

# **Measurement of free radicals and their effects on human spermatozoa**

By

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## DECLARATION

I, the undersigned, hereby declare that the work in this thesis is my own original work that I have not previously in its entirety or in part submitted it at any other university for a degree.

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## ABSTRACT

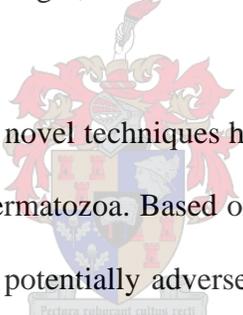
In this study, we presented data on the role of free radicals in human spermatozoa, particularly in the context of centrifugation and the potential development of defective sperm function. In order to achieve this, methods were developed to directly measure intracellular free radicals in human sperm and the effects of exogenously applied free radicals on sperm function were established. The role of brief and prolonged centrifugation and the associated generation of free radicals was also investigated.

In the first part of the study, we established flow cytometry as a reliable tool for directly measuring intracellular free radicals in human spermatozoa. It was shown that flow cytometry is an accurate, objective and relatively easy technique that can be applied to detect and measure intracellular nitric oxide (NO) and reactive oxygen species (ROS) in human spermatozoa by employing the fluorescent probes DAF-2/DA and DCFH respectively.

In the second part of the study the effects of centrifugation on free radical generation in sperm was investigated. It was shown that a brief period of centrifugation (10 min) led to increased NO and ROS generation whereas prolonged centrifugation (30 min) decreased NO generation whilst ROS generation was increased. These increases in NO and ROS generation due to centrifugation were attenuated by the addition of the NOS enzyme inhibitor, L-NAME, and the ROS scavenger, MPG, respectively. Centrifugation furthermore led to impaired sperm motility parameters and decreased cell viability, which could be restored completely by ROS scavenging (MPG), but not by NOS inhibition (L-

NAME). This suggests that the detrimental effects on sperm function may have predominantly been due to the ROS generated during centrifugation, and not NO.

The effects of exogenously administered free radicals on sperm function were investigated in the third part of the study. NO seemed to enhance sperm motility and viability at lower concentrations (30  $\mu\text{M}$  SNP), but became detrimental at higher concentrations (>100  $\mu\text{M}$  SNP). On the other hand it was observed that the addition of  $\text{H}_2\text{O}_2$  severely impaired all sperm functions measured and had no beneficial properties at any of the concentrations tested. These detrimental effects of  $\text{H}_2\text{O}_2$  could be completely abolished by the addition of its scavenger, catalase.

The image shows a faint watermark of a university crest in the center of the page. The crest features a shield with various symbols, topped with a crown and a figure holding a staff. Below the shield is a motto scroll with the Latin text "Pectora roburant cultus recti".

In conclusion, in the current study, novel techniques have been developed to successfully measure free radicals in human spermatozoa. Based on our findings, we recommend that cognisance should be taken of the potentially adverse effects of both centrifugation and free radicals on sperm function with the ultimate goal of improving the outcome of assisted reproductive technologies.

## OPSOMMING

In hierdie studie word data aangebied m.b.t. die rol van vrye radikale in menslike spermatozoa, veral in die konteks van sentrifugering en die moontlike ontwikkeling van abnormale spermfunksie. Ten einde hierdie doelwit te bereik, is metodes ontwikkel om die direkte meting van intrasellulêre vrye radikale in menslike spermatozoa moontlik te maak, en die effek van eksogeen-toegediende vrye radikale op spermfunksie te ondersoek.

In die eerste gedeelte van die studie is vloeisitometrie as 'n betroubare metode vir die direkte bepaling van intrasellulêre vrye radikale in menslike spermatozoa gevestig. Ons het aangetoon dat vloeisitometrie 'n akkurate, objektiewe en relatief maklike metode is wat vir die waarneming en meting van intrasellulêre stikstofoksied (NO) en reaktiewe suurstof spesies (ROS) in menslike spermatozoa aangewend kan word deur van die fluoreserende merkers DAF-2/DA en DCFH onderskeidelik gebruik te maak.

Die tweede gedeelte van die studie handel oor die effek van sentrifugering op die vorming van vrye radikale in sperme. Daar is aangetoon dat 'n kort periode van sentrifugering (10 min) tot verhoogde NO en ROS vorming gelei het, terwyl langer sentrifugering (30 min) tot verlaagde NO produksie en verhoogde ROS vorming aanleiding gegee het. Die verhoging in NO en ROS produksie kon deur die toediening van die NOS ensiem inhibitor, L-NAME, en die ROS opruimer, MPG, onderskeidelik opgehef word. Verder het sentrifugering ook tot verlaagde spermmotiliteit parameters en

verlaagde sel lewensvatbaarheid gelei, wat volledig deur ROS opruiming (MPG), maar nie NOS inhibisie (L-NAME) nie, omgekeer kon word. Hiervan kan afgelei word dat die nadelige effekte op spermfunksie heel moontlik hoofsaaklik aan die ROS wat tydens sentrifugering opgewek word, toegeskryf kan word.

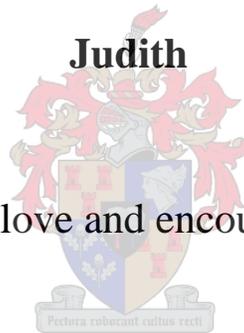
Die effekte van eksogeen toegediende vrye radikale op spermfunksie word in die derde gedeelte van die studie ondersoek. NO blyk spermmotiliteit en lewensvatbaarheid te bevorder by laer konsentrasies (30  $\mu\text{M}$  SNP), maar het nadelig begin raak by hoër konsentrasies (>100  $\mu\text{M}$  SNP). In teenstelling hiermee, het die toediening van  $\text{H}_2\text{O}_2$  nadelige effekte op al die gemete spermfunksies gehad. Hierdie skadelike effekte van  $\text{H}_2\text{O}_2$  kon volledig opgehef word deur die toediening van sy opruimer, katalase.

Die gevolgtrekking kan gemaak word dat nuwe tegnieke ontwikkel is om vrye radikale suksesvol in menslike spermatozoa te meet. N.a.v. die bevindinge van die studie, word aanbeveel dat daar op die potensieel nadelige effekte van beide sentrifugering en vry radikale op spermfunksie gelet moet word, sodat die uitkoms van geassisteerde reprodktiewe tegnologie verder verbeter kan word.

This dissertation is dedicated to

**Judith**

For your love and encouragement



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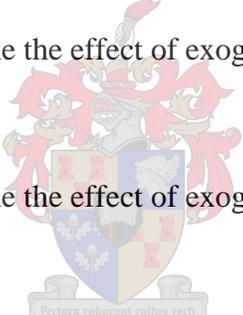
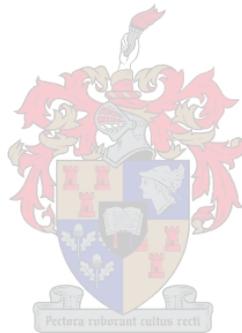
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## ALPHABETICAL LIST OF ABBREVIATIONS

AC	= Adenylate cyclase
AR	= Acrosome reaction
BSA	= Bovine serum albumin
Ca <sup>+2</sup>	= Calcium ion
cAMP	=Cyclic 3',5'adenosine monophosphate
CASA	= Computer assisted semen analysis
DAF-2/DA	= 4,5-diaminofluorescein-2/diacetate
DCFH	=2,7-dichlorofluorescein diacetate
H <sup>+</sup>	= Hydrogen cation
HCO <sub>3</sub> <sup>-</sup>	= Bicarbonate
H <sub>2</sub> O <sub>2</sub>	= Hydrogen peroxide
HTF	= Human tubal fluid
L-NAME	= N <sup>W</sup> -nitro-L-arginine methyl ester
MDA	= Malondialdehyde
MPG	= N-(2-mercaptopropionyl)Glycine
Na <sup>+</sup>	= Sodium cation
NO	= Nitric oxide
NOS	= Nitric oxide synthase
O <sub>2</sub> <sup>-</sup>	= Superoxide
ONOO <sup>-</sup>	= Peroxynitrite anion
OH <sup>-</sup>	= Hydroxyl anion
P	= Progesterone



OS	= Oxidative stress
PBS	= Phosphate buffered saline
PI	= Propidium iodide
PL	= Phospholipids
PUFA	= Polyunsaturated fatty acids
ROO <sup>-</sup>	= Peroxyl
ROS	= Reactive oxygen species
SNP	= Sodium nitroprusside
SOD	= Superoxide dismutase
VAP	= Average path velocity
VSL	= Straight-line velocity
WHO	= World Health Organization
ZP	= <i>Zona pellucida</i>
ZP3	= <i>Zona pellucida</i> glycoprotein 3

