SECTION D

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ANNEXURE A

SAR SIMULATION RESULTS

A.1 VERTICAL 900MHZ EXPOSURE CHAMBER

The irradiation system consists of a rectangular wave-guide placed vertically inside a cell culture incubator (Figure A.1). The chamber is made from aluminium and is fitted on top of a waterbed that has place for two petri dishes (outer diameter of 55 mm). Water is circulated via a pump (Figure A.1 [B-I]) from the waterbed under the petri dishes to the cooling tower behind the chamber (Figure A.1 [B-II]).

The RF-EMF signal is generated with an EDSG-1240 signal generator (Figure A.1 [C-III]) and modulated (Figure A.1 [C-V]) to match the GSM signal modulation scheme. The generated signal is amplified with RF Power Labs R720F amplifier (Figure A.1 [C-IV]) and conducted through a circulator (Mica 7Y213, Figure A.1 [C-VI]) to a power meter (Coaxial Dynamics 81000-A, Figure A.1 [C-VII]). The signal from the power meter is fed to the wave-guide chamber via a monopole type feed post. The waveguide is short-circuited at both ends, thus generating a resonant field with two electric field maximums. The power is absorbed mainly by the circulating water and cell culture medium.

A.1.1 Temperature control unit

The temperature control unit is attached to the chamber. It consists of a water pump, a waterbed (underneath the petri dishes), a water-cooling plate (behind the chamber), and a DC-power supply unit. The power supply unit provides power to the pump, which in turn circulates water from the waterbed to the water-cooling plate. The cooling plate is in direct contact with the inner wall of the incubator to provide maximal thermal exchange. Water from the cooling plate is then pumped back to the waterbed under the petri dishes.

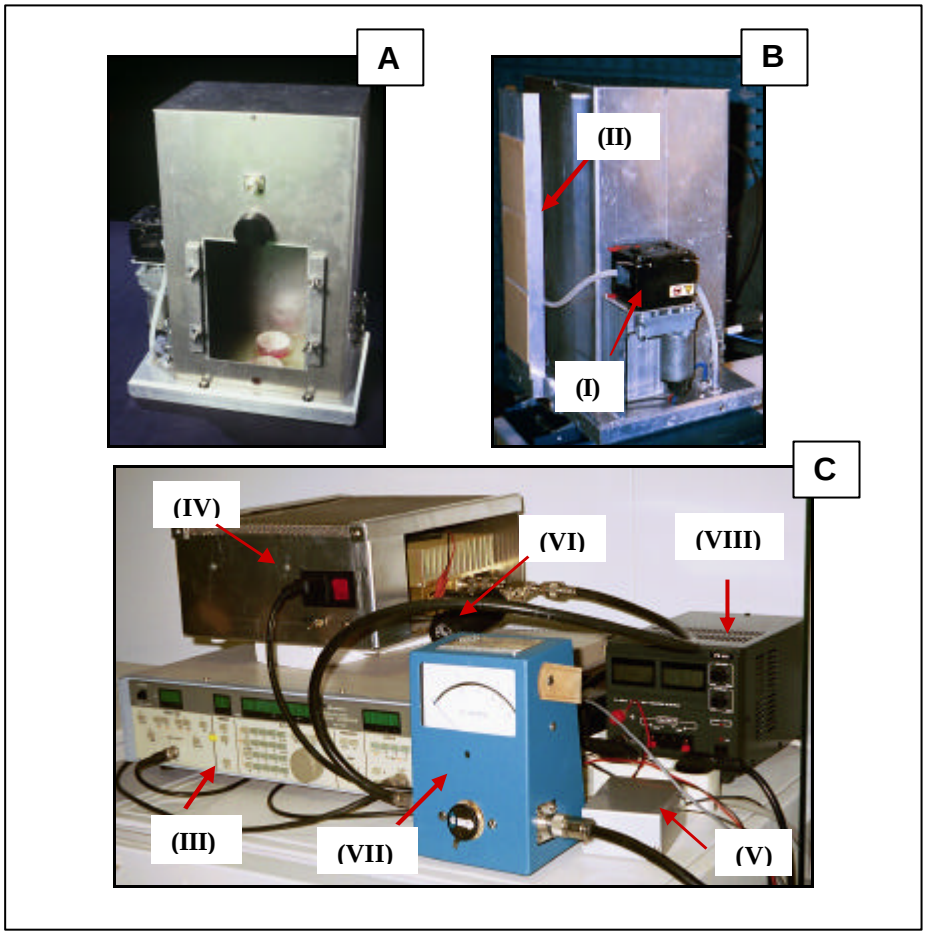

Figure A.1 **(A)** Front view of vertical exposure chamber showing the position of the petri dishes with the hatch open. **(B)** Side view of the chamber with the water pump housing (I), water is circulated from the waterbed below the petri dishes to the aluminium cooling plate (II) behind the chamber (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)** Peripheral equipment include; Signal generator (III), Amplifier (IV), GSM modulator (V), Coaxial terminator (VI), Power meter and sensor (VII), DC power supply (VIII).

The waterbed is covered with a thin layer (8 mm) of epoxy laminate. The petri dishes are positioned inside the chamber into two 'cups' (seen in Figure A.2). The height of the waterbed was designed (with APLAC simulations) so that it effectively is a quarter wavelength long, thus giving maximal E-field coupling to the cells. The temperature rise in the cells, caused by the absorbed RF-radiation, is compensated

for by the circulating water underneath the petri dishes. The local energy absorbed by the cells is transferred away in much the same way as heat is dissipated in the human body by blood circulation.

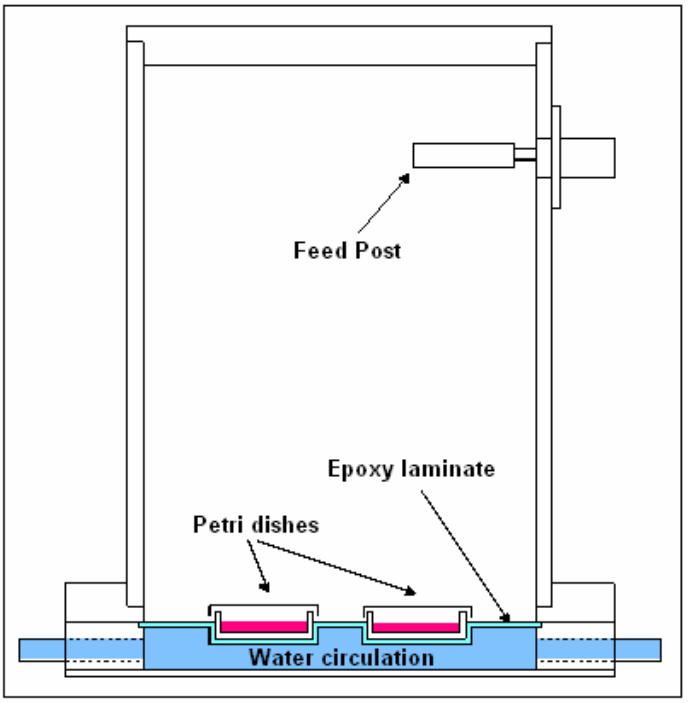

Figure A.2 Schematic drawing of the irradiation chamber. The petri dishes (diameter 55 mm) are placed in special 'cups' moulded into the epoxy laminate above the cooling water so that the medium (3 ml) is at same level than the cooling water. The water is covered with a 0.8 mm thick epoxy laminate.

A.1.2 Signal generator

The signal generator (ED-Laboratory SG1240) is used to generate the carrier signal. The frequency fed to the irradiation chamber depends on the loading of the chamber and is set so that the minimum amount of power is reflected back from chamber.

A.1.3 GSM-Modulator

The modulator in turn is connected to the signal generator. The GSM-modulator (STUK-KKS1) is used to modulate the carrier signal so that the output of the generator will match the GSM 900 MHz pulse sequence. A pulse repetition

frequency of 217 Hz (GSM modulation scheme: 0.577 ms pulse duration repeated every 4.615 ms) was used. The modulated signal is on for 1/8 of the time.

A.1.4 RF-power amplifier

The modulated signal (from the GSM modulator) generated by the signal generator is amplified by the RF-power amplifier (RF Power Labs R720F).

A.1.5 Circulator and coaxial termination

The circulator (MICA 7Y213) is used to protect the amplifier from overheating if too much power is reflected back from the chamber in the case of malfunctioning or a loose cable connection. The circulator guides the reflected power from the chamber to the Coaxial termination (50R50WCW) which turns the power to heat.

A.1.6 RF-power meter and power sensor

The RF-power meter (Coaxial Dynamics 81000-A,) is used to monitor the power fed into the chamber and power reflected back from the chamber. The power meter's sensor (820E875) shows the direction of the measured power with the arrow in front of the sensor. This means that the power fed to the chamber is measured when the arrow points to the cable connected to the chamber. When turning the sensor 180 degrees it measures the reflected power. The power meter is equipped with 2.5 W power sensor (820E875) which can measure both the forward power fed to the chamber and reflected power from chamber to the coaxial termination (50R50WCW).

A.2 SAR CALCULATION

The dosimetric evaluation of the irradiation chamber was performed at STUK (Radiation and Nuclear Safety Authority, Helsinki, Finland) and could be divided into three parts, namely, measurements, electromagnetic simulations, and thermodynamic simulations.

A.2.1 Field and Temperature measurements

SAR can be determined either by E-field measurements or by temperature rise measurements (Figure A.3) when the specific heat constant of the liquid is known. In

experiments, a total volume of 3 ml of medium per petri dish was used. E-field probes that can accurately measure electric fields in such a small volume were not commercially available at the time of measurement, thus E-field measurements were made in the air above the medium.

E-Field measurements: The E-field was measured in the air above the petri dishes. Measurement points were in the centreline of the chamber. The E-field was measured with a calibrated miniature E-field probe (Narda 8021B). E-field measurements were done at room temperature (22.5°C) and the corresponding computer simulations were done with permittivity values determined at the same temperature (measurements were also repeated with the SPEAG ET3DV6 probe and DASY4 software). Results are shown in Figure A.4.

Temperature Measurements: The absolute SAR-determination was based on temperature rise measurements done with a small Vitek-type temperature sensor. The sensor was calibrated with a calorimeter against a calibrated glass-capillar thermometer. During measurements, the sensor was placed at the centre of the petri dish at a height 1 mm from the bottom. The measurement set-up is shown in Figure A.3. Long-term temperature rise measurements were also done to evaluate the change in temperature of the cultivating medium during the exposure. Results are shown in Figure A.5.

A.2.2 Electromagnetic simulations

Computer simulations were done to evaluate the SAR-distribution inside the cultivating liquid, E-field distribution in cultivating liquid, and air and return loss of the chamber. The simulations were made using a finite difference time domain method (FDTD), with commercial SEMCAD 1.8 software (SPEAG, Switzerland). The simulation grid was graded and consisted of over 24 million cells. A total of 440 000 voxels were used to simulate the medium with the largest grid size in the culture medium being 0.1 x 0.1 x 0.1 mm. Results are shown in Figures A.6.

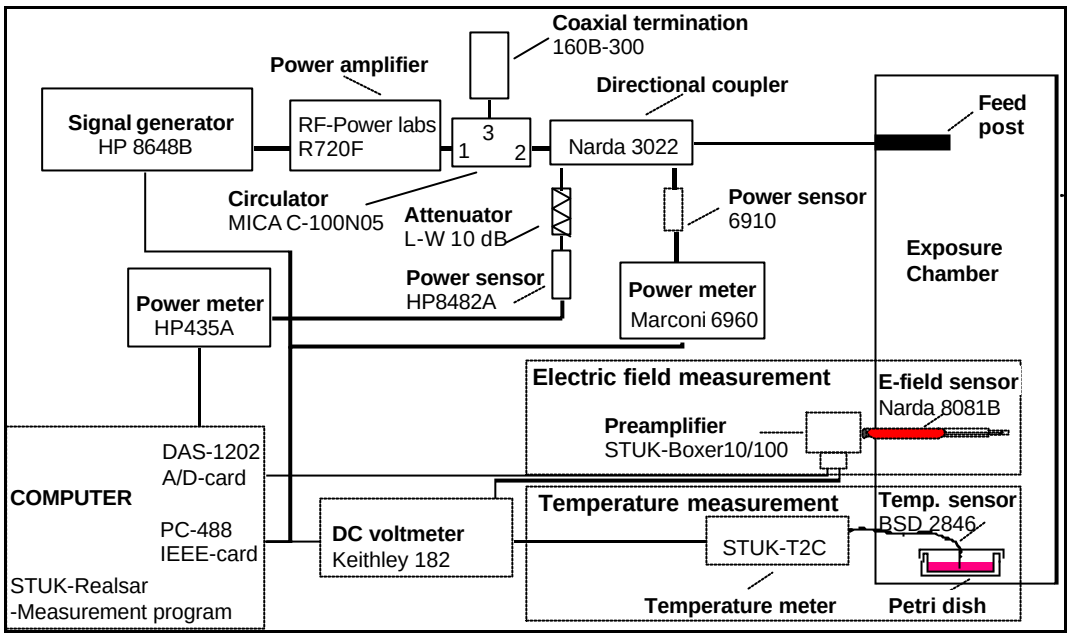

Figure A.3 Schematic diagram of temperature and electric field measurement set-up. In upper part is shown the RF-power generation and measurement part and below that, the electric field and temperature measurement parts.

A.2.3 Thermodynamic simulations

In order to investigate the accuracy of the thermal SAR measurements and to evaluate the thermal dose given to the cells, the temperature increase in the medium during irradiations was calculated using a thermal model. It was based on the conventional 3D heat transfer equation for non-homogeneous isotropic medium:

$$r\, c_p \frac{\partial T}{\partial t} = \nabla K \nabla T + r\, SAR \quad \text{, where}$$

ρ = 1000 m^3 kg^{-1} is the density, c_p = 4180 J kg^{-1} $^oC^{-1}$ is the specific heat capacity,

T is temperature (^oC), t is time (s), K= 0.56 W m^{-1} K^{-1} s^{-1} is the thermal conductivity.

Temperature measurements were performed with a thermistor sensor. The simulation and temperature measurement results are shown in Figure A7. There was a close agreement between the measured results compared to the computed results, which

indicates that the thermal model realistically describes the thermal properties of the system and increases confidence on thermal SAR assessments.

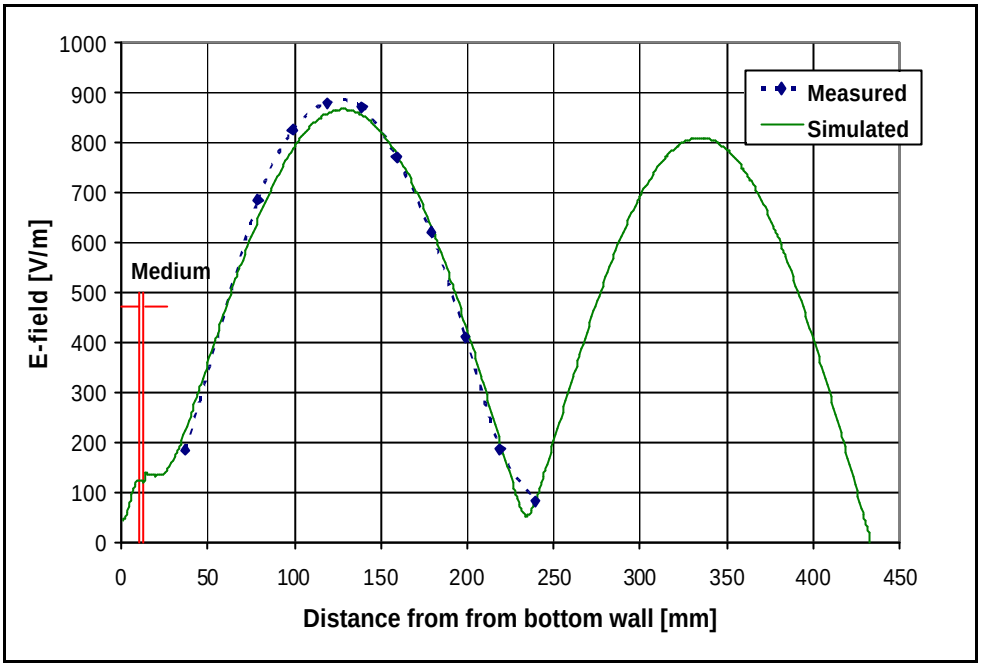

Figure A.4 Measured and simulated E-field in chamber. Measurements (blue diamonds) were made in air using the SPEAG ET3DV6 probe.

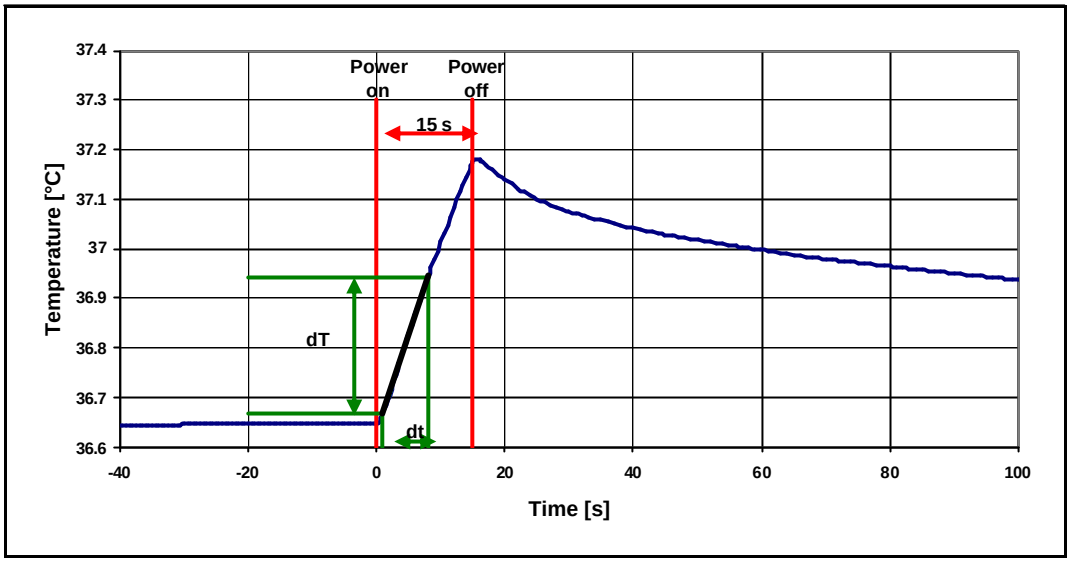

Figure A.5 Typical SAR measurements based on temperature increase using a Vitek type sensor. The SAR is evaluated from linearised temperature increase (dT) between the 1 to 8 seconds time (dt) after power on.

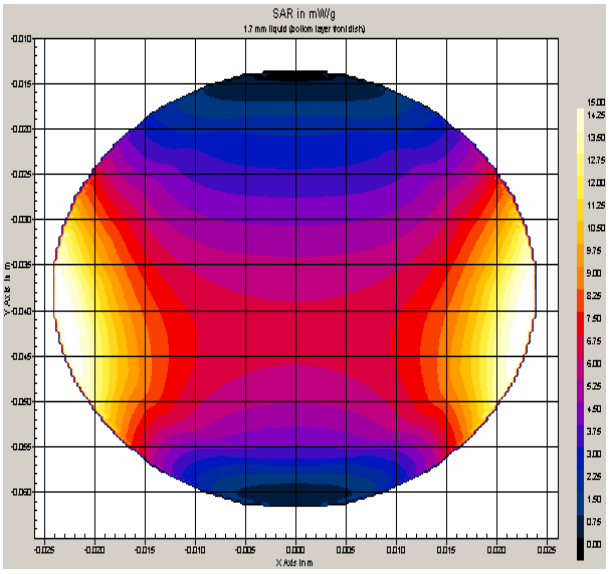

Figure A.6 Simulated (XFDTD) relative SAR distribution shown in one petri dish placed in the vertical chamber. The scale is given from 0 mW/g (black) to 15 mW/g (yellow). The inner diameter of petri dish is 50 mm.

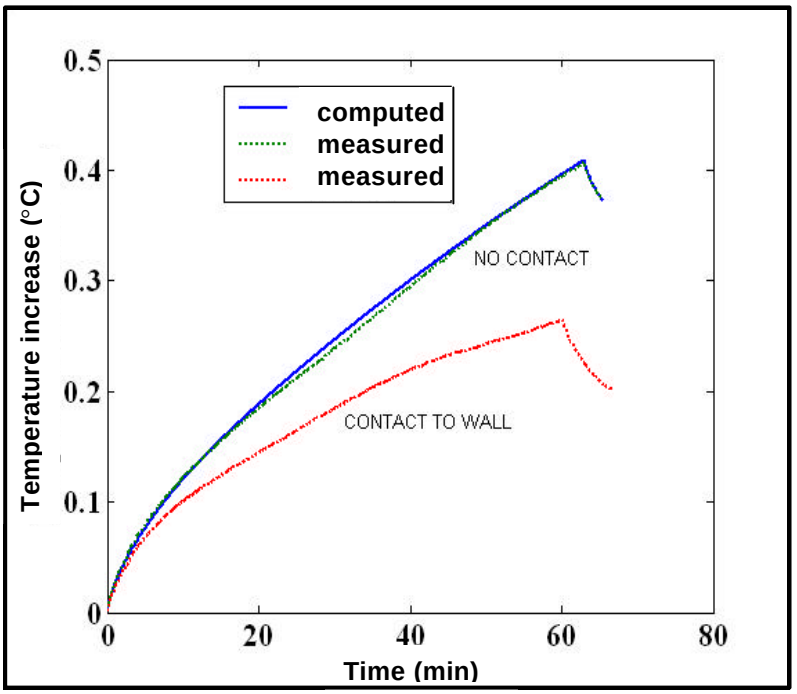

Figure A.7 Temperature increase measurements. The thermistor was either placed in the middle of the field or in contact with the petri dish wall.

The final SAR simulation and calculation results are summarized in Table A.1.

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

dBm	Average SAR (W/kg)	Temperature increase during experiments (°C)
-17.6	5.6 W/kg	± 0.3°C
-13.2	2.0 W/kg	± 0.2 °C

A.3 MAGNETIC FIELD MEASUREMENTS

During experiments, two sets of petri dishes used for control exposures were simultaneously placed inside the CO_2 incubator while an additional two sets of petri dishes were exposed to 900 MHz GSM radiation. To determine the exact placing of the control petri dishes inside the CO_2 incubator without any magnetic interference arising from the RF-exposure or the magnetic field generated by the water-pump, a magnetometer (LEMI-011, Hermanus Magnetic Observatory) was used. The results are illustrated in Figure A.8. Placing of control petri dishes directly in front of the

vertical RF-chamber hatch closely resembled conditions when the same dishes were placed inside the RF-chamber during exposures.

Magnetic field determined by magnetometer:

(1) Magnetic Field > Earth's magnetic Field

(2) Magnetic Field ˜ Earth's magnetic Field

(3) RF power on and off-Magnetic Field ˜ Earth's Magnetic Field

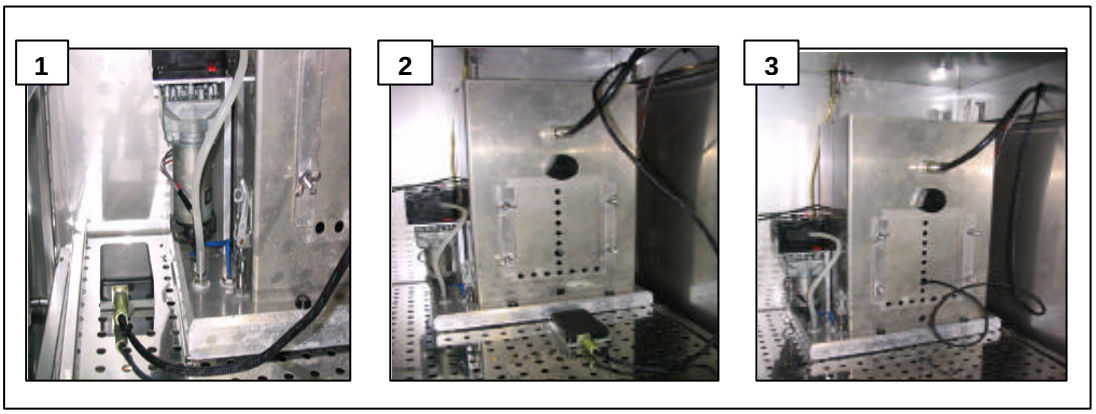

Figure A.8. Magnetometer measurement set-up. Placement of the magnetometer inside the CO_2 incubator in positions 2 and 3 closely resembled that of the earth's magnetic field.

A.4 REFERENCES

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ANNEXURE B: MACROSCOPIC AND MICROSCOPIC SPERM PARAMETERS

B.1 EVALUATION OF SPERMATOZOA

The initial step in the evaluation of spermatozoa is the determination of semen parameters followed by the assessment of sperm functionality and finally functionality is demonstrated with bioassays. The evaluation process of spermatozoa that was followed in this study is illustrated in Figure B.1. In addition to the initial macroscopic and microscopic evaluation of donors, morphology and motility were determined by computer aided sperm analysis (Chapter 3). In the following paragraphs the macroscopic and microscopic evaluation procedures followed in this study are briefly outlined.

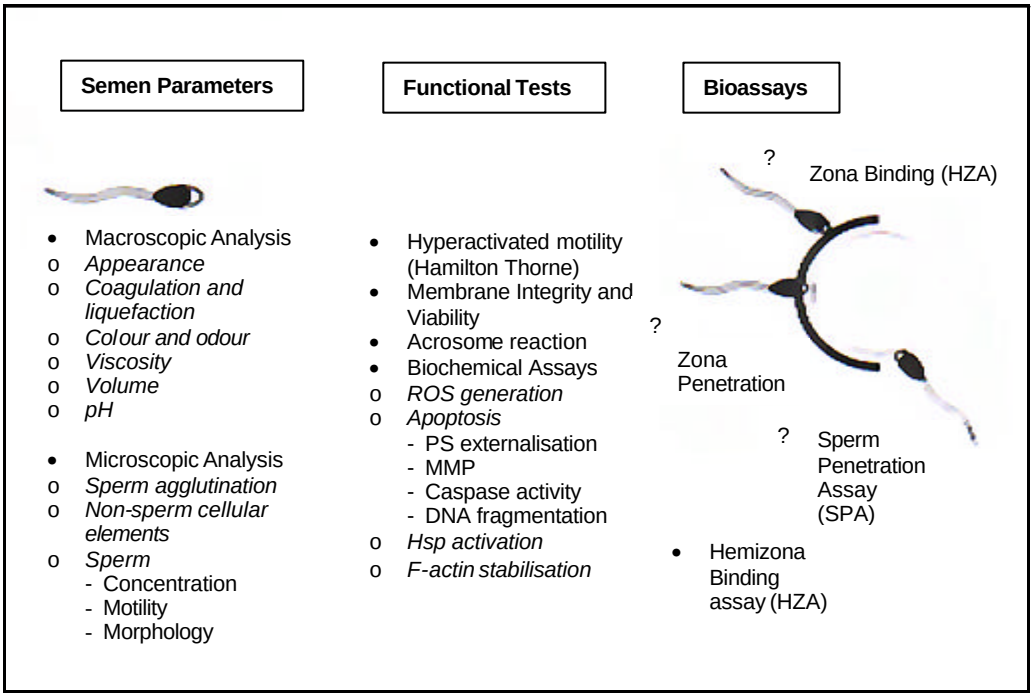

Figure B.1 Diagram illustrating different semen assessment parameters, functional tests and bioassays in the evaluation of human spermatozoa. (Adapted with permission from Oehninger *et al.*, 1991).

B.1.1 Semen parameters – macroscopic and microscopic evaluation

According to the World Health Organisation (WHO, 1999), standard human semen analysis generally assesses the semen volume, sperm concentration, the percentage of sperm showing forward progression (motility), and the percentage of sperm with normal morphology as analysed by light microscopy. There is no single sperm parameter that can accurately predict fertilisation potential, therefore a large number of diagnostic tests have been designed to determine the capability of a given individual's semen specimen to achieve fertilization of a human oocyte. These parameters as defined by the WHO (1999) are summarised in the following paragraphs.

After collection of a semen sample, semen parameters including macroscopic analysis (*appearance, coagulation and liquefaction, colour and odour, viscosity, volume and pH*) as well as microscopic analysis (*sperm agglutination - SpermMar IgG test, non-sperm cellular elements, concentration - Neubauer haemocytometer, motility - differential count, and morphology (Papanicolaou stain using Tygerberg strict criteria)*) were evaluated.

B.1.1.1 Macroscopic analysis

(i) Appearance

Normal semen consists of a mixture of spermatozoa suspended in secretions from the testis and epididymis, which at the time of ejaculation are combined with secretions from the prostate, seminal vesicles and bulbourethral glands (WHO, 1999). The appearance of normal semen when held against a light source is opaque (Jeyendran, 2003; Mortimer, 1994). Semen appearance has no apparent clinical value in human ejaculate analysis.

Method: The appearance (transparency) of the semen sample was noted. Semen was considered normal if it was opaque in appearance.

(ii) Coagulation and liquefaction

Immediately following ejaculation the specimen normally coagulates into a gelatinous mass and then liquefies (Jeyendran, 2003; Jequier, 2000; Mortimer, 1994).

Normal specimens will require less that 60 min to liquefy at 37°C. A sample is considered abnormal if it takes longer than 120 min to liquefy at 37°C.

Method: Semen samples were left for 30 min to liquefy in an incubator at 37°C.

(iii) Colour and odour

Normal ejaculate colour ranges between whitish-grey, pearl white and yellowish (Jeyendran, 2003; Jequier, 2000; Mortimer, 1994). A highly concentrated sample can be observed by the high degree of cloudiness, while a low concentrated sample will appear transparent and watery. Abnormal samples are pink, reddish, brown or yellow in colour.

Semen odour is distinctly pungent and has been compared to that of the flowers of the chestnut or St. John's beard tree. The odour is due to secretions from the prostate gland and a change in odour could be indicative of inflammation (Menkveld and Kruger, 1995). Semen colour and odour have no specific significance to sperm fertility evaluation but could be symptomatic of non-functioning accessory sexual glands (Jeyendran, 2003).

Method: The colour of the semen sample could indicate possible infection, therefore samples were considered normal when they were whitish–grey to light yellow in appearance.

(iv) Viscosity

Viscosity is a measure of the friction between various seminal fluid components as they slide by each other. This parameter is determined by appraising the length of thread formed when liquefied semen is dropped from a pipette (Jeyendran, 2003; Jequier, 2000; Mortimer, 1994). A normal sample would form small, discrete droplets, whereas in abnormal cases a tread of more than 2 cm long could form when the sample leaves the pipette (WHO, 1999; Jeyendran, 2003). Furthermore, increased viscosity could lead to the inability to determine sperm quality objectively as it can interfere with the determination of sperm motility, concentration and antibody coating of spermatozoa (WHO, 1999; WHO Seminology workshop manual, 2001).

The relationship between viscosity and fertility is unknown; however high viscosity combined with poor sperm motility could lead to a decrease in fertilisation capacity (Jeyendran, 2003; Mortimer, 1994).

Method: Semen was dropped from a wide bore disposable pipette. Semen falling in small discrete droplets was considered normal, while semen forming a thread of more than 2 cm long was noted as very viscous.

(v) Volume

Volume is defined as the quantity of ejaculate a person produces. Semen volume is primarily determined by accessory gland secretion contributions (Seminal vesicle: 75%, Prostrate: 20%, Vas deferens, epididymis and testicles: 5% and bulbourethral gland: minuscule amount to coat urethra) (Jeyendran, 2003; Jequier, 2000; Mortimer, 1994). The volume is measured to the nearest 0.1 ml with a 3-5 ml disposable syringe, graduated pipette or centrifuge tube (Jeyendran, 2003). The normal volume of an ejaculate is 2-6 ml after a sexual abstinence period of 3-5 days.

Method: The total volume of the semen sample was determined by a disposable graduated pipette.

(vi) pH

The pH of the semen sample is measured within one hour of collection using a special indicator paper (range 6.4-8.0). A drop of semen is placed on the indicator strip and after 30 seconds compared against a colour scale. Normal pH values range from 7.6 to 8.6, whereas pH values less than 7.2 or greater than 9.4 are noted as abnormal (Jeyendran, 2003; Jequier, 2000; Mortimer, 1994).

Method: The pH of the sample was determined by placing 11.5 µl of semen on a strip of litmus paper (Merck, Germany, # 9557). The colour was then immediately compared against a colour scale.

B.1.1.2 Microscopic analysis

To evaluate sperm quality microscopic analysis assesses the agglutination of spermatozoa, the presence of cellular elements other than spermatozoa, concentration, motility and morphology.

(i) Sperm agglutination - SpermMar IgG test

The term agglutination is used to describe the clumping of sperm into aggregates. There are basically two types of agglutination, non-specific agglutination and site-specific agglutination. Non-specific agglutination describes the adherence of sperm to various seminal debris, leukocytes or mucus threads and various other non-sperm elements (Jeyendran, 2003; Jequier, 2000; Mortimer, 1994). Site-specific agglutination on the other hand is used to indicate the adherence of sperm to each other in a site-specific manner, such as head-to-head, head-to-tail, tail-to tail, or any such combination (Jeyendran, 2003).

To determine agglutination status, wet microscopic smears are prepared and observed under a high-power field as described by Jeyendran (2003). Sperm is considered normal if no agglutination is observed. The sample is deemed equivocal if one or two clusters are observed and is regarded as abnormal when more than two clusters are observed per high-power field. The clinical significance of extensive agglutination is diminished availability of “free” sperm that could potentially inhibit fertilisation potential. Furthermore, site-specific agglutination could suggest immunological pathology and should be investigated by analysing anti-sperm antibodies. The SpermMar IgG test is designed indicate the presence of sperm antibodies reacting with antigens on spermatozoa. Non-specific agglutination may suggest an accessory gland infection.

Method: Site-specific agglutination of human spermatozoa was determined by the Sperm Mar IgG test (FertiproNV, Harrilabs, SA). The direct SpermMar test is performed by mixing 5 µl fresh untreated semen with 5 µl human IgG coated latex particles on a micro slide. Monospecific antihuman IgG antiserum (5 µl) was then immediately added to this mixture. The slide was then covered by a cover slip and observed under a light microscope using a 40x magnification. Agglutination will occur between IgG antibody positive spermatozoa and latex particles. If latex

particles attached to motile sperm were observed, a total of 100 sperm were counted and the percentage IgG antibody positive sperm scored.

(ii) Non-sperm cellular elements

Cellular elements such as leukocytes, erythrocytes, epithelial cells, microorganisms, and sperm precursors may be present in seminal fluid (Jeyendran, 2003; Jequier, 2000; Mortimer, 1994). These elements are observed by investigating wet microscopic smears.

A high presence of either leukocytes, microorganisms and sperm precursors (spermatids, spermatocytes and spermatogonia) are usually associated with below normal sperm count and abnormal sperm morphology and may imply an overall reduction in fertility potential (Jeyendran, 2003).

Method: A wet smear was prepared by pipetting 11.5µl of the semen sample on a microscope slide the slide was then covered by a cover slip. The wet smear was evaluated under a light microscope at 40x magnification for the presence of non-sperm cellular elements.

(iii) Concentration - Neubauer haemocytometer

Sperm concentration is measured as the number of sperm per millimetre of seminal fluid, and the sperm count is the total number of sperm in the ejaculate (Jeyendran, 2003). The most accurate measurement method is by haemocytometer. This method requires the dilution of the ejaculate with media after which two separate preparations of the semen are evaluated on each side of the counting chamber (Mortimer, 1994; WHO, 1999; Jeyendran, 2003). The WHO (1999) recommends the Neubauer Haemocytometer (100 µm depth) above other counting chambers like the Microcell (20 µm depth) and Makler (10µm depth).

The sperm count is calculated as the product of the sperm concentration and ejaculate volume. A normal ejaculate contains more than 20.0 x 10^6 sperm per ml of semen, abnormal counts are noted if there are less than 10.0 x 10^6 sperm per ml of semen (Jeyendran, 2003; Jequier, 2000; Mortimer, 1994).

Method: To count the concentration of sperm cells/ml, 50 µl of the semen was added to 950 µl trypan blue stain (Sigma Chemicals Co.) in a press top tube. The sperm were allowed to absorb the stain before both counting chambers of the Neubauer were loaded and left in a humid chamber for 10 min before scoring sperm counts in each chamber.

(iv) Motility - differential count

Sperm motility is defined as the observation of spontaneous sperm movement and is a significant factor when evaluating fertility potential (Mortimer and Mortimer, 1999; Jeyendran, 2003). It is determined by noting the ratio of motile progressive sperm to total sperm number (in a given volume) and is expressed as a percentage (Jeyendran, 2003). The WHO (1999) recommends a simple grading system for the manual evaluation of motility, however other methods such as the computer aided sperm analysis (CASA) method, although requiring more complex equipment affords a more accurate assessment. Both these methods were used in the study to evaluate sperm motility.

Sperm motility as recommended by the WHO (1999) is estimated by microscopic examination. A wet preparation is made by placing approximately 10 µl of thoroughly mixed, liquefied semen on a warm microscope slide and covering it with a warm 22 x 22 mm cover-slip (sperm motility is temperature dependent and is therefore assessed under controlled thermal conditions) (Mortimer and Mortimer, 1999; WHO, 1999). At least four to five microscopic fields are systematically examined by classifying motility parameters of no less than 200 spermatozoa (WHO, 1999; Jeyendran, 2003). Each spermatozoon is graded according to:

- Rapid progressive motility (a)
- Slow or sluggish progressive motility (b)
- Non-progressive motility (c)
- Immotility (d)

Within 60 min of ejaculation a normal sample contains 50% or more motile sperm (grades a + b) or 25% or more progressive motility (grade a) (WHO, 1999). Abnormal samples will show less than 35% overall sperm motility.

Method: The motility of unprocessed semen was determined by pipetting 11.5 µl of semen unto on a microscope slide, which was then covered by a cover slip. The slide was evaluated under a light microscope at 40x magnification and a total of 100 sperm counted with a differential counter by categorising the sperm according to WHO (1999) criteria (grades a-d). Percentages were scored and the total motility of the sample taken as the sum of the percentages from grade a and b motility.

(v) Morphology (Papanicolaou stain using Tygerberg strict criteria)

Sperm morphology defines the shape, size and surface appearance of sperm (Jeyendran, 2003). Sperm morphology is assessed by microscopic evaluation of a stained semen smear on a dry slide at high magnification and is by its very nature, highly subjective (Jeyendran, 2003; Jequier, 2000; Mortimer, 1994). The Papanicolaou stain is the preferred method used in andrology laboratories and is also recommended by the WHO (1999). Wet smears are prepared and air-dried on a warm plate before being stained (Mortimer, 1994; Jeyndran, 2003). After staining no less than 200 sperm are counted at high magnification (100x) under oil immersion using bright field optics (Mortimer and Mortimer, 1999; WHO, 1999). Sperm morphology is assessed by the Tygerberg strict criteria (Menkveld *et al*. 1990; Mortimer and Mortimer, 1999).

Method: The evaluation of sperm morphology was made according to the method described by Kruger *et al*. (1986). To determine the percentage of morphologically normal spermatozoa a wet smear was prepared by pipetting 11.5 µl of the semen sample onto a micro slide. The smear was made by pulling the semen droplet evenly along the slide (if the sample was very concentrated the droplet was smeared at a 45° angle, low concentrations were smeared at an angle of 10-30°). The slide was then allowed to air dry at room temperature. Slides were stained using a modified Papanicolau staining procedure before being mounted with DABCO.

The staining procedure is briefly summarized:

The slides were left for one day to allow the DABCO (Sigma Chemical Co.) to set, before being analysed under a light microscope by using a 100x oil immersion lens. A total of 200 spermatozoa were counted and classified as normal according to the Tygerberg strict criteria when; the head had a smooth oval configuration, the acrosome comprised 40-70% of the sperm head, no head, neck, midpiece or tail abnormalities were noted. All other sperm were scored as abnormal.

After noting all the semen parameters for each individual donor, the semen samples were processed using a density gradient centrifugation procedure. Since components from seminal fluid have been reported to influence sperm motility (Somlev *et al.*, 1997; Katkov and Mazur, 1998) experiments were conducted using processed sperm.

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ANNEXURE C

DONOR SPERM PARAMETERS - RESULTS

C.1 MACROSCOPIC SEMEN PARAMETERS

All samples were taken from healthy donors and no deviations in appearance, colour, and odour were noted. In addition, no abnormal coagulation was noted in semen samples and samples did not require an extended period to liquefy. Furthermore, viscosity of samples were considered normal (none of the samples were extremely viscous). The total volume and pH of the ejaculate for each of the twelve donors are illustrated in Figure C.1, while averages are summarised in Table C.1.

C.2 MICROSCOPIC SEMEN PARAMETERS

According to the WHO criteria (1999), normal semen parameters are defined as 'a+b' type motility or progressive motility ≥ 50% and 'a' type motility or forwards motility ≥ 25%; sperm cell concentration ≥ 20 x 10^6 cells/ml; and sperm cells with morphology < 15% abnormal forms. Based on these criteria, semen profiles were classified into three categories namely: **A** (n = 4) - normal motility and concentration with < 5% normal morphology, **B** (n = 5) - normal motility and concentration with between 5% - 10% normal morphology, and **C** (n = 3) - normal motility and concentration with > 10% normal morphology. All samples used had less than 1 x 10^6 leukocytes/ml (this was also confirmed with CD45 staining – for reference see Chapter 3) and were MAR negative. The motile sperm concentration after density purification of the total sample (total ejaculate) was ≥ 40 x 10^6 /ml in order to provide sufficient number of cells for experimentation. This formed the criteria by which donors were selected for participation in the study.

Sperm parameters determined for each of the 12 donors are presented in Figure C.1 A-E.

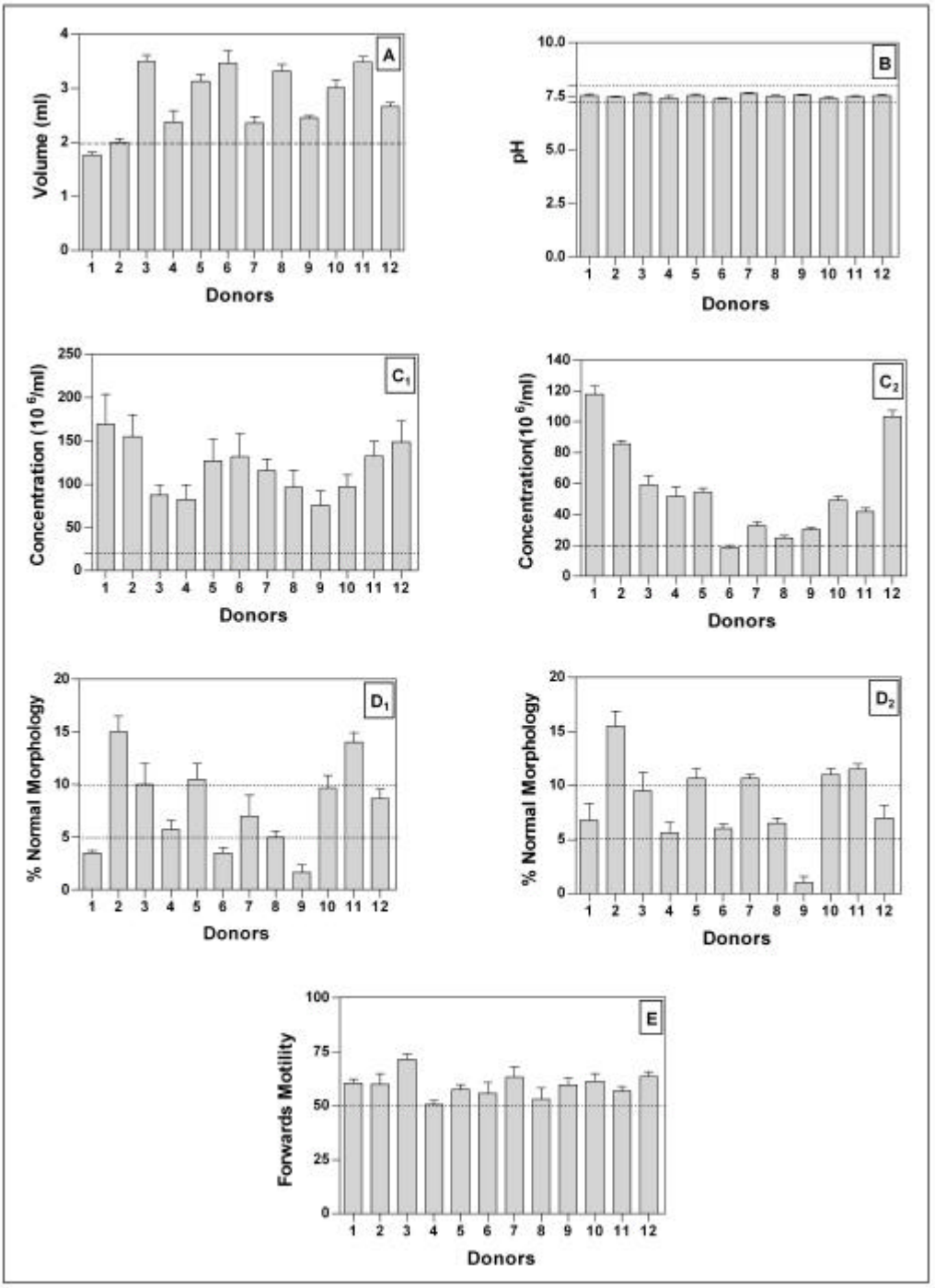

Figure C.1 Sperm parameters determined for each donor. Each end point represents the mean and standard deviation (SD) of at least 5 replicates. In all graphs WHO reference values are noted with dotted lines. **(A)** Total volume; **(B)** pH; **(C1)** Concentration pre-processing; **(C2)** Concentration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

A summary of the average sperm parameters obtained for all 12 donors (volume, pH, concentration, morphology, and motility) are noted in Table C.1. Average values for the volume, pH, and motility were obtained from the total semen sample pre density gradient purification. Both the concentration and morphology averages are supplied for pre- and post- density gradient purification.

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.

Parameter	Pre-Average ± SD	Post-Average ± SD
Volume (ml)	2.83 ± 0.34	-
pH	7.49 ± 0.08	-
Motility (%)	59.38 ± 5.43	-
Concentration (10^6/ml)	118.21 ± 30.75	55.32 ± 30.65
Morphology	7.85 ± 4.20	8.5 ± 3.80

C.3 REFERENCES

WHO (World Health Organisation) 1999. *Laboratory manual for the examination of human semen and sperm-cervical mucus interaction*. Cambridge, UK: Cambridge Univ Press, 4th Edition.