OPTIMISATION OF OOCYTE IN VITRO MATURATION USING OOCYTE SECRETED FACTORS

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This is dedicated to my beloved father who taught me that
women have the right and equal opportunity
to an education as men

Abstract

During follicular development, oocyte and somatic cells communicate by producing signals such as proteins, growth factors, hormones that interact in complex harmony. The oocyte plays an active role in this communication by secreting soluble oocyte secreted factors (OSFs) including growth differentiation factor 9 (GDF9) and bone morphogenetic factor 15 (BMP15). Those two growth factors have been implicated in follicular development and are related to female fertility. *In vitro* matured oocytes have aberrant gene expression and altered matrix protein profiles in cumulus cells compared to their *in vivo* mature counterparts, which leads to a decrease in oocyte quality and embryo development post fertilisation.

The first aim of this study was to determine the effect of exogenous native OSFs from denuded oocytes (DOs) on mouse *in vitro* maturation (IVM). In a series of experiments, native OSFs were used in various ways to observe the role of FSH, cumulus cells, and temporal effects, on production of native OSFs. Overall, co-culture of COCs with DOs improved mouse embryo development. The highest improvement in embryo development and embryo quality post co-culture of COCs with DOs was when COCs were matured in separate IVM media in the presence of FSH, then denuded at 3h to produce DOs, which were used in co-culture with COCs for another 14-15 h. Unfortunately, it is not practical to generate such large numbers of DOs in a clinical scenario. Therefore the next step was to find the functional bioactive forms of pure recombinant proteins that could improve IVM outcomes.

The above concept led to the main hypothesis of this thesis that the developmental competence of IVM oocytes can be improved by novel variants of purified recombinant GDF9 and BMP15. In these studies, GDF9 and BMP15 from various sources and forms were tested in cattle and mouse IVM. Two different forms of proteins: pro-mature complex and mature domain of GDF9 and BMP15, were used in cattle and mouse IVM. In this study, pro-mature complex of human BMP15 (hBMP15) improved cattle blastocyst development, which may, in part, be due to an increased in nicotinamide adenine diphosphate [NAD(P)H] and reduced glutathione (GSH) levels. Mature region of BMP15 had a moderate, albeit non-significant, effect on cattle embryo developmental outcomes, whilst mature region of GDF9 was

ineffective. Similar results were observed using pro-mature hBMP15, which improved the developmental competence of in vitro matured mouse oocytes, where a combination of mouse GDF9 (mGDF9) and hBMP15 (both in pro-mature complex form) produces the highest blastocyst rate.

The work presented in this thesis has provided evidence that exogenous native OSFs, and recombinant hBMP15 in its pro-mature complex form, are important for oocyte developmental programming and prove useful for improving mouse and cattle IVM oocyte developmental competence. Moreover, the source, doses and form of recombinant proteins play an important role in improving developmental competence of IVM oocytes. These results may contribute and translate to improve the success rate of *in vitro* matured human oocytes.

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.

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.

Jaqueline Sudiman

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Publications arising from this thesis

1. Sudiman, J, LJ Ritter, DK Feil, X Wang, K Chan, DG Mottershead, DM Robertson, JG Thompson, and RB Gilchrist. (2014a) Effects of differing oocyte-secreted factors during mouse in vitro maturation on subsequent embryo and fetal development. J Assist Reprod Genet 31 295-306.

2. Sudiman, J, ML Sutton-McDowall, LJ Ritter, MA White, DG Mottershead, JG Thompson, and RB Gilchrist. (2014b) Bone morphogenetic protein 15 in the pro-mature complex form enhances bovine oocyte developmental competence. PLoS One 9(7): e103563.

Conference Proceedings

1. **Sudiman J**, Ritter LJ, Mottershead DG, Thompson JG and Gilchrist RB. The Effect of Native and Recombinant Oocyte-Secreted Factors on *In Vitro* Maturation. Poster was presented at Australian Society of Medical Research in Adelaide (2011).

2. **Sudiman J**, Ritter LJ, Mottershead DG, Thompson JG and Gilchrist RB. Oocyte Secreted Factors Improve the *In Vitro* Maturation of Mouse Oocytes and Subsequent Embryo Development. Abstract was presented at Indonesian Fertility and Endocrinology Society in Bali (2011).

3. **Sudiman J**, Ritter LJ, Feil DK, Wang X, Mottershead DG, Thompson JG and Gilchrist RB. Effect of the Form and Temporal Addition of Oocyte-Secreted Factors during *In Vitro* Maturation. Poster was presented at Robinson Institute Research Symposium in Adelaide (2012), Ovarian Club in Prague (2012) and Post graduate Research Conference in Adelaide (2013).

4. **Sudiman J**, Sutton-McDowall M, Ritter LJ, White MA, Mottershead DG, Thompson JG and Gilchrist RB. Pro-mature Form of Bone Morphogenetic Protein 15 (BMP15) in Oocyte Maturation Improves Subsequent Bovine Embryo Development. Abstract was presented for Meat and Livestock finalist award at Society for Reproductive Biology in Sydney (2013).

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Abbreviations

ART	assisted reproductive technology
BSA	bovine serum albumin
BMP15	bone morphogenetic protein 15
hBMP15	human bone morphogenetic protein 15
BMPR2	bone morphogenetic protein receptor type II
cAMP	cyclic adenosine monophosphate
COC	cumulus oocyte complex
DO	denuded oocyte
ECM	extracellular matrix
EGF	epidermal growth factor
FAD	flavin adenine dinucleotide
FAF	fatty acid free
FSH	follicle stimulating hormone
FGF	fibroblast growth factor
GC	granulosa cell
GDF9	growth differentiation factor 9
hGDF9	human growth differentiation factor 9
mGDF9	mouse growth differentiation factor 9
GSH	reduced form of glutathione
GV	germinal vesicle
GVBD	germinal vesicle breakdown
GJC	gap junction communication
HEK-293T	human embryonic kidney cell line
H-αMEM	hepes-buffered minimum essential medium

H-TCM	hepes-buffered tissue culture medium
ICM	inner cell mass
IBMX	3-isobutyl-1-methylxanthine
IVC	*in vitro* culture
IVF	*in vitro* fertilization
IVM	*in vitro* maturation
IVP	*in vitro* production
KL	kit ligand
LH	luteinising hormone
LHR	luteinising hormone receptor
MI	metaphase I
MII	metaphase II
NADPH	nicotinamide adenine dinucleotide phosphate
OOX	oocytectomised complex
OSF	oocyte-secreted factor
PMSG	pregnant mare’s serum gondadotropin
PPP	pentose phosphate pathway
TCM-199	tissue culture medium 199
TCN	total cell number
TE	trophectoderm
TGFβ	transforming growth factor β
TGFβR-II	transforming growth factor β receptor type-II