specific functions, sperm functionality and induction of a stress response.

Ejaculated, density purified, human spermatozoa obtained from donors were exposed to RF-EMF at two specific absorption rate levels (SAR 2.0 and 5.7 W/kg) and examined at various time points post exposure. To determine the influence of RF exposure on sperm specific functions, sperm propensity for acrosomal exocytosis was assessed using a new technique developed to evaluate the acrosome reaction (AR) by flow cytometry. Sperm motility and morphometry were determined by computer aided sperm analysis (CASA) and sperm binding potential was evaluated by the hemi-zona assay (HZA). 900 MHz GSM exposure had no effect on the AR however some motility parameters (straight line velocity and beat cross frequency) were significantly altered. Sperm surface area and acrosomal region were also significantly reduced as a result of RF-EMF. The ability of RF-exposed sperm to bind the human oocyte evaluated with the HZA was significantly impeded.

Sperm functionality was assessed using flow cytometry; (i) the percentage of Annexin-V positive and, propidium iodide (PI) negative spermatozoa, (ii) the change in spermatozoa's mitochondrial membrane potential ($\Delta\psi m$), (iii) caspase activation, (iv) the percentage of TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling) positive spermatozoa and (v) generation of reactive oxygen species (ROS) were examined. No effect on any of the examined parameters after 900 MHz GSM exposure at either SAR level was noted. These results show that mobile phone radiation does not induce apoptosis.

The ability of 900 MHz GSM radiation to induce a stress response was evaluated by heat shock protein (Hsp) activation. Hsp27 and Hsp70 expression as well as activity were analyzed using specific antibodies with flow cytometry and Western Blot methods. Stress fibre stabilisation (F-actin polymerization) was visualized using fluorescent dye labelled phalloidin. RF-EMF had no effect on Hsp27 expression and phosphorylation nor Hsp70 expression as determined by flow cytometry and Western blot analysis. Visual assessment of stress fibre stabilization after RF exposure in sperm cells did not show any increased F-actin accumulation in the cells. RF-EMF exposure did not induce an Hsp27- or Hsp70-dependent stress response in human spermatozoa.

The effect of RF-EMF on sperm-zona binding and cell shrinkage observed at exposures of 2.0 and 5.7 W/kg, seems to be the result of an alternative non-Hsp dependent mechanism. Additional studies investigating the effect of RF-EMF on sperm-zona binding should be conducted, specifically exploring the ligand-receptor effector systems involved in sperm-zona binding. It is also suggested that electron microscopy be used to investigate conformal and structural changes as a result of RF-EMF. Considering recent reports noting an effect on sperm motility at lower SAR levels than that employed in the present study, the effect of RF-EMF on human spermatozoa motility, using the expanded analysis criteria set in this study, should be replicated at lower SAR levels that would simulate the radiation absorption from carrying the cell phone in a pocket close to the testes.

PUBLICATIONS

Submitted for publication:

1) Falzone, N., Huyser, C., Fourie, F le R., Leszczynski, D., Franken, D.R. *In vitro* effect of 900 MHz GSM radiation on mitochondrial membrane potential and motility of human spermatozoa. *Bioelectromagnetics*., submitted February 2007. (Approved for publication April 2007).
2) Falzone, N., Huyser, C., Becker, P., Fourie, F le R., Leszczynski, D., Franken, D.R. The effect of non-thermal 900 MHz GSM mobile phone radiation on the acrosome reaction, head morphometry and zona binding of human spermatozoa. *Hum Reprod*., submitted July 2007.

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1) Falzone, N., Huyser, C., Franken, D.R. Leszczynski, D. Lack of activation of Hsp27- and Hsp70-dependent stress response in human spermatozoa exposed to 900 MHz GSM radiation. *Bioelectromagnetics*., submittion September 2007.
2) Falzone, N., Huyser, C., Fourie, F le R., Cockeran, R., Leszczynski, D., Franken, D.R. *In vitro* exposure of human spermatozoa to 900 MHz GSM radiation: effect on apoptosis. *Rad Res*., submittion November 2007.
3) Falzone, N., Huyser, C., Cockeran, R., Becker, P., Franken, D.R. Significance of somatic apoptosis markers in human spermatozoa.

Conference papers:

1) Falzone, N., Huyser, C., Fourie, F le R., Franken, D.R., Leszczynski, D., 2005. Pilot study: Effects of 900 MHz GSM radiation on human sperm function. Annual Bioelectromagnetics Society meeting, Dublin, Ireland, June, 2005.
2) Falzone, N., Huyser, C., Fourie, F le R., Franken, D.R., Leszczynski, D., 2006. Effect of 900 mhz gsm radiation on apoptotic status and functionality of human spermatozoa. Annual Bioelectromagnetics Society meeting, Cancun, Mexico, June, 2006.

3) Falzone, N., Huyser, C., Fourie, F le R., Leszczynski, D., Franken, D.R., 2007. Effect of 900 MHz GSM radiation on human sperm functionality. IFFS conference, Durban, South Africa, May, 2007.

4) Falzone, N., Huyser, C., Fourie, F le R., Franken, D.R., Leszczynski, D., 2007. Lack of activation of Hsp27- and Hsp70-dependent stress response in human spermatozoa exposed to 900 MHz GSM radiation. Annual Bioelectromagnetics Society meeting, Kanazawa, Japan, June, 2007.

LIST OF ABBREVIATIONS

7-AAD - 7-amino-actinomycin D

? ψ_m - Change in the mitochondria membrane potential

aCp - Active caspase

AIF - Apoptosis inducing factor

ALH - Amplitude of lateral head displacement

AR - Acrosome reaction

ART - Artificial reproductive technologies

ATP - Adenosine triphosphate

BCF - Beat cross frequency

BSA - Bovine serum albumin

CASA - Computer aided sperm analysis

CDMA - Code Division Multiple Access

Cp - Caspase

CW - Continuous wave

DNA - Deoxyribonucleic acid

DISC - Death-inducing signalling complex

DPBS - Dulbecco's phosphate buffered saline

DMSO - Dimethyl sulfoxide

ELF - Extremely low frequencies

EM - Electromagnetic

EMF - Electromagnetic field

ER - Endoplasmic reticulum

ERK - Extracellular regulated kinases

FCM - Flow cytometry

FDMA - Frequency Division Multiple Access

FDTD - Finite Difference Time Domain

FITC - Fluorescein isothiocyanate

HSE - Heat shock element

HSF - Heat shock transcriptional factor

Hsc - Heat shock cognate protein

Hsp - Heat shock protein

HYPA - Hyper activated motility

HZA - Hemi zona assay

IVF - *In vitro* fertilization

kD - kilo Dalton

MAPK - Mitogen activated protein kinases

MMP - Mitochondrial membrane potential

MOMP - Mitochondrial outer membrane permiabilisation

PBS - Phosphate buffered saline

PCD - Programmed cell death

pCp - Pro-caspase

PBS - Phosphate buffered saline

PS - Phosphatidylserine

PI - Propidium iodide

PSA - *Pisum sativum agglutinin*

PTPC - Permeability pore complex

RF - Radio frequency

RF-EMF - Radio frequency electromagnetic field

ROS - Reactive oxygen species

SAR - Specific absorption rate

SD - Standard deviation

STR - Straightness

TDMA - Time Division Multiple Access

TUNEL - Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling

VAP - Average path velocity

VCL - Curvilinear velocity

VSL - Straight line velocity

ZP - Zona pellucida

LIST OF FIGURES

Page number

Chapter 1

Figure 1.1 Triage of the Electromagnetic spectrum indicating the position of mobile phone emissions (Adapted with permission from the EU Commission, Health and Electromagnetic fields, 2005). 3

Chapter 2

Figure 2.1 ***The stress response***: Exposure to damaging stimuli can trigger a cellular stress response resulting either in recovery or activation of the apoptotic program. Severe exposure could initiate cellular necrosis 50

Figure 2.2 ***Extrinsic Apoptotic Pathway*** (Danial and Korsmeyer, 2004 - with permission). Binding of the various trimeric ligands to death receptors, **(A)** TNF to TNRF-1, **(B)** FasL to Fas(APO-1/CD95) and **(C)** APO2L/TRAIL to DR4/5 triggers the downstream assembly of the DISC. 53

Figure 2.3 ***Intrinsic Apoptotic Pathway*** (Danial and Korsmeyer, 2004 – with permission). BH3 protein activation lead to Bax, Bak activation resulting either in the assembly of the apoptosome or ER induced apoptosis. 55

Figure 2.4. ***Major transcription factors leading to the induction of Hsp synthesis:*** **(1)** cytoplasmic complex of HSF-1 and Hsp90; **(2)** HSF-1 translocation to the nucleus; **(3)** intra-nuclear distribution of HSF-1; **(4)** nuclear complex of HSF-1 and Hsp90; **(5)** retro translocation of HSF-1 to the cytoplasm. (Adapted from Sõti *et al*., 2005). 59

Figure 2.5 ***Regulation of the intrinsic apoptotic pathway by Hsps*** (with permission - Beere 2005); **(A**) inhibiting BAX translocation to the mitochondrion, **(B)** suppression of AIF activity, **(C)** inhibiting the activation

of pro-caspase-9 by the apoptosome, **(D)** sequestering cytocrome-c release as a result of MOMP, **(E)** inhibiting Akt /BAD activation of MOMP. 63

Figure 2.6 ***Regulation of the extrinsic apoptotic pathway by Hsps*** (with permission – Beere, 2005). Hsps regulate both death receptor signalling (Hsp27 - JNK and Bax pathway; Hsp70 – Bid pathway) and cell survival pathways (Hsp90 - NF-κB pathway). 65

Chapter 3

Figure 3.1. The human fertilisation process (Adapted from "fertilization." Encyclopædia Britannica Online, 2007): **(1)** Chemoattraction, (2) specific recognition – loose association, **(3)** acrosomal exocytosis, **(4)** penetration – sperm-egg binding, **(5)** membrane fusion, **(6)** sperm invagination. 102

Figure 3.2. Front view and set-up of RF-EMF exposure chamber. Two glass Petri dishes are placed inside the chamber on top of a temperature regulated waterbed. The RF-EMF signal is fed into the chamber placed inside a CO_2 incubator via a monopole type feed post. 106

Figure 3.3 **RF-EMF exposure protocol**: Assessment of the acrosome reaction and motility characteristics post RF EMF and control exposure. 108

Figure 3.4 Metrix calculation of sperm morphometric parameters: **(A)** an abnormal spermatozoon, **(B)** a normal spermatozoon. 110

Figure 3.5 Flow cytometric assessment of 7-AAD and PI as viability probes after fixation and permeabilization of human spermatozoa. 113

Figure 3.6 The hemi-zona assay: **(1)** Oocytes from IVF were bisected and kept at room temperature while **(2)** spermatozoa were exposed for 1 hour to RF-EMF (SAR 2.0 and 5.7 W/kg). **(3)** After exposure hemi-zonae were added to 50 µl spermatozoa droplets (0.5 x 10^6 cells/ml). **(4)** Spermatozoa

and hemi-zonae were co-incubated for an additional 4 h before visual assessment of binding. 116

Figure 3.7 Velocity parameters comparing RF-EMF (dark-grey) exposed spermatozoa for SAR 2.0 W/kg (A) and 5.7 W/kg (B) with controls (light-grey) directly (T_1), 3 (T_2), and 24 (T_3) hours after a 1 hour 900 MHz GSM exposure (*$p < 0.05$). 122

Figure 3.8 Motion parameters comparing RF-EMF (dark grey) exposed spermatozoa for SAR 2.0 W/kg **(A)** and 5.7 W/kg **(B)** with controls (light grey) directly (T_1), 3 (T_2), and 24 (T_3) hours after a 1 hour 900 MHz GSM exposure (*$p<0.05$). 124

Figure 3.9 (A) Linear regression of log (mean fluorescence)$_{7\text{-AAD}}$ (R^2 = 0.93) and log (mean fluorescence)$_{PI}$ (R^2 = 0.83). **(B)** Linear regression of log (%gated)$_{7\text{-AAD}}$ fluorescence (R^2 = 0.93) and log (%gated)$_{PI}$ fluorescence (R^2 = 0.97). 127

Figure 3.10 Fluorescent staining (PSA-FITC) of the human acrosome reaction: (A) Acrosomal cap intact, **(B)** Patchy fluorescence, **(C)** Equatorial staining and **(D)** Acrosome reacted. 128

Figure 3.11 7-AAD Viability staining - Flow cytometric histograms showing overlay plots of unstained sperm (red-brown), sperm labelled with 10µl 7-AAD (blue) and sperm treated with 2 µl of Triton x-100 (green) for 15 min. prior to 7-AAD staining. 129

Figure 3.12 PSA-FITC staining - Flow cytometric histograms showing overlay plots of capacitated sperm (24 h) induced to undergo the acrosome reaction by pre-incubation with A23187 (positive control-red-brown) and sperm treated with DMSO (negative control for calcium ionophore

stimulation-green) prior to PSA-FITC staining. Fluorescence peaks note the acrosome reacted (AR) and acrosome intact (AI) sperm populations. 130

Figure 3.13 PSA-FITC staining - **(A)** Flow cytometric histograms showing overlay plots of capacitated sperm, sperm induced to undergo the acrosome reaction by pre-incubation with A23187 (positive control-red-brown); sperm incubated for 3 hours (orange) and sperm incubated for 24 hours (green) prior to PSA-FITC staining. Gating shows acrosome reacted (AR) and acrosome intact (AI) spermatozoa. **(B)** Flowcytometric dot plots and projections showing gating of PSA-FITC staining in 7-AAD$^-$ (live cells) only, region F denotes acrosome intact spermatozoa and region H acrosome reacted sperm. 131

Figure 3.14 Comparison between RF-EMF and control sperm assessed by FCM directly, 3 - and 24 - hours after a 1 hour 900 MHz GSM exposure (SAR 2.0 W/kg) **(A)** % 7-AAD$^+$ staining spermatozoa, **(B)** % acrosome intact live cells, **(C)** % acrosome reacted live. 132

Figure 3.15 Box and whiskers plot (showing medians) of number of sperm binding non-fertilized metaphase II oocytes (5 oocytes per donor, n = 10) after an hour exposure to 900 MHz GSM radiation. RF-EMF exposure caused a significant reduction in sperm bound to the hemi-zonae compared to controls (*p = 0.02). 133

Figure 3.16 Correlation of morphology and number of spermatozoa binding the hemi-zona after an hour exposure to 900 MHz GSM radiation. Number of control spermatozoa is also shown for comparison. 134

Chapter 4

Figure 4.1 RF-EMF exposure protocol: Assessment of the apoptotic status post RF-EMF and control exposure. 153

Figure 4.2 Flow cytometric histograms of total Annexin V before (red) and after (blue) addition of recombinant Annexin V- demonstrating the specificity of Annexin V staining. 164

Figure 4.3 The percentage PI, Annexin V and Annexin V^+/PI^- sperm after a 2 hour exposure to 1 µM, 5 µM and 10 µM staurosporine (STS), each datum represents the mean ± SD of three determinations. 165

Figure 4.4 Dot plot and fluorescence histograms of **(A)** Annexin V and **(B)** PI staining of human spermatozoa. 166

Figure 4.5 In $\mathbf{A_1}$ (2.0 W/kg) and $\mathbf{B_1}$ (5.7 W/kg) the total percentage Annexin V staining is noted as a function of time. $\mathbf{A_2}$ (2.0 W/kg) and $\mathbf{B_2}$ (5.7 W/kg) depict the total percentage of non-viable cells (PI^+) as a function of time. $\mathbf{A_3}$ (2.0 W/kg) and $\mathbf{B_3}$ (5.7 W/kg) show the total percentage Annexin V^+ viable cells (PI^-) as a function of time. 167

Figure 4.6 In Figures $\mathbf{A_1}$ (2.0 W/kg) and $\mathbf{B_1}$ (5.7 W/kg) the total number of apoptotic necrotic (Annexin V^+ PI^+) staining is noted as a function of time. $\mathbf{A_2}$ (2.0 W/kg) and $\mathbf{B_2}$ (5.7 W/kg) depict the total percentage of dead cells (Annexin V^- PI^+) as a function of time. 168

Figure 4.7 Cytofluorometric analysis of the depolarisation of the mitochondrial membrane potential showing a frequency histogram of processed spermatozoa (blue) stained with, MitoTracker® Red CMXRos before treatment (green) with the mitochondrial membrane potential abolisher mClCCP (red-brown). 170

Figure 4.8 The percentage of MitoTracker® Red CMXRos (150 nM) staining in RF-EMF exposed sperm at a SAR of (A) 2.0 W/kg and (B) 5.7 W/kg compared to control samples determined as a function of time 171

Figure 4.9 Cytofluorometric dot plot showing **(C)** gated sperm population, **(D)** lymphocytes, **(E)** monocytes and **(F)** granulocytes in **(A)** a processed sperm population and **(B)** a processed sperm population spiked with 2 x 10^6 white blood cells. 172

Figure 4.10 Cytofluorometric analysis of O_2^- production in human spermatozoa using hydroethidine (HE). Frequency histogram notes the increased production of ethidium (E^+) due to superoxide oxidation in processed spermatozoa incubated in the presence of 2 x 10^6/ml white blood cells (blue) compared to normal sperm (green). 173

Figure 4.11 The percentage of hydroethidine staining in sperm exposed to RF-EMF at **(A)** SAR 2.0 W/kg and **(B)** SAR 5.7 W/kg compared to control samples determined directly after exposure (T_1), 3 hours after exposure (T_2) and 24 hours after exposure (T_3). 174

Figure 4.12 The percentage of cells staining positive for activated caspase-3 determined for control and RF-EMF (SAR 2.0 W/kg) exposed cells detected at T_1 (directly after exposure), T_2 (3 hours after exposure) and T_3 (24 hours after exposure). 175

Figure 4.13. Cytofluorometric analysis of the frequency histogram of processed spermatozoa (red-brown) stained with, FITC-VAD-FMK directly after RF-EMF exposure (blue), 2 hours after exposure (green) and 24 hours after exposure (orange). 176

Figure 4.14 The percentage of cells staining positive for FITC-VAD-FMK determined for control and RF-EMF **(A)** SAR 2.0 W/kg and **(B**) SAR 5.7 W/kg exposed cells detected at T_1 (directly after exposure), T_2 (3 hours after exposure) and T_3 (24 hours after exposure). 177

Figure 4.15. Cytofluorometric analysis of the frequency histogram of processed unstained spermatozoa (red-brown), before TUNEL staining (green) and induction of DNA damage using DNAse (blue). 178

Figure 4.16 The percentage of cells staining positive for TUNEL determined for control and RF-EMF **(A)** SAR 2.0 W/kg and **(B)** SAR 5.7 W/kg exposed cells detected at T_1 (directly after exposure), T_2 (3 hours after exposure) and T_3 (24 hours after exposure). 179

Chapter 5

Figure 5.1 RF-EMF exposure protocol. Assessment of cellular stress in human spermatozoa post RF-EMF exposure and heat shock at 43ºC. 202

Figure 5.2 (**A)** Flow cytometric analysis of Hsp27 phosphorylation (dark grey) detected by anti-Hsp27P directly (T_1), 3 (T_2) and 24 hours (T_3) after an hour RF-EMF exposure at SAR 2.0 W/kg (n = 12). Baseline Hsp27 expression (white) as well as Hsp27 phosphorylation after a 1 hour heat shock at 43°C (black and white) are given at time 1. Control (light grey) samples were maintained at 37°C during the exposures. **(B)** Detection of Hsp27P expression by immunofluorescence staining directly after exposure (T_1), in (I) control and (II) RF-exposed sperm, Hsp27P fluorescence was mainly located in the neck area of the sperm. 209

Figure 5.3 (**A**) Flow cytometric analysis of Hsp70 expression detected directly (T_1), 3 (T_2) and 24 hours (T_3) after an 1 hour RF-EMF exposure at SAR 2.0 W/kg or heat shock at 43°C (n = 12). Control samples were maintained at 37°C during the exposures. **(B)** Detection of Hsp70 immunofluorescence staining directly after exposure (T_1), in (I) control and (II) RF-exposed sperm, Hsp70 fluorescence was mainly located in the neck area of the sperm. 210

Figure 5.4 Western Blot analysis of Hsp27: (A) Autoradiogram of SDS-PAGE resolved proteins for donor 2 (I), donor 12 (II) and EA.hy926 cells (III). The position of the bands correspond to heat shock proteins of Mr 27kDa, specific for Hsp27. **(B)** Densitometric analysis of Hsp27 phosphorylation status in spermatozoa directly after a 1 hour exposure to RF-EMF at SAR 2.0 W/kg or 43°C. Control samples were maintained at 37°C for the duration of the exposure. As a control Hsp27 expression in EA.hy926 cells (III) after heat shock at 43°C is also noted. 212

Figure 5.5 Western blot analysis of Hsp70: (A) Autoradiogram of SDS-PAGE resolved proteins for donor 2 (D_2- I), donor 12 (D_{12} - II) and EA.hy926 cells (III). The position of the bands correspond to heat shock proteins of M_r 70kDa, specific for Hsp70. **(B)** Densitometric analysis of Hsp70 expression in spermatozoa directly after an hour exposure to RF-EMF at SAR 2 W/kg or 43°C. Control samples were maintained at 37°C for the duration of the exposure. As a control Hsp70 expression in EA.hy926 cells (III) after heat shock at 43°C is also noted. 213

Figure 5.6 Western blot analysis of Hsps 110, 75 and 60: (A) Autoradiogram of SDS-PAGE resolved proteins for donor 2 (D2- I), and donor 12 (D12 - II). The position of the bands corresponds to heat shock proteins of Mr 110kDa, 75kDa and 60kDa specific for Hsp110, Hsp75 and Hsp60 respectively. **(B)** Densitometric analysis of Hsp110, Hsp75 and Hsp60 expression in spermatozoa directly after an hour exposure to RF-EMF at SAR 2 W/kg or 43°C. Control samples were maintained at 37°C for the duration of the exposure. 215

Figure 5.7 Western blot analysis of Hsps 90 and 40: (A) Autoradiogram of SDS-PAGE resolved proteins for donor 2 (D_2- I), and donor 12 (D_{12} - II). The position of the bands corresponds to heat shock proteins of M_r 90kDa, and 40kDa specific for Hsps 90 and 40. **(B)** Densitometric analysis of Hsps 90 and 40 expression in spermatozoa directly after a 1 hour exposure to RF-

EMF at SAR 2 W/kg or 43°C. Control samples were maintained at 37°C for the duration of the exposure. 216

Figure 5.8 Immunolocalization of F-actin. (A) AlexaFluor-labelled phalloidin stained spermatozoa predominantly in the acrosome (white arrow), post-acrosomal area (yellow arrow), as well as neck and principal tail-piece areas (red arrow). **(B)** Cellular response of spermatozoa exposed to RF-EMF, control cells were maintained at 37°C for the duration of the exposure. **(C)** Typical staining of AlexaFluor-labelled phalloidin 218

Chapter 6

Figure 6.1 The processes of homologous sperm-zona binding and penetration. 237

Annexure A

Figure A.1 (A) Front view of vertical exposure chamber showing the position of the petri-dishes with the hatch open. (B) Side view of the chamber with the water pump housing (I), water is circulated from the waterbed below the perti-dishes to the aluminium cooling plate (II) behind the chamber (thermal conducting tape attached to back of plate). The cooling plate is placed in contact with the inside wall of the incubator to allow for thermal exchange. (C) Signal generator (III), Amplifier (IV), GSM modulator (V), Coaxial terminator (VI), Power meter and sensor (VII), DC power supply (VIII). 245

Figure A.2 Schematic drawing of the irradiation chamber. The petri-dishes (diameter 54 mm) are placed in special ‘cups’ moulded into the epoxy laminate above the cooling water so that the medium (3 ml) is at same level than the cooling water. The water is covered with a 0.8 mm thick epoxy laminate. 246

Figure A.3 Schematic diagram of temperature and electric field measurement set-up. In upper part is shown the RF-power generation and

measurement part and below that the electric field and temperature measurement parts. 248

Figure A.4 Typical SAR measurements based on temperature increase using a Vitek type sensor. The SAR is evaluated from linearized temperature increase (dT) between the 1 to 8 seconds time (dt) after power on. 250

Figure A.5 Measured and simulated E-field in chamber. Measurements (blue diamonds) were made in air using the SPEAG ET3DV6 probe. 251

Figure A.6 Simulated (XFDTD) relative SAR distribution shown in one petri-dish placed in the vertical chamber. The scale is given from 0 mW/g (black) to 15 mW/g (yellow). The inner diameter of Petri dish is 50 mm. 251

Figure A.7 Temperature increase measurements. The thermistor was either placed in the middle of the field or in contact with the Petri dish wall. 252

Figure A.8 Magnetometer measurement set-up. Placement of the magnetometer inside the CO_2 incubator in positions 2 and 3 closely resembled that of the earth's magnetic field. 253

Annexure B

Figure B.1 Diagram illustrating different semen assessment parameters, functional tests and bioassays in the evaluation of human spermatozoa. (Adapted with permission from Oehninger *et al*., 1991). 255

Annexure C

Figure C.1 Sperm parameters determined for each donor. Each end point represents the mean and standard deviation (SD) of at least 5 replicates. In all graphs WHO reference values are noted with dotted lines. **(A)** Total volume; **(B)** pH; **(C1)** Concentration pre-processing; **(C2)** Concentration post-processing; **(D1)** Morphology pre-processing; **(D2**) Morphology post-processing and **(E)** Forwards motility, of each semen sample. 267

LIST OF TABLES

Chapter 1

Table 1.1 Summary of biophysical mechanisms of RF-EMF. 11

Table 1.2 Summary of IEGMP (2000) report on biological effects of mobile phone exposure in cellular systems. 16

Table 1.3A Genotoxic effects from mobile phone exposure 17

Table 1.3B Effect of mobile phone exposure on Apoptosis. 22

Table 1.3C Effect of mobile phone exposure on gene and protein expression. 26

Table 1.3D Effect of mobile phone exposure on male germ cells ..... .32

Chapter 2

Table 2.1 Heat shock protein families, their expression and functions…….. 90

Table 2.2 Conditions that lead to the activation of heat shock proteins (from Prohászka and Füst, 2004)…….. 61

Chapter 3

Table 3.1 Percentage progressive motility after **(A)** RF-EMF (2.0 W/kg) and **(B)** RF-EMF (5.7 W/kg) exposure in exposed and control spermatozoa determined directly (T_1), 2 h (T_2) and 24 h (T_3) after exposure. 120

Table 3.2 Linear regression results of percentage rapid-, slow-, non-progressive and immotile spermatozoa after RF-EMF (2.0 and 5.7 W/kg)

exposure compared to control spermatozoa. Spermatozoa exposed at 2.0 W/kg are also compared to sperm exposed at 5.7 W/kg. 120

Table 3.3 Summary of morphometric results of sperm exposed to **(A)** 2.0 W/kg and **(B)** 5.7 W/kg compared to control values. 125

Table 3.4 Summary of p values of the linear regression analysis results of sperm morphology determined by CASA. RF exposed sperm were firstly compared to controls, morphometric parameters were compared directly (T_1) and 2 hours (T_2) after exposure and the number of normal and abnormal forms were compared. 125

Table 3.5 Linear regression analysis comparing sperm morphometric parameters of sperm exposed at 2.0 W/kg to sperm exposed at 5.7 W/kg are summarised as p values. 126

Chapter 4

Table 4.1 Summary of paired t-test results comparing viability (PI-fluorescence) and PS externalisation (Annexin V fluorescence) of RF-exposed spermatozoa at SAR 2.0 W/kg with SAR 5.7 W/kg. 169

Table 4.2 Comparison between Annexin V^+ PI^-, Annexin V^+ PI^+ and Annexin V^- PI^+ staining cells as a progression of time. 169

Table 4.3 A Correlations between apoptotic biomarkers of RF-EMF (SAR 2.0 W/kg) exposed spermatozoa evaluated directly (T_1), 3 hours (T_2) and 24 hours (T_3) after exposure. 180

Table 4.3 B Correlations between apoptotic biomarkers of RF-EMF (SAR 5.7 W/kg) exposed spermatozoa evaluated directly (T_1), 3 hours (T_2) and 24 hours (T_3) after exposure. 181

Chapter 6

Table 6.1 Summary of effects of RF-EMF on sperm specific and functional assays in highly motile human spermatozoa. 234

Annexure A

Table A.1 SAR distribution results used in experimentation. 252

Annexure C

Table C.1 Average sperm parameters: Volume, pH and motility averages ± SD of the semen sample is noted. Concentration and morphology averages ± SD are given both pre- and post- density gradient purification. 268