The Role of the Cumulus Oocyte Complex During Ovulation

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"Just because something doesn't do what you planned it to do doesn't mean it's useless."

Thomas Edison

“Somewhere, something incredible is waiting to be known.”

Carl Sagan

Abstract

Ovulation is fundamentally crucial to the reproductive success of all mammals. Despite this fact there remain major knowledge gaps in our understanding of how the Luteinizing Hormone (LH) surge, which initiates ovulation, controls this process. There have been numerous theories regarding this phenomenon, yet the underlying mechanisms involved remain relatively unknown. In this thesis I sought to elucidate mechanisms involved in ovulation, with a particular focus on the role played by the expanded cumulus oocyte complex (COC). Specifically, I investigate whether the cumulus cells and their associated matrix following expansion could contribute actively to its own extrusion from the ovarian follicle during ovulation.

I developed a novel hypothesis whereby the cumulus cells transition to an adhesive, motile and invasive cell phenotype in response to an ovulatory stimulus, hCG an analog of LH. I investigate whether the cumulus cells from expanded COCs are capable of cell adhesion to various extracellular matrices found in the follicle wall, and whether this is dependent upon hormonal stimulation by comparison to cumulus cells from unexpanded COCs, not receiving such stimulation.

Further, I investigate whether the cumulus oocyte complex is capable of transitioning to a migratory cell phenotype. I tested this with established methods used in the study of cancer cell metastasis. I determine whether this phenotype is firstly dependent on an ovulatory stimulus, and whether it is cumulus cell specific. I attempt to elucidate the molecular mechanisms involved by investigating expression of the well-characterised CD44 cell migration pathway in COCs, during an ovulation time-course. I then use specific antagonists to this pathway, to inhibit cell migration.

The final step in our hypothesis involves the investigation of the invasive capacity of the expanded COC. I analyse whether the expanded COCs are capable of degrading an extracellular matrix barrier during migration assays, and I compare this ability to characterised invasive and non-invasive breast cancer cell lines. I also investigate possible mechanisms involved in the invasive phenotype by inhibiting the matrix metalloprotease system, proposed to play an important role in the degradation of the follicle wall during follicle rupture, and by examining the *Adamts1* null mouse, as Adamts1 is a protease shown to be crucial during ovulation.

This thesis demonstrates novel and exciting properties of the cumulus oocyte complex during ovulation; offering new insight into our understanding of this complex process. It shows that the oocyte and its surrounding cumulus cells are not merely a passive entity, as previously thought, but rather may play an active role during this vital reproductive process.

Declaration

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.

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Abstracts arising from this thesis

2009

ALVINO ER, Robker RL and Russell DL. An *active role for the Cumulus Oocyte Complex During Ovulation*. *Biol Reprod*, June 15, 2009 81 (1 Supplement) 51 Annual meeting of the Society for the Study of Reproductive Biology, International Conference, 18 – 21 July ,Pittsburgh, Pennsylvania, USA

ALVINO ER, Robker RL and Russell DL. CD44 *Signalling in the Cumulus Oocyte Complex during Ovulation*. *Reproduction, Fertility and Development*, Vol. 21 No. 9 Pages 73 – 73. Published: 26 August 2009 . Australian Society for Reproductive Biology, Annual meeting of the Endocrine Society of Australia (ESA) & Society of Reproductive Biology, National Conference, 23-26 August, Adelaide, South Australia, Australia

ALVINO ER, Robker RL and Russell DL. (2009) *An Active Role for the Cumulus Oocyte Complex During Ovulation.* Australian Society for Medical Research, State Conference, 4 June, Adelaide South Australia, Australia

2008

ALVINO ER, Robker RL and Russell DL. *CD44 signaling in mouse ovulatory cumulus–oocyte complexes*. *Reproduction, Fertility and Development,* Vol. 20 No. 9 Pages 69 - 69, Published 29 August 2008 Australian Society for Reproductive Biology, Annual meeting of the Endocrine Society of Australia (ESA) & Society of Reproductive Biology, National Conference, 25-28 August, Melbourne, Victoria, Australia.

ALVINO ER, Robker RL and Russell DL. (2008) *Demonstrating an active role for the cumulus oocyte complex during ovulation in Mice.* Australian Society for Medical Research, State Conference, 4 June, Adelaide South Australia, Australia

2007

ALVINO ER, Robker RL and Russell DL. (2007) *An Active Role for the Cumulus Oocyte Complex During Ovulation.* Australian Society for Medical Research, State Conference, 6 June, Adelaide South Australia, Australia

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Abbreviations

αMEM	Minimum Essential Medium alpha
Adamts	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif
Ambp	alpha 1 microglobulin/bikunin
ANOVA	analysis of variance
Ar	Androgen receptor
ART	artificial reproductive technology
bp	base pairs
BSA	bovine serum albumin
Bmp15	bone morphogenetic protein 15
CD44	CD44 antigen
cAMP	cyclic adenosine monophosphate
cDNA	Complementary DNA
Cebpb	CAAT/enhancer binding protein (C/EBP), beta
COC	cumulus oocyte complex
Csf2	colony stimulating factor 2 (granulocyte-macrophage)
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
dNTP	Deoxyribonucleotide
DNA	Deoxyribonucleic acid
eCG	equine chorionic gonadotropin
ECM	extracellular matrix
Egf	epidermal growth factor

Egf-L	Egf-like ligand
Egfr	epidermal growth factor receptor
EMT	epithelial to mesenchymal transition
ERK1/2	Extracellular-signal-regulated kinase 1 and 2
FCS	Fetal calf serum
F1	first filial
FSH	follicle stimulating hormone
GC	granulosa cell
Gdf9	growth differentiation factor 9
GDP	guanosine diphosphate
GEF	Guanine nucleotide exchange factor
GTP	guanosine triphosphate
GTPase	guanosine triphosphatase
h	hour
HA	hyaluronan
Has2	hyaluronan synthase 2
HC	heavy chain
hCG	human Chorionic Gonadotropin
Hmmr	hyaluronan mediated motility receptor (RHAMM)
HSC-3	human head and neck squamous carcinoma cell line
IαI	inter-α trypsin inhibitor
Ifna	interferon alpha
IL	interleukin
i.p.	intraperitoneal

IU	international units
IVF	invitro fertilisation
IVM	in vitro maturation
KO	knock out
LB	luria broth
LH	Luteinizing hormone
Lhcgr	luteinising hormone/choriogonadotropin receptor
LPS	lipopolysaccharide
Lyve1	lymphatic vessel endothelial hyaluronan receptor 1
MAPK	Mitogen-activated protein kinase
MI	metaphase I
MII	metaphase II
min	minute
mIU	milli international units
MMP	matrix metalloproteinase
mRNA	Messenger RNA
Nrip1	Nuclear receptor interacting protein 1
^{o}C	degrees Celsius
OSF	oocyte seceted factor
OSE	ovarian surface epithelium
PB	polar body
PBS	Phosphate Buffered Saline
Ptg	prostaglandin
Ptger2	prostaglandin E receptor 2 (subtype EP2)

PCR	polymerase chain reaction
Pde4d	phosphodiesterase 4D, cAMP specific
Pgr	progesterone receptor
Plg	plasminogen
Plat (tPA)	plasminogen activator, tissue
Plau (uPA)	plasminogen activator, urokinase
PGRKO	Progesterone receptor knockout
Ptgr2	prostaglandin E receptor 2, subtype EP2
Ptgs2	prostaglandin-endoperoxide synthase 2
Ptx3	pentraxin related gene
PVDF	polyvinylidene difluoride
Rac1	RAS-related C3 botulinum substrate 1
Rcf	Relative centrifugal force
RhoA	ras homolog gene family, member A
RNA	ribonucleic acid
Rock	Rho-associated coiled-coil containing protein kinase
Rpl19	ribosomal protein L19
Rpm	revolutions per minute
RT	reverse transcription
RT-PCR	reverse transcription polymerase chain reaction
SEM	standard error of the mean
SDS	Sodium Dodecyl sulphate
SDS-PAGE	Sodium Dodecyl sulphate - polyacrylamide gel electrophoresis
TBE	tris borate EDTA

Tgfb	transforming growth factor, beta
Tiam1	T-cell lymphoma invasion and metastasis 1
TIMP	tissue inhibitor of metalloproteinase
TLR	toll like receptor
Tnfaip6	Tumor necrosis factor alpha-induced protein 6
Tnfa	tumour necrosis factor alpha