THE UNIVERSITY OF ADELAIDE
AUSTRALIA

The Effect of Paternal Obesity on Sperm Function, Embryo Development and Subsequent Pregnancy Outcomes

Hassan Waleed Bakos

B.Hlth.Sc.(Hons.)

Discipline of Obstetrics and Gynaecology

School of Paediatrics and Reproductive Health

Faculty of Health Sciences

The University of Adelaide

Thesis submitted in fulfilment of the requirements for the admission to the

Degree of Doctor of Philosophy in Medicine

December 2010

Table of contents

Thesis abstract

Obesity and its health consequences are an increasing health burden for Australian society, with more than seven million adults in Australia being overweight or obese. According to the Burden of Disease and Injury in Australia (BoD) study, high body mass was responsible for 7.5% of the total burden of disease and injury and is increasing. It is now clear that maternal obesity reduces fertility, in part through actions on the egg, which affect the health of the resultant pregnancy. However, the potential role of male obesity in infertility has been essentially ignored. This is surprising, as the male gamete contributes half of the genetic material of the embryo. Furthermore, sperm function constitutes the single most common cause of infertility. Therefore, factors influencing the health of the sperm and the underlying mechanisms behind any pathology are paramount.

The overall aim of this thesis was to determine the relationship between paternal obesity and male fertility. A novel and unique animal model was developed to elucidate the effects of paternal obesity on embryo development and pregnancy. Results showed that paternal obesity has negative effects on both sperm quality as well as embryo development and quality.

To confirm the results seen in the mouse, the pregnancy and live birth outcomes of a large cohort of patients undergoing assisted reproductive technology were assessed in relation to male obesity. Confirming the results observed in the mouse, it was clearly demonstrated that both sperm function parameters as well as embryo development were significantly reduced where the male partner is obese. This observation was evident after controlling for important factors such as maternal and paternal age as well as maternal body mass index (BMI). Furthermore, clinical pregnancy rates were reduced, miscarriage rates increased and live birth rates were reduced.

Moreover, analysis revealed that as paternal BMI increased, both seminal plasma glucose as well as insulin levels also increased, implicating the role of glucose, in particular, as detrimental to sperm function.

To further elucidate the metabolic markers involved, additional in-vitro studies were conducted whereby factors such as elevated glucose were shown to negatively affect sperm quality in vitro with elevated reactive oxygen species levels and DNA damage observed. These results are consistent with the effects seen on obese men and mice.

In summary, the current studies have shed some light on some longstanding questions regarding the effects of paternal obesity on fertility. Additional questions have been raised as to further understanding the mechanisms behind these effects (and their potential reversibility). This thesis has shed some much-needed light on a major knowledge gap in men‘s heath and will undoubtedly stimulate further interest in this very important area of applied science and medicine.

Declaration

This work contains no material that 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. The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University‘s digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Hassan W. Bakos

30 September 2010

Acknowledgements

Firstly, I would like to sincerely thank my primary supervisor Dr Michelle Lane for her guidance and support throughout my PhD. She has always encouraged me to take ownership of my work and allowed me to put my own ideas into practice. Her combined background of basic research as well as clinical practice has certainly given me a more comprehensive perspective on reproductive biology. A special thank you for being a mentor, not just a supervisor. At times when I felt things weren‘t going well, she has always made me focus on what I‘ve done well, a quality of hers, which certainly encouraged me to persevere and keep going. Thank you Michelle for accepting me as your first Andrology student! I do remember when my project was almost going to be on oocytes and embryos! But I‘m glad I persevered! Who would have thought that sperm function and beyond is now a major aspect of the Gamete and Embryos Biology Group; yes gamete not just oocytes and embryos. Finally, thank for reading my chapters so quickly, especially the last few! It has been an honour to work under your supervision.

My thanks also go to my co-supervisor Emeritus Professor Brian P. Setchell for being incredibly generous with his time and for making himself available whenever I needed him. Thank you for all the references, which I would never have read if it wasn‘t for his library collection. Professor Setchell is a wealth of knowledge and I‘m proud to have been able to work under his guidance.

I would like to express my gratitude to some key people throughout my PhD. These include Associate Professor Jeremy Thompson for his encouragement and belief in me. Many thanks to Dr Megan Mitchell who guided me through many aspects of the embryology work. This work

was definitely worth doing and clearly has resulted in many other collaborative projects and publications.

Thanks to members of the Gamete and Embryo Biology Group who helped in one way or another (especially on those long days when the animal embryology was being carried out). These include Alicia Filby, Sarah Wakefield, Nicole Palmer and Samantha Schulz. A special thanks also to David Froiland for his technical support.

Thanks also to members of the Discipline of Obstetrics and Gynaecology, School of Paediatrics and Reproductive Health, the Research Centre for Reproductive Health and the Robinson Institute, particularly for the nominations, invitations to special events and the Robert Seamark Award! I appreciate the support of my postgraduate coordinators Dr David Mottershead, Associate Professor David Kennaway, Dr Carmella Riccadelli and other members of the postgraduate committee.

I would like to thank another special organisation, without which I would not have been able to conduct much of the work in this PhD: Repromed. Thank you to the clinical staff and members of the scientific advisory committee for encouraging research at the clinic. Many thanks to all the nurses who were involved in the BMI measurements. A special thanks to members of the Andrology Laboratory, Margaret Szemis, Melissa Heinze and Ozlem Tunc. I would also like to thank Dr Tim Harwood, Professor David Handelsman and Professor Gail Risbridger for their assistance and advice with testosterone measurements and Dr Miles De Blasio for the metabolite measurements. Many thanks also to Ms Nena Bierbaum for her grammatical and formatting assistance.

Finally, to my family, particularly my mother and brother, my enduring thanks. To my mother who supported me in every possible way to ensure that I was able to complete my studies. To my brother for being very supportive throughout the last four years. To my late father for his

encouragement, inspiration and words of wisdom to achieve the best in my career. Thank you all from the bottom of my heart. Thanks to all my other extended family members and friends who have kept me in their thoughts and prayers. Your prayers have finally been answered!

Publications arising from this thesis

Bakos HW, Mitchell M, Setchell BP and Lane M. The effect of paternal diet induced obesity on sperm function and fertilisation in a mouse model. *International Journal of Andrology;* 2010 Jul 23. [Epub ahead of print].

*****Mitchell M, ***Bakos HW** and Lane M. Paternal diet induced obesity impairs embryo development and implantation in the mouse. *Fertility and Sterility;* 2010 Nov 1. [Epub ahead of print].

Bakos HW, Henshaw RC, Mitchell M and Lane M. Paternal body mass index is associated with decreased blastocyst development and reduced live birth rates following assisted reproductive technology. *Fertility and Sterility;* 2010. [In press].

*** Equal contribution**

Abstracts arising from this thesis

Published abstracts

Bakos HW (2010) Obesity and sperm. *Australian and New Zealand Journal of Obstetrics and Gynaecology*; **50** (Suppl. 1): 15-15

Invited Abstract

Bakos HW, Palmer NO and Lane M (2010) The effect of paternal diet induced obesity on sperm capacitation, acrosome reaction, binding and fertilisation in mouse model. *Reproduction, Fertility and Development*; **22** (Suppl.): 41–41

Awarded Society for Reproductive Biology Student Travel Grant

Bakos HW, Mitchell M and Lane M (2009) Paternal obesity decreases clinical pregnancy rates following ART: The missing piece of the puzzle. *Australian and New Zealand Journal of Obstetrics and Gynaecology*; **49** (Suppl. 1): A2-A2

Bakos HW, Mitchell M, Setchell BP and Lane M (2009) Paternal obesity impairs sperm function and subsequent embryo and pregnancy outcomes. *Reproduction, Fertility and Development*; **21** (Suppl.): 65–65

Awarded the Society for Reproductive Biology Oozoa Award

Awarded Finalist Young Investigator Award

Bakos HW, Mitchell, M and Lane M (2009) Male obesity induces oxidative stress and DNA damage in mouse spermatozoa. *Human Reproduction*; **46** (Suppl. 1): i224-i224

Awarded Faculty of Health Sciences Travel Fellowship

Awarded Research Centre for Reproductive Health Travel Grant

Mitchell M, **Bakos HW**, and Lane M (2009**)** Male obesity: Consequences for embryo development and fetal outcomes. *Journal of Developmental Origins of Health and Disease*; **1** (Suppl. 1): S43–S43

Bakos HW and Lane, M (2008) Differential effects of hexoses on sperm metabolism and function in culture. *Reproduction Fertility and Development*; **20** (Suppl.): 83–83

Awarded Society for Reproductive Biology Student Travel Grant

Awarded Research Centre for Reproductive Health Travel Award Grant

Bakos HW, Thompson JG and Lane M (2007) Sperm DNA damage: Is carbohydrate metabolism to blame? *Fertility and Sterility*; **88** (Suppl. 1): S48-S49

Awarded the Society for Male Reproduction & Urology Travelling Scholar Award

Awarded Research Centre for Reproductive Health Travel Grant

Bakos HW, Froiland DA, Thompson JG and Lane M (2007) Elevated glucose levels induce lipid peroxidation and DNA damage in human spermatozoa. *Australian and New Zealand Journal of Obstetrics and Gynaecology*; **47** (Suppl. 1): A1-A1

Other abstracts

Bakos HW (2009) Obesity and male fertility: Effects and potential mechanisms. Proceedings of the International Student Research Forum (ISRF), Griffith University, Gold Coast

Selected to represent the University of Adelaide by the Head of School of Paediatrics and Reproductive Health

Later awarded ISRF Best Presentation Award

Bakos HW, Thompson JG and Lane M (2007) Sperm DNA damage and carbohydrate metabolism: Is there a link? Proceedings of the South Australian Annual Scientific Meeting of the Australian Society for Medical Research, Adelaide

Awarded Most Outstanding Presentation of Clinical Research by the Australian Society for Medical Research

List of figures

List of tables