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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Emily Renee Alvino

October 2010
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Abstracts arising from this thesis

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2008


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2007

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<thead>
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<th>Description</th>
</tr>
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<tbody>
<tr>
<td>αMEM</td>
<td>Minimum Essential Medium alpha</td>
</tr>
<tr>
<td>Adamts</td>
<td>a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif</td>
</tr>
<tr>
<td>Ambp</td>
<td>alpha 1 microglobulin/bikunin</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>Ar</td>
<td>Androgen receptor</td>
</tr>
<tr>
<td>ART</td>
<td>artificial reproductive technology</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>Bmp15</td>
<td>bone morphogenetic protein 15</td>
</tr>
<tr>
<td>CD44</td>
<td>CD44 antigen</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>Cebpb</td>
<td>CAAT/enhancer binding protein (C/EBP), beta</td>
</tr>
<tr>
<td>COC</td>
<td>cumulus oocyte complex</td>
</tr>
<tr>
<td>Csf2</td>
<td>colony stimulating factor 2 (granulocyte-macrophage)</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>dNTP</td>
<td>Deoxyribonucleotide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>eCG</td>
<td>equine chorionic gonadotropin</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>Egf</td>
<td>epidermal growth factor</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>Egf-L</td>
<td>Egf-like ligand</td>
</tr>
<tr>
<td>Egfr</td>
<td>epidermal growth factor receptor</td>
</tr>
<tr>
<td>EMT</td>
<td>epithelial to mesenchymal transition</td>
</tr>
<tr>
<td>ERK1/2</td>
<td>Extracellular-signal-regulated kinase 1 and 2</td>
</tr>
<tr>
<td>FCS</td>
<td>Fetal calf serum</td>
</tr>
<tr>
<td>F1</td>
<td>first filial</td>
</tr>
<tr>
<td>FSH</td>
<td>follicle stimulating hormone</td>
</tr>
<tr>
<td>GC</td>
<td>granulosa cell</td>
</tr>
<tr>
<td>Gdf9</td>
<td>growth differentiation factor 9</td>
</tr>
<tr>
<td>GDP</td>
<td>guanosine diphosphate</td>
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<td>GEF</td>
<td>Guanine nucleotide exchange factor</td>
</tr>
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<td>GTP</td>
<td>guanosine triphosphate</td>
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<tr>
<td>GTPase</td>
<td>guanosine triphosphatase</td>
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<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HA</td>
<td>hyaluronan</td>
</tr>
<tr>
<td>Has2</td>
<td>hyaluronan synthase 2</td>
</tr>
<tr>
<td>HC</td>
<td>heavy chain</td>
</tr>
<tr>
<td>hCG</td>
<td>human Chorionic Gonadotropin</td>
</tr>
<tr>
<td>Hmmr</td>
<td>hyaluronan mediated motility receptor (RHAMM)</td>
</tr>
<tr>
<td>HSC-3</td>
<td>human head and neck squamous carcinoma cell line</td>
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<tr>
<td>I(\alpha)I</td>
<td>inter-(\alpha) trypsin inhibitor</td>
</tr>
<tr>
<td>Ifna</td>
<td>interferon alpha</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
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<td>i.p.</td>
<td>intraperitoneal</td>
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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
°C  degrees Celsius
OSF  oocyte secreted factor
OSE  ovarian surface epithelium
PB  polar body
PBS  Phosphate Buffered Saline
Ptg  prostaglandin
Ptger2  prostaglandin E receptor 2 (subtype EP2)
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>Pde4d</td>
<td>phosphodiesterase 4D, cAMP specific</td>
</tr>
<tr>
<td>Pgr</td>
<td>progesterone receptor</td>
</tr>
<tr>
<td>Plg</td>
<td>plasminogen</td>
</tr>
<tr>
<td>Plat (tPA)</td>
<td>plasminogen activator, tissue</td>
</tr>
<tr>
<td>Plau (uPA)</td>
<td>plasminogen activator, urokinase</td>
</tr>
<tr>
<td>PGRKO</td>
<td>Progesterone receptor knockout</td>
</tr>
<tr>
<td>Ptgr2</td>
<td>prostaglandin E receptor 2, subtype EP2</td>
</tr>
<tr>
<td>Ptgs2</td>
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<td>Ptx3</td>
<td>pentraxin related gene</td>
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<td>polyvinylidene difluoride</td>
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<tr>
<td>Rac1</td>
<td>RAS-related C3 botulinum substrate 1</td>
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<tr>
<td>Rcf</td>
<td>Relative centrifugal force</td>
</tr>
<tr>
<td>RhoA</td>
<td>ras homolog gene family, member A</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>Rock</td>
<td>Rho-associated coiled-coil containing protein kinase</td>
</tr>
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<td>Rpl19</td>
<td>ribosomal protein L19</td>
</tr>
<tr>
<td>Rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RT</td>
<td>reverse transcription</td>
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<tr>
<td>RT-PCR</td>
<td>reverse transcription polymerase chain reaction</td>
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<td>SEM</td>
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<td>SDS</td>
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<tr>
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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