Xenobiotics; Effects on Female Fertility

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BSc (Biotech) (Hons) Class I

Doctor of Philosophy

April, 2012

Declaration

I hereby certify that this thesis is submitted 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; and endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

(Signed)…….

Acknowledgments

First of all I'd like to thank the University of Newcastle's Reproductive Science group for all of its support and assistance over the past four years. I would also like to express my deepest gratitude to my supervisors: Prof. Eileen Mclaughlin, Dr Shaun Roman and A/Prof. Brett Nixon. Your sponsorship, academic example and independent style of supervision have all been invaluable to me throughout my candidature. A very special thanks goes out to my fellow "lab monkeys" and research assistants. You made the monotonous exciting and the difficult doable with friendly advice, humour, and companionship. In particular, I'd like to acknowledge my good friend Kate Redgrove; thanks for taking the journey with me. I'd also like to thank my "school friends" for all their support and approval. More importantly I'd like to thank them for providing me with a distraction from the lab, without which I'm sure I'd never have reached this point.

Like almost every other aspect of my life, my family has been crucial to my success during my candidature. Thankyou Mum for all your encouragement (99.9!), Pup for your grounding, Andrew for your loyalty and Elizabeth for your laughter; I love you all very much. Finally, I'd like to thank the most important person in the world to me, my gorgeous wife Jacqueline Elizabeth Sobinoff. Your love inspires me to new heights everyday; without you none of this would be possible.

Publications included as part of this thesis

Literature review:

Sobinoff, A. P., Bernstein, I. R., and McLaughlin, E. A. (2012). All Your Eggs in One Basket; Mechanisms of Xenobiotic Induced Female Reproductive Senescence. In Senescence (T. Nagata, ed., pp. 559-584. InTech Press Rijeka, Croatia.

Chapter 1:

Sobinoff, A. P., Pye, V., Nixon, B., Roman, S. D., and McLaughlin, E. A. (2010). Adding Insult to Injury: Effects of Xenobiotic-Induced Preantral Ovotoxicity on Ovarian Development and Oocyte Fusibility. *Toxicological Sciences* **118**, 653-666.

Chapter 2:

Sobinoff, A. P., Mahony, M., Nixon, B., Roman, S. D., and McLaughlin, E. A. (2011). Understanding the Villain: DMBA-Induced Preantral Ovotoxicity Involves Selective Follicular Destruction and Primordial Follicle Activation through PI3K/Akt and mTOR Signaling. *Toxicological Sciences* **123**, 563-575.

Chapter 3:

Sobinoff, A. P., Pye, V., Nixon, B., Roman, S. D., and McLaughlin, E. A. (2012). Jumping the gun: Smoking constituent BaP causes premature primordial follicle activation and impairs oocyte fusibility through oxidative stress. *Toxicology and Applied Pharmacology* **260**, 70-80.

Chapter 4:

Sobinoff, A. P., Mahony, M., Nixon, B., Roman, S. D., and McLaughlin, E. A. (2012). Staying alive: PI3K pathway promotes primordial follicle activation and survival in response to 3-MC induced ovotoxicity. *Toxicological Sciences*, doi:10.1093/toxsci/kfs137.

Statements of Contribution

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

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Prof. Eileen Anne McLaughlin

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A/Prof. Jenny Cameron (Assistant Dean Research Training)

Date……..

Additional Publications with Relevance to this Thesis

Chapter in book:

McLaughlin Eileen Anne, Sobinoff Alexander (2010) 'Contraception targets in mammalian ovarian development', *Fertility Control*, Springer, Berlin, 45-66

Conference Publications:

A. P. Sobinoff, V. Pye, B. Nixon, S. D. Roman and E. A. McLaughlin (2009) 'Xenobiotics; Influence on ovarian follicular development', *Reproduction, Fertility and Development*, Adelaide, SA

Pye Victoria Jayne, Sobinoff Alexander, Nixon Brett, Roman Shaun Daryl, McLaughlin Eileen Anne (2010) 'Xenobiotics; influence on long term oocyte viability', *OzBio 2010: The Molecules of Life - from Discovery to Biotechnology. Poster Abstracts*, Melbourne, VIC

Sobinoff Alexander, Pye Victoria Jayne, Nixon Brett, Roman Shaun Daryl, McLaughlin Eileen Anne (2010) 'Consistent mechanism of primordial follicle activation in neonatal mouse ovotoxicity', *OzBio 2010: The Molecules of Life - from Discovery to Biotechnology. Poster Abstracts*, Melbourne, VIC

Sobinoff Alexander, Pye Victoria Jayne, Nixon Brett, Roman Shaun Daryl, McLaughlin Eileen Anne (2010) 'Short Term Xenobiotic Exposure Compromises Long Term Oocyte Viability', *Reproduction, Fertility and Development*, Sydney, NSW

Sobinoff Alexander, Nixon Brett, Roman Shaun Daryl, McLaughlin Eileen Anne (2011) 'Evidence of selective follicular destruction and primordial follicle activation in DMBA induced ovotoxicity' *Reproduction, Fertility and Development*, Cairns, QLD

Sobinoff Alexander, Pye Victoria Jayne, Nixon Brett, Roman Shaun Daryl, McLaughlin Eileen Anne (2011) 'Neonatal xenobiotic exposure compromises adult oocyte viability' *Reproduction, Fertility and Development*, Cairns, QLD

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Abstract

Over the course of the 20th century there has been an increasing trend in western women opting to delay childbirth in the pursuit of social and economic stability. This development has highlighted the need to identify and characterise ovotoxic xenobiotics (foreign chemical compounds) which threaten their fertility. Arguably the most insidious ovotoxic xenobiotics are those which target the irreplaceable primordial follicle pool for destruction, resulting in pre-mature ovarian senescence. Although many of these xenobiotics have been identified, the molecular mechanisms behind their ovotoxicity remain largely unknown. Employing a neonatal mouse model rich in primordial follicles, the studies presented in this thesis were aimed at characterising the mechanisms of ovotoxicity for six xenobiotics known to target immature follicles (4-vinylcyclohexene diepoxide; Methoxychlor; Menadione; Benzo-a-pyrene, 7,12-Dimethylbenz-[a]anthracene; 3-Methylcholanthrene). The effects of short term xenobiotic exposure on long term oocyte viability were also examined to determine whether follicles which survive ovotoxic destruction were still functionally viable.

Microarray analysis and quantitative PCR revealed a unique ovarian response to each xenobiotic involving a number of genes linked to follicular growth/development, cell death and tumorigenesis *in vitro*. Immunohistological and histomorphological analysis confirmed the microarray data, and revealed a consistent mechanism of ovotoxicity involving primordial follicle activation alongside developing follicle atresia *in vitro* and *in vivo*. Immunohistological and pharmacological inhibition studies also revealed an essential role for the PI3K/Akt/mTOR signalling pathways in 3-Methylcholanthrene and 7,12-Dimethylbenz-[a]anthracene induced primordial follicle activation and survival. Studies into the effects of short term neonatal exposure on long term female fertility revealed no difference in the number of healthy oocytes ovulated in neonatally treated adults compared to controls. However, comprehensive oocyte viability analysis revealed a decreased capacity for fertilisation caused by oxidative damage to the oolemma membrane due to mitochondrial electron transport chain leakage.

The studies conducted in this thesis have identified a common mechanism of xenobiotic induced primordial follicle depletion via a homeostatic mechanism of developing follicle recruitment, with some ovotoxic xenobiotics inducing primordial follicle survival as opposed

to atresia. In addition, the contents of this thesis also provide the first documented evidence of short term neonatal exposure causing long term oocyte dysfunction through xenobiotic induced oxidative stress.

Aims and Hypothesises

The overall aim of this thesis was to determine the molecular mechanisms behind xenobiotic induced follicular depletion and to examine the long term effects of ovotoxic xenobiotic exposure after its removal from the follicular environment. Given the discrepancies seen in the current literature, it was hypothesised that xenobiotic induced primordial follicle depletion is not solely due to follicular atresia, and that short term xenobiotic exposure has long term effects on female fertility. The first aim was investigated through a series of in vitro and in vivo culture experiments using histological/immunohistological techniques combined with microarray analysis. The second involved an extensive in vivo experiment where neonatal mice were dosed with varying concentrations of xenobiotic over several days. These neonatal mice were then allowed to reach sexual maturity, and parameters of female fertility were assessed 6 weeks after they were last treated with xenobiotics. Overall six xenobiotics were assayed. These were 4-vinylcyclohexene diepoxide, methoxychlor, menadione, 9:10-dimethyl-1:2-benzanthracene, benzo[a]pyrene, and 3-methylcholanthrene.