
“INVESTIGATION OF THE ROLE OF OXIDATIVE STRESS IN MALE INFERTILITY”

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DECLARATION

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Ozlem Tunc
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PUBLICATIONS AND ABSTRACTS ARISING FROM THIS THESIS

PUBLICATIONS

- 1 - "Development of the NBT assay as a marker of sperm oxidative stress"
Ozlem Tunc, Jeremy Thompson, Kelton Tremellen
International Journal of Andrology 2010 Feb; 33(1):13-21

- 2 - "Improvement in sperm DNA quality using an oral antioxidant therapy"
Ozlem Tunc, Jeremy Thompson, Kelton Tremellen
Reproductive BioMedicine Online 2009 Jun; 18(6):761-8.

- 3 - "Oxidative DNA damage impairs global sperm DNA methylation in infertile men"
Ozlem Tunc, Kelton Tremellen
Journal of Assisted Reproduction and Genetics 2009 Sep-Oct 26(9-10):537-44

- 4 - "Macrophage activity in semen significantly correlated with sperm quality in infertile Men "
Kelton Tremellen, Ozlem Tunc
International Journal of Andrology 2010 Dec; 33(6):823-31

- 5 - "Impact of Body Mass Index on sperm oxidative stress"
Ozlem Tunc, Hassan Bakos, Kelton Tremellen
Andrologia (accepted in Aug 2009, anticipated online publication in December 2010)

ABSTRACTS

"A novel assay for identification of oxidative stress related male infertility"
Poster presentation The Fertility Society of Australia 9-12 September 2007
Hobart, Tasmania

"Optimization of sperm DNA quality by using an oral antioxidant therapy"
Gene, environment, lifestyle interaction and Human reproduction 7-10
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"Optimization of sperm DNA quality with the use of an oral antioxidant therapy"
Oral Presentation in the Fertility Society of Australia 19-22 October 2008
Brisbane, Australia

ABBREVIATIONS

8-OHdG	8-hydroxy-20-deoxyguanosine
A.U	Arbitrary Units
AMH	Anti-mullerian hormone
ANOVA	Analysis of Variance
ART	Assisted Reproductive Technology
BSA.....	Bovine serum albumin
°C	Degrees celsius
CK	Creatine kinase
DMSO.....	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothretiol
ELISA	Enzyme-linked immunosorbent assay
FSH.....	Follicle stimulating hormone
GPx	Glutathione peroxidase
GSR	Glutathione reductase
H ₂ O ₂	Hydrogen peroxide
HBSS	Hanks buffered salt solution
hCG	Human chorionic gonadotrophin
Hcy	Homocysteine
HNE	Hydroxynonenal
HPLC	High Performance Liquid Chromatography
HRP	Horse Radish Peroxidase
IFN γ	Interferon gamma
IU	International unit
KOH	Potassium hydroxide
kg.....	Kilogram
LDH	Lactic acid dehydrogenase
LH.....	Luteinizing Hormone
MDA	Malondialdehyde
μ g	Microgram
μ l	Microliter
mL	Milliliter
mg	Milligram
NaCl	Sodium Chloride

NAG	neutral alpha glucosidase
NBT	Nitroblue Tetrazolium
NO	Nitric oxide
NOS	Nitric oxide synthase
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
PMN	Polymorphonuclear Neutrophils
Rcf	Relative centrifugal force
ROC	Receiver operating characteristic
ROS	Reactive oxygen species
SD	Standard Deviation
SDS	Sodium dodecyl sulphate
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TBAR	Thiobarbituric acid
TBARS	Thiobarbituric acid-reacting substances
TNF α	Tumor Necrosis Factor Alpha
X	Xanthine
XO	Xanthine oxidase

ABSTRACT

In recent years, there has been some suggestion of an increase in male factor infertility in the industrialized countries with a decline in sperm counts and a rise in sperm pathology. Male factor infertility is a multifactorial phenomenon that is observed in approximately half of infertile couples and affects one man in 20 in the general population. The potential causes of male infertility arise from a number of factors including genetic, lifestyle factors and chronic diseases. However, a high proportion of infertile male patients have now been shown to have defective sperm functions related to oxidative stress.

Oxidative stress in semen has been speculated as one of the major factors causing male infertility and has been identified in 30-80% of cases of male infertility. While oxidative stress is accepted as a significant pathology, there is currently an inadequate knowledge of the exact mechanisms by which oxidative stress develops in male infertility, as well as a lack of an easy and reliable method for the measurement of seminal oxidative stress in routine clinical use.

The main objective of this doctoral thesis is to investigate the underlying causes for oxidative stress in infertile men and the mechanisms by which oxidative stress develops. Furthermore it will also examine the effectiveness of an oral antioxidant therapy for treatment of seminal oxidative stress.

During these doctoral studies experiments were designed with the aims of:

- Developing a standardized protocol for the measurement of seminal oxidative stress, that can be conducted in the average clinical laboratory with minimal additional equipment (NBT Assay)
- Examining the causes for oxidative stress in semen. Obesity has previously been identified as a cause of systemic oxidative stress. Therefore I examined if obesity causes oxidative stress to sperm. Seminal inflammation and its role in oxidative damage in semen are also investigated.
- Determination if antioxidant supplementation is an effective treatment of oxidative sperm damage.
- Assessment of the relation between Oxidative stress and sperm DNA methylation. Previous studies have linked male infertility with epigenetic abnormalities of the male genome. Since oxidative stress has been shown to interfere with somatic cell epigenetic programming I investigated the possibility of a similar link in sperm.

It is hoped that advances outlined in this thesis will have made a significant contribution to the diagnosis, prevention and treatment of the male infertility.