

The Impact of Maternal Ageing and Chemotoxicants on Female Fertility

Nicole Jacqueline Camlin

B. Biomedical Science (Hons Class I)

*Thesis submitted to the Faculty of Science and Information
Technology, The University of Newcastle, Australia in fulfilment of
the requirement of the degree of the Doctor of Philosophy*

Friday 7th October 2016



THE UNIVERSITY OF
NEWCASTLE
AUSTRALIA

Declaration

I hereby declare that all material found within this thesis to the best of my knowledge has not been published or written by another person, except where due reference is given. In addition, no part of this dissertation has been previously submitted to The University of Newcastle or any other tertiary institution in order to obtain a degree or diploma. I also hereby consent to copy/copies of this thesis to be made available for both loan and photocopying from the University Library subject provisions set forth in the Copyright Act 1968.

Statement of Authorship

I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publication/s/scholarly work.

Signed

Nicole Jacqueline Camlin

Date: 6th October 2016

Dr Janet Holt

(Primary Supervisor)

Date: 6th October 2016

Prof. Eileen McLaughlin

(Secondary Supervisor)

Date: 6th October 2016

Acknowledgments

Firstly, I would like to thank my supervisor Janet; you have truly been an inspiration throughout my PhD. I am so thankful for all the guidance and support you have given me. Even during your maternity leave I never felt like I didn't have support and you were always a text message away when I needed you. You have truly helped me grow into the scientist I am today, and words cannot express to you how thankful I am for that. To my co-supervisor Eileen, thank you for the support throughout the years. You have been a huge encouragement throughout my candidature freely offering your years of wisdom and knowledge.

To the rest of the reproductive science group, especially the PhD and honours students thank you for all the fun times and encouragement. You guys really have made these years fly by and without you it just wouldn't have been as enjoyable. In particular I would like to thank Bettina and Aimee. Aimee, your constant enthusiasm and excitement for any small achievement I made has truly been a blessing throughout the years. Furthermore, your "Haten Katen" ways have brought much laughter and joy into the office. Bettina, you have truly become one of my best friends. I can't image my PhD without you. You have been a shoulder to cry on, an ear to complain to, someone to laugh (and sing with), thank you for all the good times. I would also like to thank Jessie, Shaun and Kate for your advice, assistants and words of encouragement throughout my PhD.

To my family, you guys have always been amazing. To my Mum and Dad, you are the best parents I could have. You have always been crazy supportive of anything I have wanted to do. Your support, encouragement, love and unwavering belief that I can do anything I put my mind to has allowed me to become the person I am today, thank you. To my sister, brother-in-law and beautiful nephew, thanks for all the support, visits and photos. Having a nephew is truly the best distraction on bad days. To Andrew, who has, in the last decade become a large part of my family. Meeting you in first year was one of the best things to have happened.

Your love of science and your desire for research inspired me to begin this PhD path, you helped me discover my passion, so thank you.

Finally, to my loving partner, Mitch, you have been amazing. Your patience and calmness help ground me. Thank-you for all the weekends keeping me company as I imaged, the late-night injection trips and the endless listening to practice talks. You have been my rock throughout my PhD, listening to the endless complaints, enduring my stressful weeks and celebrating my wins. Your love and support is truly unbelievable and I can't thank you enough for just being you.

Manuscripts Included as Part of Thesis

Camlin, N. J., McLaughlin, E.A., and Holt, J.E. (2014). “Through the smoke: Use of in vivo and in vitro cigarette smoking models to elucidate its effect on female fertility.” Toxicology and Applied Pharmacology **281**(3): 266-275.

Camlin, N.J., Sobinoff, A.P., Sutherland, J.M., Beckett, E.L., Jarnicki, A.G., Vanders, R.L., Hansbro, P.M., McLaughlin E.A., Holt, J.E. (2016). “Maternal smoke exposure impairs the long term fertility of female offspring in a murine model.” Biology of Reproduction **94**(2):39.

Conference Proceedings Relevant to Thesis

Camlin, N. J., Sobinoff, A.P., Sutherland, J.M., Hansbro, P.M., McLaughlin, E.A., and Holt, J.E. In utero exposure to mainstream cigarette smoke: long term effects on reproductive potential of female pups. 44th annual meeting for the Society of Reproductive Biology, August 2013, Sydney, Australia. (Oral)

Camlin, N. J., Sobinoff, A.P., Sutherland, J.M., Hansbro, P.M., McLaughlin, E.A., and Holt, J.E. Long-term reproductive effects of in utero exposure to mainstream cigarette smoke on female offspring. 18th Annual RHD conference. Newcastle, Australia. November, 2013. (Oral)

Camlin, N. J., Sobinoff, A.P., Sutherland, J.M., Hansbro, P.M., McLaughlin, E.A., and Holt, J.E. Maternal and Grand-maternal exposure to cigarette smoke: The long term effects on female fertility. 45th annual meeting for the Society of Reproductive Biology, August 2014, Melbourne, Australia. (Oral)

Camlin, N.J., Sobinoff, A.P., Sutherland, J.M., Hansbro, P.M, McLaughlin E.A., Holt, J.E. Maternal and Grand-maternal exposure to cigarette smoke: The long term effects on oocyte quality. The EMBO Conference on Meiosis, August-September 2015, Oxford, England. (Poster)

Camlin, N.J., Redgrove, K.A., McLaughlin, E.A., and Holt, J.E. Motoring through: The role of Kif4 in female meiosis. 20th Annual RHD conference, November 2015, Newcastle, Australia. (Oral)

Camlin, N.J., Sobinoff, A.P., Sutherland, J.M., Hansbro, P.M, McLaughlin E.A., Holt, J.E. Maternal and Grand-maternal exposure to cigarette smoke: The long term effects on oocyte quality. 20th Annual RHD conference, November 2015, Newcastle, Australia. (Poster)

Camlin, N. J., McLaughlin, E.A., and Holt, J.E. A kinesin motor protein is essential for normal mammalian oocyte meiosis. 47th annual meeting for the Society of Reproductive Biology, August 2016, Gold Coast, Australia. (Oral)

Awards

Finalist for the Oozoa Award for best oral student presentation, 45th Annual Conference for Society of Reproductive Biology, Melbourne, Australia (2014)

Faculty of Science and I.T Conference Scholarship, University of Newcastle, Australia (2015)

Best Poster Presentation, 20th Annual RHD Conference, Newcastle, Australia (2015)

Finalist for the Oozoa Award for best oral student presentation, 47th Annual Conference for Society of Reproductive Biology, Gold Coast, Australia (2016)

Winner of Science Meets Publican 60 Second Elevator Pitch, 47th Annual Conference for Society of Reproductive Biology, Gold Coast, Australia (2016)

Additional Publications and Conference Proceedings

Kumar, M., **Camlin, N.J.**, Holt, J.E., Teixeira, J.M., McLaughlin, E.A., and Tanwar, P.S. (2016). Germ cell specific overactivation of WNT/ β catenin signaling has no effect on folliculogenesis but causes fertility defects due to abnormal foetal development. Scientific Reports **6**:27273. (Journal Article)

Mihalas, B.P., **Camlin, N.J.**, McLaughlin, E.A., and Nixon B. The Impact of oxidative stress on oocyte meiosis. 3rd Annual Australian Society for Medical Research Satellite Scientific Meeting, April 2016, Newcastle, Australia. (Poster)

McLaughlin, E.A., Bernstein, I.R., Mihalas, B.P., Pye, V., **Camlin, N.J.**, McCluskey, A., Nixon, B., Holt, J.E., and Redgrove, K.A. Dynammin 2: Roles in female fertility. 49th Annual Conference for the Society for the Study of Reproduction, July 2016, San Diego, USA. (Oral)

Mihalas, B.P., **Camlin, N.J.**, McLaughlin, E.A., and Nixon B. Lipid peroxidation contributes to the ROS mediated deterioration of meiotic competency and quality of oocytes. 47th annual meeting for the Society of Reproductive Biology, August 2016, Gold Coast, Australia. (Poster)

Table of Contents

Declaration.....	II
Acknowledgments.....	III
Manuscripts Included as Part of Thesis	V
Conference Proceedings.....	VI
Awards	VII
Additional Publications.....	VIII
Abstract.....	10
Chapter 1: Through the smoke: Use of in vivo and in vitro cigarette smoking models to elucidate its effect on female fertility.....	12
1.1: Statement of Contribution.....	13
1.2: Overview.....	14
1.3: <i>Through the smoke: Use of in vivo and in vitro cigarette smoking models to elucidate its effect on female fertility</i>	15
Chapter 2: Maternal smoke exposure impairs the long-term fertility of female offspring in a murine model.....	25
2.1: Statement of Contribution.....	26
2.2: Overview.....	27
2.3: <i>Maternal smoke exposure impairs the long-term fertility of female offspring in a murine model</i>	28
Chapter 3: Grandmaternal but not great-grandmaternal smoke exposure reduces female fertility in a murine model.....	40
3.1: Statement of Contribution.....	41
3.2: Overview.....	42
3.3: <i>Grandmaternal but not great-grandmaternal smoke exposure reduces female fertility in a murine model</i>	43
Chapter 4: The role of kinesin motor protein Kif4 in female meiosis.....	76
4.1: Statement of Contribution.....	77
4.2: Overview.....	78
4.3: <i>The role of kinesin motor protein Kif4 in female meiosis</i>	79
Chapter 5: Motoring through: The role of kinesin superfamily proteins in female.....	104
5.1: Statement of Contribution.....	105
5.2: Overview.....	106
5.3: <i>Motoring through: The role of kinesin superfamily proteins in female meiosis</i>	107
Concluding Remarks and Future Directions.....	136
Appendices.....	142
Appendix A: Chapter 2 Supplementary Data.....	143
Appendix B: Chapter 3 Supplementary Data.....	146
Appendix C: Chapter 4 Supplementary Data.....	148
Appendix D: Abstract and Chapter Overview References.....	153
Appendix E: Additional Publication	157

Abstract

Mammalian females are born with all the oocytes they will ever have. The quality and quantity of these gametes dictates the reproductive life span of a woman. In recent decades it has been well established that oocyte quality decreases with increasing maternal age primarily as a result of oocytes failing to separate chromosomes correctly. The outcome is a condition known as aneuploidy, which can result in increased rates of miscarriage and birth defects such as Down's Syndrome. Additionally, exposure of oocytes to chemotoxicants, including those found in cigarette smoke, has been found to prematurely reduce oocyte quality in women leading to early onset menopause. The aim of this thesis is to investigate potential causes of female fertility decline, including ageing and multigenerational exposure to cigarette smoke.

Research within this thesis highlights the role of *in utero* and multigenerational smoke exposure in reducing female fertility. Approximately 12% of Australian and American women smoke throughout their pregnancy (Australia's mothers and babies, 2015; Tong et al., 2009). This has been linked to decreased birth weight, increased risk of sudden infant death syndrome, childhood cancers and asthma in *in utero* exposed offspring (maternal smoke exposed). Additionally, a growing body of evidence suggests that these maternal smoke exposed females have a reduction in fecundability in later life (Weinberg et al., 1989; Ye et al., 2010). However, the cause of this reduced fertility, and the fertility of the subsequent generations is largely unknown. Throughout this thesis, I detail how maternal and grandmaternal smoke exposure decreases female fertility in a mouse model, whilst great-grandmaternal smoke exposure appears to have little effect.

In addition to investigating the effects of multigenerational smoke exposure on female fertility, this thesis also explores the impact of maternal ageing on oocyte quality. It has been

well established that oocyte quality decreases with increasing age (Hassold et al., 2007; Jones, 2008). However, the molecular mechanisms underpinning this phenomenon are still being unravelled. In order to gain further insight into the fidelity of the oocyte cell cycle, I chose to examine a member of the kinesin motor protein family, Kif4. Kinesins are known to be important for the mitotic cell cycle, but little is known about how they function in mammalian oocytes. In mitosis Kif4 is involved in chromosome condensation and separation, metaphase and midzone spindle formation and cytokinesis (Hu et al., 2011; Mazumdar et al., 2004; Samejima et al., 2012). I show here for the first time that Kif4 has dynamic localisation throughout meiosis, and importantly, that it has essential roles in female meiosis, including spindle formation and polar body extrusion (cytokinesis). Furthermore, Kif4 appears to have roles in trafficking kinetochore proteins Ndc80 and CENP-C under the control of Aurora Kinase B and Cdk1. Finally, I show that Kif4 protein levels are elevated in metaphase I and II oocytes from reproductively aged mice, implicating a role for this protein in age related oocyte quality decline.

Collectively the data presented in this thesis helps build a clearer picture of the role of multigenerational smoke exposure and/or maternal ageing on reduced female fertility.