Importance of Oocyte to Cumulus Cell Bi-directional Signalling on Oocyte and Subsequent Embryo and Foetal Development and Viability

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ABSTRACT

Oocyte in vitro maturation (IVM) possesses significant scientific and clinical benefits such as the elimination of dangerous side-effects like ovarian hyperstimulation syndrome. Unfortunately, due to a lack of understanding of the intricate processes involved, IVM success rates are low and cannot rival that of current IVF protocols involving hormonal stimulation. Recent scientific advancement has unveiled the existence of the oocyte-cumulus cell (CC) bi-directional regulatory loop and its importance to the development and survival of both cell types during folliculogenesis. This thesis therefore aimed to investigate the significance of these communication axes during IVM on oocyte and CC functions such as cumulus expansion, metabolism and oxidative stress levels and oocyte developmental competence into foetal development.

To target oocyte to CC signalling, growth differentiation factor 9 (GDF9), the primary identified oocyte paracrine factor in the mouse, and its SMAD2/3 signalling pathway were investigated. FSH/EGF were examined as modulators of CC to oocyte signalling, as these can only exert their effects on oocyte maturation through the CCs.

Maturation of mouse cumulus-oocyte complexes (COCs) in the absence of FSH/EGF and/or in the presence of SMAD2/3 inhibition resulted in the ablation of cumulus expansion whereas the addition of exogenous GDF9 to intact COCs significantly increased cumulus expansion. To assess the importance of cumulus expansion independent of oocyte and cumulus communications to embryo development, azaserine, an inhibitor of hyaluronan synthesis was used. Azaserine did not successfully attenuated cumulus

expansion in this system however subsequent blastocyst formation was severely diminished. Cumulus expansion was therefore not indicative of oocyte developmental competence.

Meiotic maturation was only affected by FSH/EGF but both signalling pathways affected sperm penetration. While blastocyst formation was unaffected, disrupted oocyte or cumulus signalling significantly decreased blastocyst inner cell mass (ICM) numbers. Conversely, addition of exogenous GDF9 with FSH/EGF during IVM increased ICM numbers. Significantly, exogenous supplementation of GDF9 during IVM increased foetal survival while inhibition of SMAD2/3 signalling had the opposite effect. Implantation rates and foetal weights were unaffected in both treatments.

The effect of GDF-9 and SMAD 2/3 signalling on metabolism of the COC was examined. The existence of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFB) isoforms were discovered in COCs and along with other metabolic gene transcripts, were significantly altered in the absence of FSH/EGF and with exogenous GDF9 and FSH/EGF although SMAD2/3 inhibition had no effect. Glycolytic activity however, was decreased in the absence of FSH/EGF and with SMAD2/3 inhibition but increased with exogenous GDF9. TCA cycle activity was only affected by FSH/EGF. The absence of FSH/EGF, SMAD2/3 inhibition and azaserine during IVM all resulted in increased oxidative stress levels in the oocyte.

The work in this thesis also demonstrated that oocyte and CC signalling are co-dependent on each other as apart from cumulus expansion and slower developmental rates,

perturbations of both signalling pathways simultaneously did not have additive effects on oocyte developmental competence or on CC metabolic functions.

This thesis has therefore provided significance to the field of oocyte IVM through the evidence that oocyte-CC bi-directional communication during IVM is essential to oocyte viability and foetal outcomes.

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 to Christine Xueling Yeo 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 text.

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* Data presented in Chapter 5 and 6 have published as listed respectively

a. **Yeo CX**, Gilchrist RB, Lane M. Disruption of Bi-directional Oocyte-Cumulus Paracrine Signalling during In-Vitro Maturation Reduces Subsequent Mouse Oocyte Developmental Competence. Biology of Reproduction 2009 May; 80(5):1072-80.
b. **Yeo CX**, Gilchrist RB, Thompson JG, Lane M. Exogenous Growth Differentiation Factor 9 in Oocyte Maturation Media Enhances Subsequent Embryo Development and Fetal Viability in Mice. Human Reproduction 2008 Jan;23(1):67-73.

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GLOSSARY/ ABBREVIATIONS

293H	293 human embryonic kidney cell line
ALK	Activin receptor like kinase
BMP	Bone morphogenetic protein
BMPRII	Bone morphogenetic protein receptor type II
cAMP	Cyclic adenosine monophosphate
CC	Cumulus cell
CEEF	Cumulus expansion enabling factor
COC	Cumulus oocyte complex
DO	Denuded oocyte
EGF	Epidermal growth factor
EMP	Embden-Myerhof pathway
FCS	Foetal calf serum
FGO	Fully grown oocyte
FSH	Follicle stimulating hormone
GC	Granulosa cell
GDF9	Growth differentiation factor 9
GFPT1	Glutamine-fructose-6-phosphate transaminase 1
GV	Germinal vesicle
GVBD	Germinal vesicle breakdown
HA	Hyaluronic acid
Has2	Hyaluronan synthase 2
HBP	Hexosamine biosynthesis pathway
ICM	Inner cell mass
IVF	In vitro fertilisation
IVM	In vitro maturation
Ldh	Lactate dehydrogenase
LH	Luteinising hormone
MAPK	Mitogen activated protein kinase
mGC	Mural granulosa cell

MI	Meiosis metaphase I
MII	Meiosis metaphase II
OHSS	Ovarian hyperstimulation syndrome
OOX	Oocytectomised cumulus complex
OSF	Oocyte secreted factor
PDE	Phosphodiesterase
PFKFB	6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase
PFKP	Phosphofructokinase platelet
PGE2	Prostaglandin E_2
PI3K	Phosphatidylinositol 3-kinase
PKA	cAMP dependent protein kinase A
PKC	cAMP dependent protein kinase C
PTGS2	Prostaglandin endoperoxide synthase 2
TCA	Tricarboxylic acid cycle
TE	Trophectoderm
TGFβ	Transforming growth factor beta
TGFBR1	Transforming growth factor beta 1 receptor

PUBLICATIONS AND CONFERENCE

PROCEEDINGS

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