Functional Characterisation of the Cumulus Oocyte Matrix during Maturation of Oocytes

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SUB CRUCE LUMEN

“The majority of people meet with failure because of their lack of persistence in creating new plans to take the place of those which fail. “

Napoleon Hill

“Results! Why, man, I have gotten a lot of results. I know several thousand things that won't work.“

Thomas A. Edison

Opportunity is missed by most people because it is dressed in overalls and looks like work.

Thomas A. Edison

Abstract

Female gametes, or oocytes grow and mature in a niche environment maintained by the somatic cells of the ovarian follicle. At ovulation ovarian follicle cells respond to the luteinising hormone (LH) surge coordinating the final maturation, meiotic resumption and release of oocytes. Simultaneously, production of a unique "mucified" extracellular matrix surrounding the oocyte through synthesis of Hyaluronan (HA) and HA cross-linking proteins produces an "expanded" and stabilised cumulus oocyte matrix with a specific composition, structure and function.

In vitro maturation (IVM) of oocytes is a procedure by which cumulus oocyte complexes (COCs) are stimulated to produce cumulus matrix and undergo oocyte maturation *ex* vivo. *In vitro* maturation is a useful procedure for studying oocyte competence as well as offering health benefits for patients undergoing assisted reproduction. Oocytes derived from IVM have much lower developmental competence than *in vivo* matured oocytes, likely as a result of altered environmental conditions and gene expression leading to suboptimal maturation and/or inappropriate metabolic control in oocytes. Cumulus matrix expansion is widely used as an indicator of good oocyte developmental potential, however, the mechanism(s) that endow oocyte quality and how these may be influenced by the cumulus matrix are poorly understood.

To better understand the process by which cumulus matrix is linked to the final stages of oocyte maturation, I undertook investigation of mouse COC matrix composition and function after *in vivo* maturation in comparison to IVM. The gene responsible for Hyaluronan synthesis, *Has2*, was not impaired under IVM conditions. In contrast, two key extracellular matrix proteins; Versican and Adamts1, which are normally selectively incorporated into periovulatory COCs *in vivo*, were greater than 10-fold reduced in IVM whether stimulated with Egf and/or FSH. This work is the first to show that commonly used IVM conditions result in altered gene expression in cumulus cells. Furthermore, the absence of Adamts1 and Versican suggest that COC matrix may be functionally insufficient.

Although associated with good developmental potential, the function of the COC matrix in oocyte maturation is unknown. I assessed the properties of COC matrix that control metabolite supply to oocytes by examining transport of fluorescently labelled glucose and cholesterol across mouse COCs. Profound differences in the control of metabolite supply to oocytes in IVM were observed. *In vivo*

matured complexes were capable of excluding glucose from the entire COC and cholesterol was excluded from oocytes. Conversely IVM COCs were more permissive to rapid equilibration of glucose and cholesterol concentrations across the complex and in oocytes. In fact both metabolites accumulated rapidly in IVM oocytes resulting in inverse gradient patterns of glucose and cholesterol abundance with highest concentrations accumulating in the oocyte after IVM vs highest concentrations surrounding the COC after *in vivo* maturation conditions. As oocytes are highly sensitive to high glucose my results indicate that metabolic balance in IVM may be disrupted due to impaired molecular filtration properties of the mucified COC matrix that controls supply of hydrophilic and lipophylic substrates. Importantly these novel findings can explain the glucose sensitivity of IVM oocytes and identifies a mechanism by which IVM may lead to poorer oocyte developmental competence.

To translate these findings into the improvement of IVM I generated recombinant expression plasmid constructs for several *Adamts1* and *Versican* functional domains. The efficacy of Versican as an IVM supplement that activates cumulus cell signal transduction was proved in principle, by showing enhanced COC matrix expansion when added to mouse IVM cultures. Similar mechanisms are likely to be functional in human COCs since I demonstrated *VERSICAN* and *ADAMTS1* expression in human *in vivo* matured cumulus and granulosa cells. This work has advanced our understanding of oocyte maturation and will lead to improvements in IVM and healthier outcomes from reproductive therapies.

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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Kylie Renee Dunning

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2. Dunning KR, Brown HM, Thompson JG, Robker RL, Russell DL. Cumulus Oocyte Matrix Function during Oocyte Maturation and Ovulation. *In preparation*

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2. **Dunning KR,** Lane M, Brown HM, Yeo C, Thompson JG, Robker RL and Russell DL. *Altered Composition of the Cumulus Oocyte Complex Matrix During in vitro Maturation of Oocytes.* Podium presentation at the 2007 Society for the Study of Reproduction, International Conference, July, Texas, USA.
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Abbreviations

αMEM	Minimum Essential Medium alpha
ADAM	A Disintegrin and Metalloprotease
Adamts1	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motifs
Ambp	alpha 1 microglobulin/bikunin
ANOVA	analysis of variance
Ar	Androgen receptor
ART	artificial reproductive technology
bp	base pairs
BSA	bovine serum albumin
CBP	complement binding protein
cDNA	Complementary DNA
CEI	cumulus expansion index
CIP	Calf Intestinal Alkaline Phosphatase
COC	Cumulus Oocyte Complex
CRP	complementary regulatory protein
CS	chondroitin sulphate
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
E. coli	Escherichia coli
eCG	Equine chorionic gonadotropin
ECM	extracellular matrix
Egf	Epidermal growth factor
Egf-L	Egf-like peptide
EgfR	Egf receptor
ErbB2	erythroblastic leukemia viral oncogene homolog 2
ERK	Extracellular signal-regulated kinase
F1	first filial
FAK	focal adhesion kinase
FCS	fetal calf serum

FF-MAS	Follicular fluid-meiosis-activating sterol
FSH	Follicle Stimulating Hormone
G	globular
G1	globular domain 1
G2	globular domain 3
GAG	glycosaminoglycan
GC	granulosa cell
GEC	glomerular endothelial cell
GLUT	glucose transporter
GREM1	gremlin
GV	germinal vesicle
h	hour
HA	Hyaluronan
Has1	Hyaluronan synthase 1
Has2	Hyaluronan synthase 2
Has3	Hyaluronan synthase 3
HC	heavy chain
hCG	human Chorionic Gonadotropin
HS	heparin sulphate
HSPG	heparin sulphate proteoglycans
IαI	inter-α trypsin inhibitor
i.p.	intraperitoneal
ITS	insulin transferrin selenium
IU	international units
IVM	*in vitro* maturation
kDa	kilodalton
KO	knock out
LB	luria broth
LH	Luteinizing hormone
Lhcgr	luteinizing hormone/choriogonadotropin receptor
mGC	mural granulosa cell

MI	metaphase I
MII	metaphase II
min	minute
MMP	Matrix Metalloproteinase
mRNA	Messenger RNA
Nrip1	Nuclear receptor interacting protein 1
^{o}C	degrees Celsius
OHSS	ovarian hyperstimulation syndrome
PB	polar body
PBS	Phosphate Buffered Saline
PCOS	polycystic ovarian syndrome
PCR	Polymerase Chain Reaction
Pgr	Progesterone receptor
PRKO	Progesterone receptor knockout
Ptger2	prostaglandin E receptor 2, subtype EP2
Ptgs2	prostaglandin-endoperoxide synthase 2
Ptx3	Pentraxin 3
PVDF	polyvinylidene difluoride
Rac	RAS-related C3 botulinum substrate 1
RHAMM	receptor for HA-mediated motility
RhoA	ras homolog gene family, member A
RNA	ribonucleic acid
ROI	region of interest
ROS	reactive oxygen species
Rpm	revolutions per minute
RT	reverse transcription
RT-PCR	realtime reverse transcription polymerase chain reaction
S.E.M.	standard error of the mean
SDS	Sodium Dodecyl sulphate
SDS-PAGE	Sodium Dodecyl sulphate - polyacrylamide gel electrophoresis
Tnfaip6	Tumor necrosis factor alpha-induced protein 6
TSP-1	thrombospondin type I

VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
ZP	zona pellucida