EPIDERMAL GROWTH FACTOR-LIKE PEPTIDE SIGNALLING AND OOCYTE IN VITRO MATURATION

Dulama Richani, B.Btec (Hons)

The Robinson Institute,
Research Centre for Reproductive Health,
Discipline of Obstetrics and Gynaecology,
School of Paediatrics and Reproductive Health,
The University of Adelaide, Australia.

Thesis submitted to the University of Adelaide in total fulfilment of the requirements for the degree of Doctor of Philosophy in Medicine.

February 2014

THE UNIVERSITY of ADELAIDE

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

A growing body of evidence has recently implicated follicular epidermal growth factor (EGF)-like peptide signalling as essential for the propagation within the ovarian follicle of the LH stimulus that induces oocyte maturation and ovulation. The EGF-like peptides amphiregulin, epiregulin, and betacellulin are produced in mural granulosa and cumulus cells in response to LH, and signal via the EGF receptor (EGFR) in these cells to ultimately induce oocyte maturation, cumulus expansion, and ovulation. Although the function and impact of EGF-like peptide signalling on oocyte maturation *in vivo* has been characterised, little is currently known about the effect of oocyte *in vitro* maturation (IVM) on this important signalling network. This thesis aimed to investigate the regulation of EGF-like peptide signalling in mouse cumulus cells and the effect of various IVM models on this network.

FSH is a universal IVM additive, and EGF is occasionally used in animal IVM. The effect of FSH-stimulated IVM, EGF-stimulated IVM versus *in vivo* maturation (IVV) on cumulus cell EGF-like peptide mRNA and/or protein expression, the activity of EGFR, and its classic downstream effector, ERK1/2, were examined. EGF-like peptide mRNA expression, amphiregulin protein expression, and EGFR phosphorylation were significantly lower using FSH-stimulated IVM than during IVV. EGF stimulated significantly lower EGFR phosphorylation, but not EGF-like peptide mRNA expression. These data demonstrate that this signalling network is perturbed in IVM cumulus cells.

The effect of FSH, EGF, amphiregulin and epiregulin in IVM on subsequent blastocyst development revealed that epiregulin and amphiregulin significantly increased blastocyst yield and/or the proportion of inner cell mass in blastocysts, than FSH or EGF. Examination of the metabolic profiles of IVM cumulus-oocyte complexes (COCs) matured in the presence of these stimulants revealed that EGF-like peptides and EGF induced significantly higher COC glucose metabolism via the hexosamine biosynthesis pathway than FSH, consequently enabling more hyaluronic acid synthesis and protein β-O-linked glycosylation in the cumulus cells. Epiregulin significantly increased intra-oocyte FAD^{++} and the REDOX ratio compared to FSH, and all three EGF-like peptides induced more oocyte mitochondrial activity than EGF or FSH.

Evidence has shown that increasing 3'-5'-cyclic adenosine monophosphate (cAMP) using pharmacological agents significantly increases IVM oocyte developmental competence. This concept, in the form of a pre-IVM culture period with cAMP modulators, was examined in conjunction with IVM in the presence of epiregulin, amphiregulin, EGF or FSH. A pre-IVM phase in conjunction with IVM with EGF-like peptides endowed greater oocyte developmental competence than with FSH or EGF, as evidenced by increased embryo yield and/or quality, which were comparable in embryo development and/or quality rates from IVV oocytes.

This thesis provides the physiological basis for, and evidence that, EGF-like peptides are more appropriate IVM additives than FSH or EGF. EGF-like peptides endow greater oocyte developmental competence than FSH, possibly by regulating important aspects of COC metabolism. Combining this concept with cAMP modulation of IVM COCs may represent a more physiological IVM system than existing IVM approaches, as it yields more blastocysts of higher quality. The knowledge provides new opportunities for the treatment of infertility in women and for the *in vitro* production of embryos and advanced breeding in animals.

DECLARATION

I certify that this work contains no material which 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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 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 and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

February 2014

Dulama Richani

ACKNOWLEDGEMENTS

As I sit down to write these acknowledgements, the one person at the forefront of my mind is my wonderful husband, Adnaan Baraky. I could not have wished for a more supportive partner as I travelled down this road. Adnaan, you have always lifted me up, supported me, consoled me, encouraged me, inspired me, and made me believe I can achieve anything. You have gone on this journey with me and filled every second of it with love and encouragement. I can never thank you enough for what you give me and what you do for me on a daily basis. I love you!

I owe a great deal of thanks to my supervisors Associate Professors Robert Gilchrist and Jeremy Thompson. I very honestly say that I couldn't have asked for better supervisors. Thank you for your mentorship, friendship, and guidance. Thank you for giving me the freedom to pursue my own interests within my project. I know you probably had no idea what I was up to in the lab at times but you had faith in me. Most of all, thank you for teaching me how to be a good researcher and for nurturing my love of research. You have always had your doors open to me and always went above and beyond the call of duty to help me. I am forever grateful!

A special thanks goes to Lesley Ritter for so many things. Lesley, thank you for your support, advice, and guidance throughout my PhD. Thank you for patiently answering the *thousands* (maybe even hundreds of thousands?) of questions I asked you over the years, for your support in the lab, and for our chats.

I would really like to thank my fellow lab members who became my friends and laughed a lot with me: Laura Frank, Mel White, Ryan Rose, Marie Anastasi, Jacky Sudiman, Xiaoqian Wang, Dianne Feil, Annie Whitty, Mel McDowall, and all those I have not mentioned by name. I will miss all our weird and wonderful conversations and our outings to the pub. Laura, you helped me keep my sanity when things weren't going well in the lab and you have been my PhD soul mate. Mel White and Marie, thanks for all the wild times (you know what I'm talking about). Ryan and Jacky, thanks for all the silly times in our PhD room; you made our office fun.

Last, but certainly not least, I would like to thank my highly supportive family who have been a source of encouragement though out my PhD. You all light up my life. I would like

to especially thank Mum and Nick for all that they have done for me and given me. Thank you for babying and spoiling me, even to this day, and for always being there. I love you all!

As I reflect back, I feel very grateful and lucky to have completed my PhD surrounded by such wonderful people. Although it was a rollercoaster ride at times, you are all the reason I loved going to work every day. I am so happy to see how much I have grown as a scientist since the beginning of my PhD studies.

ABBREVIATIONS

AC	adenylate cyclase
ANOVA	analysis of variance
AREG	amphiregulin
ART	assisted reproductive technology
ATP	adenosine triphosphate
BMP15	bone morphogenetic factor 15
BSA	bovine serum albumin
BTC	betacellulin
cAMP	cyclic adenosine monophosphate
CEI	cumulus expansion index
CC	cumulus cell
cGMP	cyclic guanine monophosphate
CNP	C-type natriuretic peptide
COC	cumulus-oocyte complex
DNA	deoxyribonucleic acid
DO	denuded oocyte
eCG	equine chorionic gonadotropin
ECL	enhanced chemiluminescence
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EREG	epiregulin
ERK	extracellular signal-regulated kinase
ErbB	avian erythroblastosis oncogene B
FAD^{++}	flavin adenine dinucleotide
FSH	follicle stimulating hormone
FSK	forskolin
GC	granulosa cell
GDF9	growth differentiation factor 9
GFPT	glutamine:fructose-6-phosphate dehydrogenase
GlcNAc	N-acetylglucosamine
GV	germinal vesicle
GVBD	germinal vesicle breakdown
HBP	hexosamine biosynthesis pathway

hCG	human chorionic gonadotropin
IBMX	3-isobutyl-1-methylxanthine
ICM	inner cell mass
ICSI	intracytoplasmic sperm injection
IVF	*in vitro* fertilization
IVM	*in vitro* maturation
JNK	c-Jun N-terminal kinase
LH	luteinising hormone
MI	metaphase I
MII	metaphase II
mg	milligram(s)
MGC	mural granulosa cell
mL	millilitre(s)
mM	millimolar
mRNA	messenger RNA
mTOR	mammalian target of rapamycin
NAD^+/NADH	nicotinamide adenine dinucleotide
$NADP^+$/NADPH	nicotinamide adenine dinucleotide phosphate
NAD(P)H	combined NADH and NADPH
NS	non siginificant
O-GlcNAcase	β-N-acetylglucosaminidase
OGT	β-*O*-linked glycosylation
P	probability
PBS	phosphate buffered saline
PDE	phosphodiesterase
PDE3	phosphodiesterase subtype 3
PFK	phosphofructokinase
PI	propidium iodide
PI3K	phosphoinositide-3-kinase
PPP	pentose phosphate pathway
REDOX	reduction-oxidation
RIPA	radioimmunoprecipitation
RNA	ribonucleic acid
RT	reverse transcription
SD	significant difference

SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	standard error of the mean
TCA	tricarboxylic acid
TE	trophectoderm
µL	microliter
µM	micromolar

PUBLICATIONS

Scientific publications generated throughout PhD candidature:

1. **Dulama Richani**, Lesley J. Ritter, Jeremy G. Thompson and Robert B. Gilchrist. 2013. Mode of oocyte maturation affects EGF-like peptide function and oocyte competence. *Molecular Human Reproduction* 19(8):500-509. {Appendix 1}

2. Robert B. Gilchrist and **Dulama Richani**. 2013. Somatic guidance for the oocyte. *Developmental Cell* 27:603-605. {Appendix 4}

3. **Dulama Richani**, Melanie L. Sutton-McDowall, Laura A. Frank, Jeremy G. Thompson and Robert B. Gilchrist. 2014. Effect of epidermal growth factor-like peptides on the metabolism of *in vitro*-matured mouse oocytes and cumulus cells. *Biology of Reproduction* 90(3):49, 1–10. {Appendix 2}

4. **Dulama Richani**, Xiaoqian Wang, Hai-tao Zeng, Johan E.J. Smitz, Robert B. Gilchrist and Jeremy G. Thompson. 2014. Pre-maturation with cAMP modulators in conjunction with EGF-like peptides during IVM enhances mouse oocyte developmental competence. *Molecular Reproduction and Development* 81:422-435. {Appendix 3}

5. Hai-tao Zeng, **Dulama Richani**, Melanie L. Sutton-McDowall, Zi Ren, Johan E.J. Smitz, Yvonne Stokes, Robert B. Gilchrist and Jeremy G. Thompson. 2014. Pre-maturation with cyclic adenosine monophosphate modulators alters cumulus cell and oocyte metabolism and enhances developmental competence of *in vitro* matured mouse oocytes. *Biology of Reproduction* [In Press, BIOLREPROD/2014/118471].

6. Hannah Brown, Marie Anastasi, Laura Frank, Karen Kind, **Dulama Richani**, Rebecca Robker, Darryl Russell, Robert Gilchrist and Jeremy Thompson. 2014. Haemoglobin: A gas-transport molecule that is hormonally regulated in the ovarian follicle. *Human Reproduction* [Submitted 11 June 2014, HUMREP-14-0702].

CONFERENCE PROCEEDINGS

Abstracts (published)

D. Richani, M.L. Sutton-McDowall, L.A. Frank, J.G. Thompson and R.B. Gilchrist (2013) *'Effect of epidermal growth factor-like peptides on the metabolism of in vitro matured mouse oocytes and cumulus cells'*, Society for Reproductive Biology, Sydney, Australia

D. Richani, L.J. Ritter, J.G. Thompson and R.B. Gilchrist (2012) *'Consequences of in vitro maturation of oocytes on cumulus cell EGF-like peptide signalling'*, Society for the Study of Reproduction, Pennsylvania, USA

D. Richani, L.J. Ritter, J.G. Thompson and R.B. Gilchrist (2011) *'Cumulus cell EGF-like peptide and receptor signalling during oocyte in vitro maturation'*, The Society of Reproductive Biology, Cairns, Australia

Meeting abstracts (unpublished)

D. Richani, M.L. Sutton-McDowall, L.A. Frank, J.G. Thompson and R.B. Gilchrist (2013) *'Effect of EGF-like peptides on the metabolism of oocytes and their associated somatic cells'*, Australian Society for Medical Research, Adelaide

D. Richani, L.J. Ritter, J.G. Thompson and R.B. Gilchrist (2012) *'Consequences of in vitro maturation of oocytes on cumulus cell EGF-like peptide signalling'*, Faculty of Health Sciences Postgraduate Research Conference, the University of Adelaide

D. Richani, L.J. Ritter, J.G. Thompson and R.B. Gilchrist (2011) *'The effect of oocyte in vitro maturation on EGFR pathway signalling'*, Australian Society for Medical Research, Adelaide

Conference presentations

Oral presentations

2013 Annual meeting of Society of Reproductive Biology, Sydney, Australia
'Effect of EGF-like peptides on the metabolism of oocytes and their associated cumulus cells'

2013 Annual meeting of Australian Society for Medical Research, Adelaide, Australia
'Effect of EGF-like peptides on the metabolism of oocytes and their associated somatic cells'

2011 Annual meeting of Society of Reproductive Biology, Cairns, Australia
'Cumulus cell EGF-like peptide and receptor signalling during oocyte in vitro maturation'

Poster presentations

2012 Annual meeting of the Society for the Study of Reproduction, Pennsylvania, USA, *Consequences of in vitro maturation of oocytes on cumulus cell EGF-like peptide signalling'*

2012 Faculty of Health Sciences Postgraduate Research Conference, the University of Adelaide, *'Consequences of in vitro maturation of oocytes on cumulus cell EGF-like peptide signalling'*

2011 Annual meeting of Australian Society for Medical Research, Adelaide
'The effect of oocyte in vitro maturation on EGFR pathway signalling'

AWARDS & SCHOLARSHIPS

Competitions

2013 Finalist in the David Healy New Investigator Award, 44th Annual Meeting of the Society for Reproductive Biology, Sydney, Australia

2012 Finalist in the Adelaide Research & Innovation Pty Ltd Award, Faculty of Health Sciences Postgraduate Research Conference, the University of Adelaide

Visit to overseas group

2012 Professor Marco Conti, Department of Obstetrics/Gynaecology & Reproductive Sciences, University of California, San Francisco (UCSF), San Francisco, USA

Scholarships and Grants

Scholarships

2010-2013	Australia Postgraduate Award Research Scholarship
2010-2013	PhD Top-up Scholarship ($15,000)
2013	PhD Top-up Scholarship ($14,222)

Travel grants

2013	Robinson Institute, the University of Adelaide
2013	School of Paediatrics and Reproductive Health, the University of Adelaide
2013	Annual Meeting of Society of Reproductive Biology, Sydney
2012	Faculty of Health Sciences, the University of Adelaide
2012	Robinson Institute, the University of Adelaide
2012	Research Centre for Reproductive Health, the University of Adelaide
2011	Research Centre for Reproductive Health, the University of Adelaide
2011	Annual Meeting of Society of Reproductive Biology, Cairns