The role of reactive oxygen species and oxidative stress in post-ovulatory ageing and apoptosis of the mammalian oocyte

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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: 15th July, 2015
Declaration

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution.

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…………………

Tessa Lord
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Publications and awards arising from work in this thesis

1. Publications

Chapter 1


Chapter 2


Chapter 3


Chapter 4

2. Statements of contribution

I attest that the Research Higher Degree candidate Tessa Lord 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.

L. Prof. John Aitken
Date: 8 July 2015

A. Prof. Brett Nixon
Date: 8 July 2015

Prof. Keith Jones
Date: 8 July 2015

Jacinta Martin
Date: 8 July 2015

Frances Martin (ADRT)
Date:
3. Conference Proceedings


Lord, T., Aitken, R. J. Post-fertilization changes improve the capacity of the oocyte to cope with oxidative stress. Australian Society for Medical Research NSW Scientific Meeting. Sydney, Australia. June 2014.


Lord, T., Aitken, R. J. Fertilization prevents entry of the MII oocyte into senescence by improving the capacity to cope with oxidative stress and subsequent damage. 44th annual conference of the Society for Reproductive Biology. Sydney, Australia. August 2013.


4. Awards

‘Best Presentation by a PhD Student’ prize - Australian Society for Medical Research Satellite Scientific Meeting, Newcastle, Australia (2014)

‘Faculty of Science and I.T. Conference Scholarship’ – University of Newcastle, Australia (2014)

‘Oozoa award’ for best student presentation - Society of Reproductive Biology, Sydney, Australia (2013)

Finalist for the Oozoa award for best student presentation, Society of Reproductive Biology, Gold Coast, Australia (2012)
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Abstract

Following ovulation, the MII stage oocyte awaits fertilization in the oviduct, or, in the case of assistive reproductive technologies (ART), in *in vitro* culture medium. In the absence of fertilization, however, the oocyte experiences a time-dependent deterioration in quality referred to as post-ovulatory ageing. Post-ovulatory ageing is associated with a decline in fertilization rate, as well as the production of poor quality embryos, an increased risk for post-implantation errors and production of offspring with compromised health. Although the consequences associated with post-ovulatory ageing are well defined, the molecular mechanisms which orchestrate this decline in oocyte quality, or conversely, act to prevent post-ovulatory ageing in the event that timely fertilization has occurred, are not well understood. In this thesis we decipher a critical role for reactive oxygen species (ROS) in the initiation of post-ovulatory ageing and apoptosis of the mammalian oocyte. Using a mouse model, we have characterised a time–dependent accumulation of intracellular ROS following retrieval of the ovulated oocyte. This elevation in ROS was found to instigate a self-perpetuating cycle of lipid peroxidation, electrophilic aldehyde production and mitochondrial damage; resulting in the initiation of an intrinsic apoptotic cascade. The elevation in levels of ROS and electrophilic aldehydes within the oocyte were directly associated with a decreased capacity to participate in fertilization and support embryo development.

Importantly, research within this thesis has demonstrated that timely fertilization of the oocyte is associated with an up-regulation of glutathione peroxidase activity, and accelerated DNA repair by the base excision repair (BER) pathway. These post-fertilization changes in oocyte biochemistry aide in circumventing the otherwise
inevitable initiation of post-ovulatory ageing by preventing the accumulation of ROS and oxidative DNA damage.

In identifying the critical role for ROS and electrophilic aldehydes in post-ovulatory ageing and apoptosis of the oocyte, it was possible to select antioxidant and aldehyde-reactive compounds to attenuate the onset of these processes. Specifically, melatonin was found to significantly improve fertilization rate, embryo formation rate and embryo quality in oocytes aged for 8 and 16 h in vitro, as well as delay the initiation of apoptosis. Similarly, penicillamine was found to prevent the decline in fertilization rate and embryo formation rate associated with elevated levels of electrophilic aldehydes. The adaptation of these supplementation techniques for use in a human ART setting would be advantageous in lengthening the optimal window of time in which oocytes must be inseminated post-retrieval, as well as increasing the viability of re-insemination techniques such as rescue-ICSI; potentially minimising the likelihood that further ovarian stimulation cycles would be necessary following a failure to fertilize by IVF.

Collectively, these data provide a significant contribution to the field of knowledge surrounding degeneration and apoptosis of the mammalian oocyte, and provide novel methodologies for attenuating these events in an in vitro setting.