

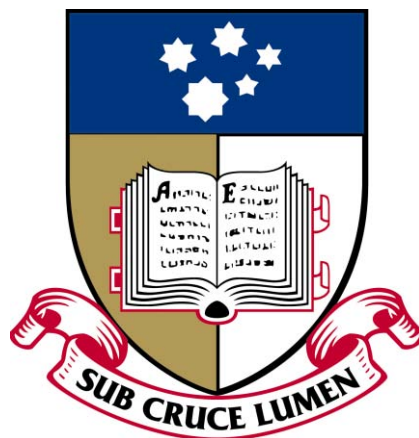
# The Impact of Exogenous TGF $\beta$ 1 on Male Reproductive Function

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Thesis submitted for the total fulfilment for the degree of  
Doctor of Philosophy (PhD)

April 2008



*For Paul*

*None of the work presented in this thesis would have been possible without his generous love, encouragement and support.*

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*For Aaron*

*His enduring love and patience throughout all of my studies is deeply appreciated.*

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*For my Family and Friends*

*Their ability to highlight the lighter side of this project has been uplifting.*

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## ABBREVIATIONS

°C	Degrees celsius
ANOVA	Analysis of variance
APES	3-aminopropyltriethoxysilane
Bp	Base pair
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumin
cDNA	Complimentary deoxyribonucleic acid
CFSE	Carboxyl fluorescein succinimide ester
CSF-1	Colony-stimulating factor-1
CT	Cycle threshold
DAB	3,3'-diaminobenzidine
dFUR	Fluorodeoxyuridine
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
FACS	Fluorescence activated cell sorter analysis
FSH	Follicle stimulating hormone
g	Grams
HBSS	Hanks buffered salt solution
hCG	Human chorionic gonadotropin
HSD3 $\beta$ 1	3 $\beta$ -hydroxysteroid dehydrogenase-1
IFN $\gamma$	Interferon gamma
Ig	Immunoglobulin
iNOS	Inducible nitric oxide synthase
IU	International unit
KCl	Potassium chloride
kDa	kilo Dalton
KH <sub>2</sub> PO <sub>4</sub>	Potassium di-hydrogen orthphosphate
kg	Kilogram
LAP	Latency associated peptide
LH	Luteinizing hormone
mRNA	Messenger ribonucleic acid
mL	Millilitre

mg.....	Milligram
Na <sub>2</sub> HPO <sub>4</sub> .....	di-Sodium hydrogen orthophosphate
NaCl.....	Sodium chloride
NaH <sub>2</sub> PO <sub>4</sub> .....	Sodium di-hydrogen phosphate
NCBI .....	National Centre for Biotechnology Information
NCE .....	Non contact erections
NMS.....	Normal mouse serum
nNOS.....	Neuronal nitric oxide synthase
NOS.....	Nitric oxide synthase
PBS .....	Phosphate buffered saline
PCR.....	Polymerase chain reaction
PE .....	Phycoerythrin
PFA.....	Paraformaldehyde
PMN.....	Polymorphonuclear neutrophils
PMSG .....	Pregnant mare serum gonadotropin
PVP .....	Polyvinylpyrrolidone
Rcf .....	Relative centrifugal force
rhLTGFβ1 .....	Recombinant human latent transforming growth factor beta 1
RIA.....	Radioimmunoassay
rpm.....	Revolutions per minute
RPMI.....	Roswell Park Memorial Institute
RT-PCR.....	Reverse transcription - polymerase chain reaction
SCID .....	Severe combined immune deficiency
SDS .....	Sodium dodecyl sulphate
SEM.....	Standard Error of the Mean
TGFβ1-/-.....	Null mice without the TGFβ1 gene
TGFβ1+/- .....	Heterozygote mice with the TGFβ1 gene on one allele
TGFβ1+/+ .....	Wild type mice with the TGFβ1 gene on both alleles
TNFα.....	Tumor Necrosis Factor Alpha
TβRI.....	TGFβ receptor 1
μg.....	Microgram
μL.....	Microlitre

## ABSTRACTS AND PUBLICATIONS ARISING FROM THIS THESIS

### ABSTRACTS

L. McGrath, R. Robker, SA Robertson. *TGF $\beta$ 1 is not responsible for erection dysfunction TGF $\beta$ 1 null mice*. Australian Society for Medical Research. June 2005

L. McGrath, R. Robker, SA Robertson. *Influence of exogenous TGF $\beta$ 1 on reproductive performance in TGF $\beta$ 1 null male mice*. Society of Reproductive Biology. July 2005

### PUBLICATIONS

L. McGrath, R. Robker, SA Robertson. *Effect of TGF $\beta$ 1 null mutation on structure and function of the penis in mice* (manuscript in preparation).

L. McGrath, R. Robker, SA Robertson. *Influence of exogenous TGF $\beta$ 1 replacement on reproductive performance in TGF $\beta$ 1 null mutant mice* (manuscript in preparation).

## ABSTRACT

The TGF $\beta$  family of cytokines are potent signalling molecules that regulate tissue development, inflammation and immunity. Previous studies in mice with a null mutation in the *Tgfb1* gene (TGF $\beta$ 1<sup>-/-</sup> mice) implicate a key role for TGF $\beta$ 1 in male reproductive function. These mice show profound infertility due to an inability to copulate successfully, associated with reduced testosterone and sperm production. The focus of this project was to 1) further characterize mechanisms underpinning reproductive deficiency in male TGF $\beta$ 1<sup>-/-</sup> mice, 2) identify a reliable physiological marker of TGF $\beta$ 1 availability in vivo, and 3) to determine whether exogenous TGF $\beta$ 1 administration influences TGF $\beta$ 1 availability and restores fertility.

To investigate the causes of unsuccessful copulation by TGF $\beta$ 1<sup>-/-</sup> mice, penis morphometry was examined. Penile organ structure, as assessed by scanning electron microscopy, was comparable between genotypes however a superfluous epidermal covering that impeded penile spine protrusion was evident in TGF $\beta$ 1<sup>-/-</sup> mice. The epidermal covering was not due to increased epithelial cell proliferation, as measured by Brdu labelling and immunohistology. Behavioural observations of erectile activity showed that TGF $\beta$ 1<sup>-/-</sup> mice achieved spontaneous erections albeit at reduced frequency compared to TGF $\beta$ 1<sup>+/+</sup> mice.

The efficacy of exogenous TGF $\beta$ 1 replacement was evaluated by first identifying measures of in vivo TGF $\beta$ 1 availability and/or function and selecting an effective route of administration. Serum TGF $\beta$ 1 and testosterone levels were reliable discriminators of TGF $\beta$ 1 genotype. Gene expression and phagocytic function of peritoneal macrophages revealed no differences between genotypes. Exogenous sources of TGF $\beta$ 1 for replacement studies included colostrum, naturally occurring in breast milk and recombinant human latent TGF $\beta$ 1 (rhLTGF $\beta$ 1). Colostrum did not increase circulating levels and rhTGF $\beta$ 1 injection caused only transient elevation of serum levels. Thus mini-osmotic pumps were used to deliver a constant supply of cytokine to TGF $\beta$ 1<sup>-/-</sup> mice.

The fertility status of TGF $\beta$ 1<sup>-/-</sup> mice receiving exogenous TGF $\beta$ 1 was investigated. Reproductive behaviour in response to normal receptive female mice was assessed twice during treatment, on day 7 and day 14. Blood, liver and reproductive tissues were collected at sacrifice. Circulating TGF $\beta$ 1 was increased in TGF $\beta$ 1 treated TGF $\beta$ 1<sup>-/-</sup> mice above TGF $\beta$ 1<sup>-/-</sup> control levels, although this did not affect circulating testosterone. Erectile activity and sperm production were unchanged. Videotaping behaviour with estrous females revealed that the TGF $\beta$ 1<sup>+/+</sup> mice successfully mounted and intromitted, unlike the TGF $\beta$ 1<sup>-/-</sup> controls. The TGF $\beta$ 1<sup>-/-</sup> mice receiving exogenous TGF $\beta$ 1 displayed moderately enhanced mounting and intromission behaviour although this remained less frequent than in the TGF $\beta$ 1<sup>+/+</sup> controls. Ejaculation behaviour was not observed in any TGF $\beta$ 1<sup>-/-</sup> mice regardless of TGF $\beta$ 1 replacement, compared to TGF $\beta$ 1<sup>+/+</sup> controls where >90% mice displayed ejaculated.

Modest improvement in the copulation activity of the TGF $\beta$ 1<sup>-/-</sup> mice receiving exogenous TGF $\beta$ 1 suggests that systemic TGF $\beta$ 1 availability can influence reproductive performance in male TGF $\beta$ 1<sup>-/-</sup> mice. However since fertility was not restored, locally produced TGF $\beta$ 1 in the reproductive tract and/or hypothalamic pituitary axis are also implicated in regulating fertility. These findings advance our knowledge of the role of the TGF $\beta$ 1 cytokine in male reproductive physiology and may have relevance for devising new treatments for infertility and erectile dysfunction in men.



## DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

.....  
Leanne Jane McGrath

April 2008

## ACKNOWLEDGMENT

It's a pleasure to finally be able to thank those that have helped me complete this project. I thank Associate Professor Sarah Robertson for providing me with such a significant opportunity, for steering the project and for critically assessing this thesis. Sarah also enhanced my awareness of the effort needed to achieve quality, completeness and relevance, an invaluable lesson. I sincerely thank my supervisor Dr Rebecca Robker for providing the clarity needed on many aspects of this work, she simplified the complexities and priorities that such a broad project can generate. I am also grateful to Becky for providing a kind and sympathetic ear during the more difficult times and for her considerable assistance in reviewing this thesis.

I would like to thank the staff and students of the Department of Obstetrics and Gynaecology for their advice and help with technical and administrative issues. Laboratory Animal Services provided much assistance by maintaining the mouse colony on a daily basis and staff within The Adelaide Microscopy Centre taught me the techniques of scanning electron microscopy. Thank-you.

In particular I would like to acknowledge the assistance of Alison Care for her technical assistance in preparing samples for the phagocytosis experiment and gathering data using FACS analysis. I would sincerely like to thank both Leanne Srpek for sharing her expertise in small animal surgery and Pat Grant for her advice with molecular biology protocols. A special thanks is extended to Associate Professor Bill Breed for imparting his expertise concerning the reproductive tract of male mice and for excising the tissues needed for my work on penile structure.

This research was undertaken with the support of an ARC Discovery Grant and Associate Professor Sarah Robertson's budget. It is gratefully acknowledged.