Quality Control Mechanisms Responsible for the Maintenance of Genomic Integrity in the Female Germline

Jacinta Martin
Bachelor of Biotechnology (Honours 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

Date: 31st August 2018

This research was supported by an Australian Government Research Training Program (RTP) Scholarship

DECLARATION

Statement of Originality

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, 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. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

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.

Thesis by Publication

I hereby certify that this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

Signed: ….....

Jacinta Hope Martin

ACKNOWLEDGEMENTS

I would like first like to begin by expressing my deepest gratitude to my supervisors, Brett, John and Liz. To Brett, words are inadequate to express how truly grateful I am for your supervision. You have granted me the freedom to follow my ideas and provided me with endless opportunities along the way. You have quickly become my mentor and friend and I'm truly very grateful to be a part of the 'Nixon Family'. John, you sincerely are one of the most inspiring individuals I have ever met. From the moment we met you have shared a passion for scientific investigation and reproductive biology, your enthusiasm and encouragement have been a cornerstone to my candidature and I'm so grateful to have had the opportunity to work alongside you. Liz amongst everyone you have been the biggest asset to this experience. I'm so grateful for your unwavering support, advice and mentorship but above all else I have immensely appreciative the friendship we have forged. You really are the most incredible person I have met. Your kindness, passion for life and strength are unmatched and are going to take you so very far. We have already shared so many crazy adventures and I know that no matter where we end up that there will be many more to come. I'm excited to see what the future holds for you and to hear about every new accomplishment and success that is undoubtedly coming your way.

Shaun, while only an unofficial supervisor you have in your own way taught me more than I could have imagined. You have challenged me to be better than I am, to take more risks and to be confident. You have also been there when times have been tough and I'm so thankful for this. Tessa, you have been a constant through my experience with the Reproductive Science Group. You dutifully took up your role as my mentor through placement and quickly became an irreplaceable friend. Over the years you have provided me with a wealth of unwavering support and encouragement. Alle, I really could not have imaged this experience without you. You have one of the most wonderful minds that inspires a deeper understanding of the world. I'm so grateful for your friendship and I can't see what the future hold for you!

Kasey, you have been with me since the beginning of my time in the Reproductive Science group and I could not have asked for a better friend to share, learn and grow alongside of during these past few years. Brendan, you too have been significant throughout this journey. I'm so grateful for your crazy ways and ability to light up a room, you have added so much joy to this experience. To the rest of the PhD

crew Roisin, David, Heather, Natalie, Jess, Bettina, Caitlin, Emma, Sarah, Wei and Jacob it has truly been a pleasure sharing this experience with you all and I can't wait to see what the future holds for us all.

There has been a great deal of many other people whom without this work would not have been possible and while it isn't possible to name them all I especially wanted to acknowledge the incredible support of Amanda, Haley, Sara, Ilana, Mona Kristen and Sarah. You are all so immensely talented and a credit to the lab. Jessie and Kate, you have become a part of my science family and for that I am extremely grateful. Thank you for your advice, mentorship, friendship and laughter. Angela, you are an especially important member of the group. Thank you for always going above and beyond for me and NEVER forget that I am your favourite.

Finally my greatest thanks go to my family. My Mum, Dad, and brothers you have had to endure the ups and downs of PhD life alongside me but have always unwaveringly met the challenge. Your support has been instrumental in this achievement and I'm so appreciative to have had you along for the ride.

PUBLICATION AND AWARDS ARISING FROM THIS THESIS

1. Publications

Chapter 1*:*

1. **Martin, J.H**., Bromfield, E.G., Aitken, R.J., Nixon, B., (2017). Biochemical alterations in the oocyte in support of early embryonic development. Cellular and Molecular Life Sciences 74: 469-485. DOI: 10.1007/s00018-016-2356-1.

Published | Cellular and Molecular Life Sciences

Chapter 2:

2. **Martin, J.H.,** Nixon, B., Lord, T., Bromfield, E.G., Aitken, R.J., (2016). Identification of a key role for permeability glycoprotein in enhancing the cellular defense mechanisms of fertilized oocytes. Developmental Biology. 1;417(1):63-76. DOI:10.1016/j.ydbio.2016.06.035.

Published | Developmental Biology

3. **Martin, J.H.,** Bromfield, E.G., Aitken, R.J., Lord, T., Nixon, B., (2016). Data on the concentrations of etoposide, PSC833, BAPTA-AM, and cycloheximide that do not compromise the vitality of mature mouse oocytes, parthenogenetically activated and fertilized embryos. Data in Brief; 8: 1215–1220. DOI: 10.1016/j.dib.2016.07.046.

Published | Data in Brief

Chapter 3:

4. **Martin, J.H.,** Bromfield, E.G., Aitken, R.J., Lord, T. and Nixon, B. (2018). Double strand break DNA Repair occurs via Non-Homologous End-Joining in Mouse MII Oocytes. Scientific Reports; 8 (1); 9685. DOI: 10.1038/s41598-018-27892-2

Published | Scientific Reports.

Chapter 4:

5. **Martin, J.H**., Aitken, R.J., Bromfield, E.G., Cafe, S.L., Frost, E.R., Sutherland, J.M, Nixon, B and Lord, T (2018). Investigation into the presence and functional significance of proinsulin C-peptide in the female germline.

Submitted 26/08/18| Biology of Reproduction

Chapter 5:

6. **Martin, J.H.,** Aitken, R.J., Bromfield, E.G. and Nixon, B. (2018). DNA damage and repair in the female germline; contributions to assisted reproductive technologies

Submitted June 2018 | Human Reproduction Update

2. Statements of Contribution

I attest that the Research Higher Degree candidate Jacinta Martin has contributed upward of 50% towards data collection/analysis and manuscript preparation for all the publications included in this thesis for which I am a co-author.

……

Professor Brett Nixon
Date: 16/08/18

……

Laurate Professor. John Aitken
Date: 19/08/18

…… ……

Dr. Elizabeth Bromfield
Date: 14/08/18

……

Dr. Tessa Lord
Date: 08/08/18

……

Dr Jessie Sutherland
Date: 15/08/18

……

Miss Emily Frost
Date: 09/08/18

……

Miss Shenae Cafe
Date: 14/08/18

……

Professor Frances Martin
Assistant Dean of Research Training
Date: 27/08/2018

3. Conference proceedings relevant to this thesis

2018:

1. **Martin, J.H,** Bromfield, E.G , Lord, T., Aitken, R.J., and Nixon, B. DNA repair and protection in the female germline. 50th Annual Scientific Meeting of the Society for Reproductive Biology, Adelaide, Australia. August 2018.

   Oral Presentation/ Finalist for the Oozoa award

2. **Martin, J.H,** Bromfield, E.G , Lord, T., Aitken, R.J., and Nixon, B. DNA repair and protection in the female germline. Gordon Research Seminar- Mammalian Reproduction, Renaissance Tuscany Il Ciocco, Italy, August 2018.

   Poster presentation

3. **Martin J.H,** Bromfield, E.G, Lord, T., Aitken, R.J and Nixon, B. DNA repair and protection in the female germline. Australian Society for Medical Research (ASMR) Satellite Scientific Meeting, Newcastle, Australia. June 2018.

   Poster presentation

2017:

1. **Martin, J.H,** Bromfield, E.G, Aitken, R. and Nixon, B. Double Strand Break Repair occurs via Non Homologous End Joining in mouse MII oocytes. 49th Annual Scientific Meeting of the Society for Reproductive Biology, Perth, Australia. August 2017.

   Oral presentation

2. **Martin, J. H,** Lord, T., Nixon B., and Aitken, R. J. Identification of a key role for permeability glycoprotein in enhancing the cellular defence mechanisms of fertilized oocytes. The 20th Annual Frontiers in Reproduction Symposia, Marine Biological institute, Woods Hole, Massachusetts, USA. June 2017.

   Oral presentation

3. **Martin, J. H**, Lord, T., Nixon B., Aitken, R. J. Identification of a key role for permeability glycoprotein in enhancing the cellular defence mechanisms of fertilized oocytes. The Australia and New Zealand Society for Cell and Developmental Biology-Cell & Developmental Biology Meeting. The University of New South Wales Sydney, Australia, USA. April 2017

   Oral presentation

2016:

1. **Martin, J. H**, Lord, T., Nixon B., Aitken, R. J. Identification of a key role for permeability glycoprotein in enhancing the cellular defence mechanisms of fertilized oocytes. 48th Annual Scientific Meeting of the Society for Reproductive Biology, Gold Coast, Australia. August 2016.

   Oral presentation

2. **Martin, J. H**, Lord, T., Nixon B., Aitken, R. J. Identification of a key role for permeability glycoprotein in enhancing the cellular defence mechanisms of fertilized oocytes. NSW Reproduction Forum, Newcastle, Australia. Invited speaker: December 2016.

   Invited speaker | Oral presentation

3. **Martin, J. H,** Lord, T., Nixon B., Aitken, R. J. Identification of a key role for permeability glycoprotein in enhancing the cellular defence mechanisms of fertilized oocytes. Australian Society for Medical Research (ASMR) Satellite Scientific Meeting, Newcastle, Australia. April 2016.

   Poster presentation

2015:

1. **Martin, J. H.,** Lord, T., Nixon B., Aitken, R. J. Permeability glycoprotein enhances cellular drug exclusion in the fertilised oocyte; upholding DNA integrity. 20th Annual Newcastle University Research Higher Degree conference. Newcastle, Australia. December 2015.

   Oral presentation | Winner of the 'Best presentation prize'

2. **Martin, J. H.,** Lord, T., Nixon B., Aitken, R. J. Permeability glycoprotein enhances cellular drug exclusion in the early embryo, upholding DNA integrity. 47th Annual Scientific Meeting of the Society for Reproductive Biology. Adelaide, Australia. August 2015.

   Oral presentation | Finalist in the Oozoa award

3. **Martin, J. H.,** Lord, T., Nixon B., Aitken, R. J. Permeability glycoprotein enhances cellular drug exclusion in the fertilised oocyte; upholding DNA integrity. Australian Society for Medical Research (ASMR) Satellite Scientific Meeting, Newcastle, Australia. April 2015.

   Poster presentation

4. Invited Seminars:

2018

1. **Southhampton University**, South Hampton**, UK|** July 2018

   Seminar title: 'DNA repair and protection in the female germline'

2017

1. **Brown University**, Rhode Island**, USA|** June 2017

   Seminar title: 'How oocytes and embryos protect their genetic integrity'.

2. **Northwestern University,** Chicago, **USA|** June 2017

   Seminar title: 'How oocytes and embryos protect their genetic integrity'.

3. **University of Montreal Hospital Centre**, Montreal, **Canada|** June 2017

   Seminar title: 'How oocytes and embryos protect their genetic integrity'.

4. **McGill University,** Quebec, **Canada|** June 2017

   Seminar title: 'How oocytes and embryos protect their genetic integrity'.

2016

1. **The NSW Reproduction Forum, Hunter Medical Research Institute, Newcastle, Australia |** December 2016

   Seminar title: Seminar title: **'**Identification of a key role for permeability glycoprotein in enhancing the cellular defence mechanisms of fertilized oocytes'.

2. **Newcastle University, Newcastle, Australia |** July 2016

   Seminar title: **'**A PhD in reproductive science: Investigating oocyte and embryo genetic integrity'.

5. Additional Publications

2018

1. Nixon, B., Johnston, S.D., Skerrett-Byrne, D.A., Anderson, A.L., Stanger, S.J., Bromfield, E.G., **Martin, J.H.,** Hansbro, P.M., Dun, M.D. (2018). Proteomic profiling of Australian saltwater crocodile (*Crocodylus porosus*) spermatozoa refutes the tenet that post-testicular maturation is restricted to mammals**|** (2018). **Molecular and Cellular Proteomics**; doi: 10.1074/mcp.RA118.000904

2. Heat exposure induces oxidative stress and DNA damage in the male germ line. Houston B.J, Nixon B, **Martin J.H**, De Iuliis G.N, Bromfield E.G, McEwen K.E, Aitken R.J. **Biology of Reproduction** (2018). Jan 17. doi: 10.1093/biolre/ioy009.

3. Houston, B.J., Nixon, B., **Martin, J. H,** Mcewan, K.E, King, B.V., Aitken, R.J, De Iuliis, G.N. (2018). Whole body exposure to radiofrequency electromagnetic radiation induces DNA fragmentation in mouse spermatozoa. **Manuscript in preparation | PLOS One.**

2017

1. Flourishing follicles: Overview of ovarioles. Kelleher A.M, Khalaj K, **Martin J.H**, Scaia M.F, Wilson R.L. **Molecular Reproduction and Development** (2017). Dec;84(12):1237. doi: 10.1002/mrd.22858.

2015

1. Murine inhibin α-subunit haploinsufficiency causes transient abnormalities in prepubertal testis development followed by adult testicular decline. Itman C., Bielanowicz A., Goh H., Lee Q., Fulcher A.J., Moody S.C., Doery, J.C., **Martin J.,** Eyre S., Hedger M.P., Loveland K.L (2015). **Endocrinology**; 156(6). DOI: 10.1210/en.2014-1555.

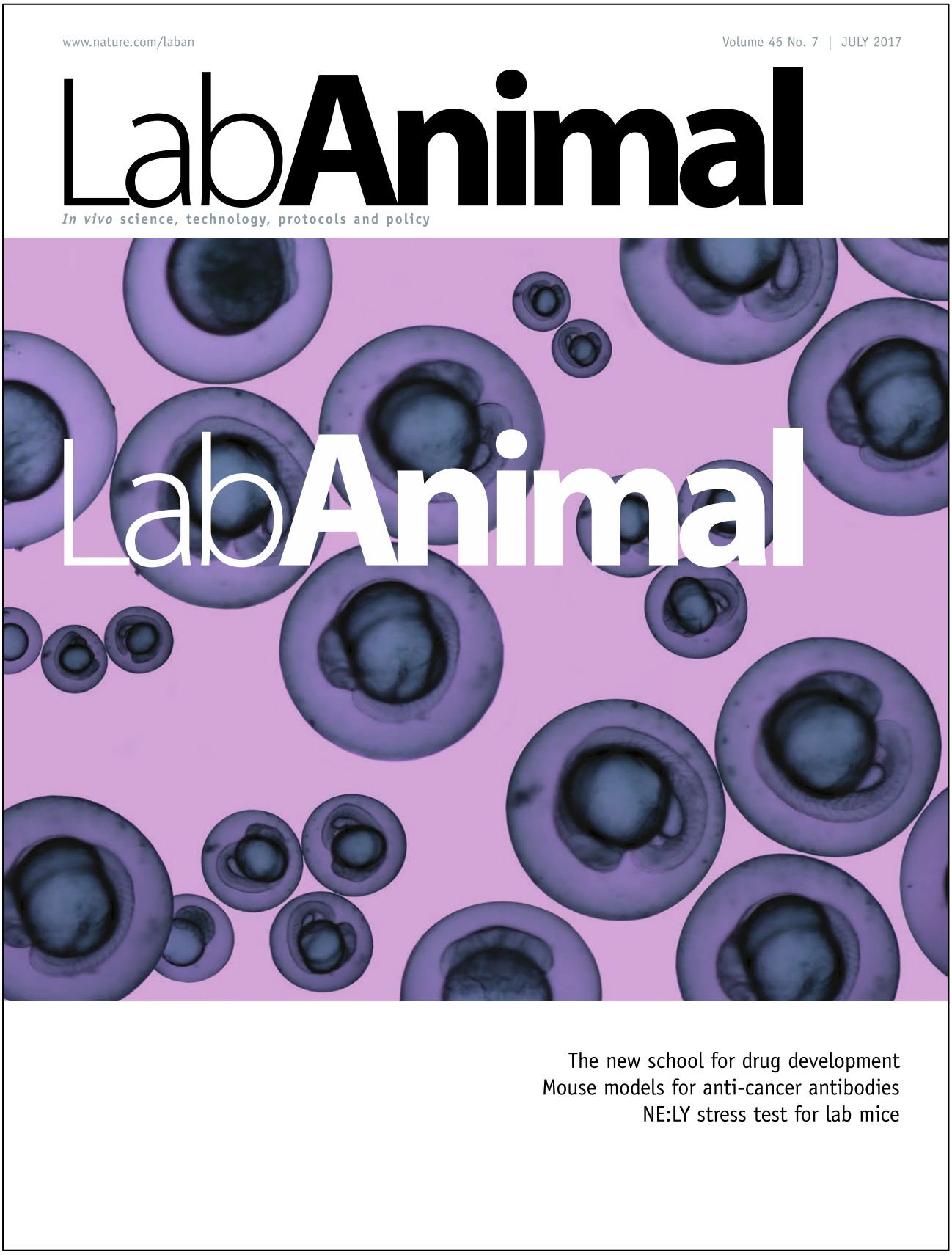
2014

1. Accumulation of electrophilic aldehydes during post- ovulatory ageing causes reduced fertility, oxidative stress and apoptosis. Lord, T., **Martin, J. H**., Aitken, R. (2014). **Biology of Reproduction** December; 92(2). DOI: 10.1095/biolreprod.114.122820.

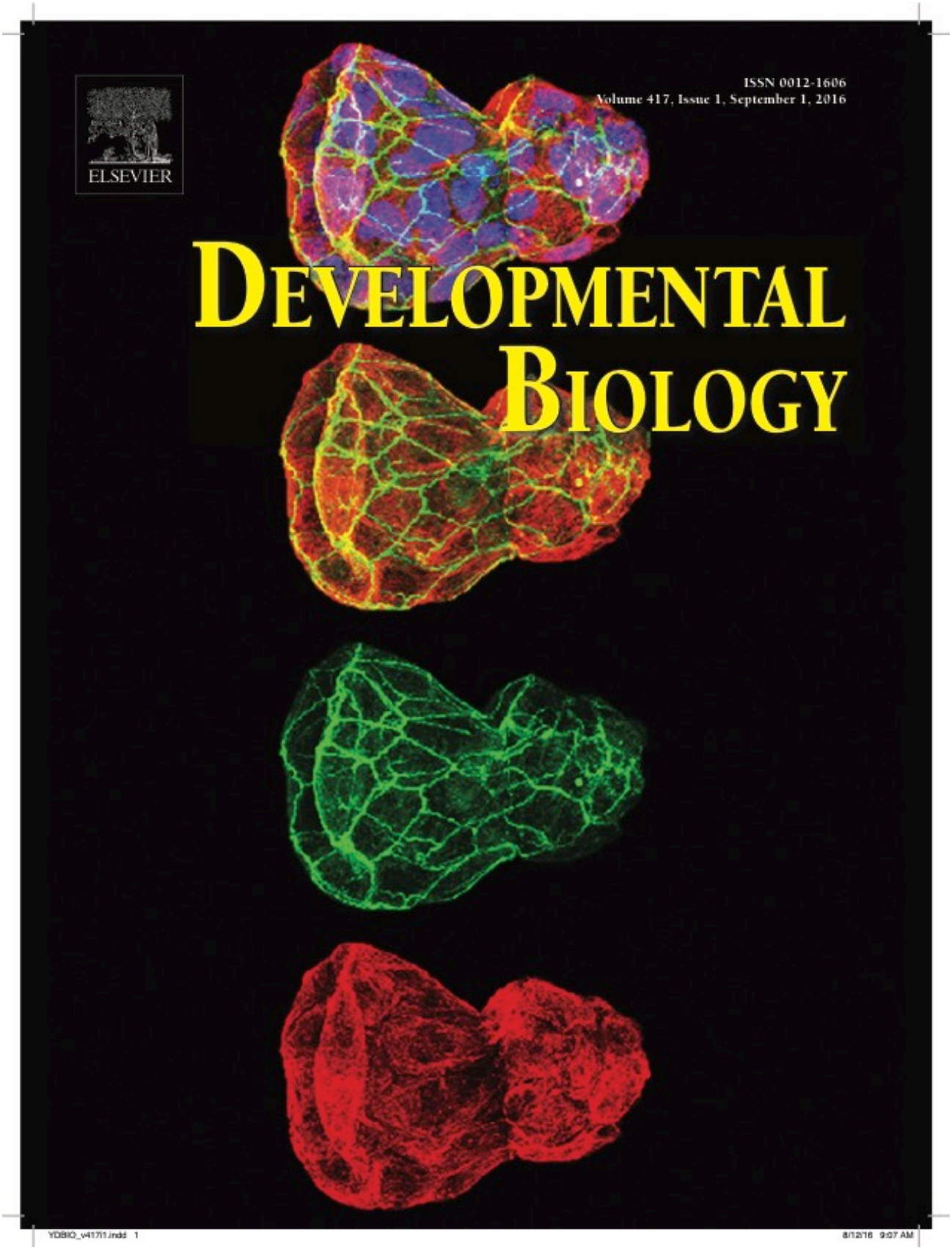
2. Accumulation of electrophilic aldehydes during postovulatory ageing causes reduced fertility, oxidative stress and apoptosis. Lord, T., **Martin, J. H.,** Aitken, R. (2014). **Fertility and Sterility**; 102(3):e330. DOI: 10.1016/j.fertnstert.2014.07.1118.

6. Cover Images

Nature Lab Animal, Volume 46, No. 7, July 2017



Developmental Biology, Volume 417, Issue 1 (1st September 2016).



http://www.journals.elsevier.com/developmental-biology/cover-of-the-week/volume-417-issue-1-1st-september-2016

7. Distinctions and awards

Tuition scholarship| University of Newcastle for the PhD2Postdoc course (2018)| **$180**

Jennie Thomas Medical Research Travel Grant (2017)| HMRI | **$8900**

Greaves Family Postgraduate Scholarship in Medical Research (2017)| HMRI | **$5000**

Frontiers in Reproduction Tuition Scholarship (2017)| Marine Biological Institute and the University of Chicago| **$ 8500**

Higher Degree Research International Conference Scholarship (2017)| UON |**$2500**

Travel Grant (2017)| Faculty of Science, UON to attend Frontiers in Reproduction| **$1500**

Travel Grant (2017)| Australia and New Zealand Society for Cell and Developmental Biology-Cell and 'The company of biologists' |UNSW **$100**

Best Science image (2017)| Australia and New Zealand Society for Cell and Developmental Biology-Cell| UNSW | **$100**

Newcastle University Faculty of Science and IT Best HDR Publication Award (2016)| UON| **$1000**

Newcastle University School of Environmental and Life Sciences 'Excellence Award for First Year Presentation' 20th annual RHD conference| UON| December 2015| **$100**

Newcastle University School of Environmental and Life Sciences Priority Research Centre in Reproductive Science research in the molecular basis of sperm-egg interaction vacation scholarship 2013/2014. Effective date 11/04/2014| UON | **$1500**

Australian Postgraduate PhD Award in the Faculty of Science and Information Technology. 22/02/2015- 22/02/2018

Award Finalist:

Oozoa Award 'Best student presentation' |Society for Reproductive Biology | 2018

Travel Award | Reproductive Biology Journal| 2018

Travel Award | Biology Journal| 2018

HMRI-SA 'Future' Medical Research Travel Grant | 2016

Oozoa Award 'Best student presentation' |Society for Reproductive Biology | 2015

Table of contents

ABSTRACT

DNA is the genetic repository containing the necessary information for cellular viability, fate decisions and development. In the female germline, genetic integrity also underpins successful conception, embryonic development, pregnancy and the future health of the offspring. In spite of its importance, DNA remains a chemical entity prone to structural alteration. If left unresolved, these structural lesions have the potential to lead to mutation and broader-scale genomic aberrations, which may elevate the predisposition of individuals to non-communicable diseases in later life. While it is therefore likely that female germ cells possess a sophisticated suite of quality control mechanism to defend their genome, the precise nature of these defence systems is not well understood. Given this knowledge gap, the overall aim of the studies described within this thesis was to explore the endogenous DNA protection and repair machinery present in the mammalian oocyte and early embryo.

In completing these studies, we have uncovered several novel protective strategies employed by the oocyte and early preimplantation embryo to safeguard their genomic integrity. These include the first evidence for a critical link between fertilisation and the synthesis of transmembrane transporter molecules belonging to the multidrug resistant protein family. Specifically, we implicate permeability glycoprotein (PGP) in increasing the bi-directional transport capacity of the zygote immediately following fertilisation. We posit that the activity of membrane bound PGP counters the influx of genotoxic agents, shielding the embryonic pronuclei from the induction of DNA damage. Excitingly, we also demonstrate that the preservation of the maternal genome, prior to fertilisation, is enhanced by an endogenous store of DNA repair proteins accumulated during oogenesis, providing the first evidence of an active DNA repair program in the post-ovulatory (MII) oocyte. Accordingly, we

demonstrate a role for non-homologous end joining (NHEJ) as a repair platform for correcting damage of the maternal DNA prior to fertilisation.

Having demonstrated that the oocyte and preimplantation embryo contain a sophisticated suite of defence strategies for the detection, repair or prevention of DNA damage, we hypothesized that the efficacy of these defences may be augmented by pro-survival factors. We therefore explored the capacity of C-peptide, a hormone implicated in the regulation of intracellular signalling pathways, to modulate oocyte and early embryo biology. Through this work, we observed a previously unappreciated abundance of C-peptide within the mouse ovary, oocyte and follicular fluid and uncovered a putative interaction between C-peptide and the DNA repair enzyme, breast cancer type 2 susceptibility protein (BRCA2) following oocyte activation. Collectively, these findings lend support to a novel role for C-peptide in the female germline and raise the prospect that C-peptide may exert direct physiological effects within the female reproductive system.

Taken together, the findings reported in this thesis have enhanced our understanding of the maintenance of genetic stability in the female germline. Importantly, this collection of studies offers a molecular understanding of the endogenous capacity of the oocyte and preimplantation embryo to detect and subsequently respond to DNA damage and, in turn, identifies novel clinical targets to enhance oocyte competence *in vitro* and potentially improve assisted reproductive technologies.

FOREWORD

The uptake of assisted reproductive technologies has exponentially increased since the first successful cycle in 1978, such that 40 years on, 1 in 6 couples routinely seek recourse in assisted reproductive technologies (ART) to achieve pregnancy (Australian Institute of Health and Welfare, 2015). Today, ART accounts for approximately 5.8% of all births in Australia (Australian bureau of Statistics, 2017). Yet, in spite of its routine use, ART remains invasive, expensive and not without risk. Moreover, ART does not address the specific cause of a couple's infertility (Aitken and De Iuliis, 2010). While numerous pathologies can contribute to infertility and its adverse sequelae, mounting evidence suggests that a substantial proportion of assisted reproductive cycles with poor prognoses are associated with reduced genetic integrity of the gamete(s).

Indeed, genomic instability in the germline has the potential to alter embryonic gene expression and drive modified developmental programmes with every subsequent cell cycle (Fleming et al., 2018). In recognition of this, there is an impetus to explore new ways to protect the female germline from genomic damage and thus preserve fertility. Currently however, little is known regarding the innate mechanisms of DNA protection and repair employed by the post-ovulatory oocyte and early embryo. Given this, characterisation of the cellular, metabolic, and physiological mechanisms safeguarding the female germline is key to understanding how to enhance these processes.

In this thesis, we have surveyed the quality control mechanisms utilised by the oocyte and early preimplantation embryo to ensure genomic fidelity. Specifically, the publications arising from this work have focused on the protective strategies of: (i)

efflux transporter proteins (Chapter 2), non-homologous end joining DNA repair pathways (Chapter 3) and novel pro-survival factors, such as C-peptide, which may facilitate the correct localisation of DNA repair machinery in the zygote (Chapter 4). The collective findings described in these publications provide a critical framework for informing novel strategies to preserve oocyte/embryo quality *in vitro* and thus contribute to improvements in contemporary assisted reproductive technologies.