"INVESTIGATION OF THE ROLE OF OXIDATIVE STRESS IN MALE INFERTILITY"

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DECLARATION

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Ozlem Tunc
November 2010

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TABLE OF CONTENTS

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 Genetics2009 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
February 2008 Malmo, Sweden

"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 ..... Arbitary Units
AMH ..... Anti-mullerian hormone
ANOVA .....Analysis of Variance
ART ..... Assisted Reproductive Technology
BSA..... Bovine serum albumin
^{o}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 peroxidise
GSR ..... Glutathione reductase
H_2O_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
IFNγ ..... Interferon gamma
IU ..... International unit
KOH ..... Potassium hydroxide
kg..... Kilogram
LDH ..... Lactic acid dehydrogenase
LH..... ..... Luteinizing Hormone
MDA ..... Malondialdehyde
µg .....Microgram
µl .....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
TNFα .....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.