Induced IVM: A New Approach to Oocyte Maturation *in vitro*

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Abstract

Oocyte *in vitro* maturation (IVM) is a technique that would alter the management of human infertility if success rates were notably higher. Oocyte maturation *in vivo* is a highly orchestrated, induced process, whereby 3'-5'-cyclic adenosine monophosphate (cAMP)-mediated meiotic arrest is overridden by the gonadotrophin surge. However, using standard IVM, oocytes resume maturation spontaneously hence compromising developmental competence. The aim of this thesis was to establish an improved system for mammalian oocyte IVM by studying the inclusion of various forms of cAMP modulators during IVM and examine oocyte quality and developmental capacity.

Firstly, this thesis includes a series of experiments designed to examine the effect of specific inhibition of phosphodiesterase type 8 (PDE8) during IVM of bovine oocytes on cAMP levels, meiotic and developmental capacity. The inhibition of PDE8 degradation resulted in a dose-dependent increase in cAMP levels and delayed oocyte meiotic resumption. However, the inhibition of PDE8 degradation failed to enhance oocyte developmental competence.

This thesis includes an extensive series of studies designed to establish a novel induced-IVM system. Firstly, a pre-IVM phase was developed where immature bovine or mouse oocytes were briefly treated with the adenylate cyclase activator, forskolin and a non-specific PDE inhibitor, IBMX, which substantially increased intra-oocyte cAMP to *in vivo* physiological levels. Secondly, to maintain oocyte cAMP levels and prevent precocious oocyte maturation, oocytes were then matured with an oocyte-specific PDE 3 inhibitor, cilostamide and simultaneously induced to mature by FSH. The net effect of this system was an increase in oocyte-somatic cell gap-junctional communication and a delay in meiotic progression through prophase I to metaphase II, extending the standard IVM interval. Moreover FSH-induced maturation was prevented by an epidermal growth factor receptor inhibitor, AG1478, demonstrating that induced oocyte maturation functions via secondary autocrine signalling within the cumulus cell compartment.

Results from the present thesis also demonstrated that induced-IVM leads to a substantial improvement in oocyte quality, which in turn had long-term developmental consequences improving embryo/fetal yield and pregnancy outcomes. The work presented in this thesis validates this technology using two mammalian models. In the bovine, induced-IVM more than doubled embryo yield (27% to 69%), relative to standard-IVM. Similarly in the mouse, induced-IVM substantially increased fertilization rate (55% vs. 82%), embryo yield (55% vs. 86%), embryo quality, implantation rate (28% vs. 53%), fetal yield (8% vs. 26%) and fetal weights (0.5g vs. 0.9g). All these embryo and fetal readouts using induced-IVM in mice were equivalent to those using *in vivo* matured oocytes (conventional IVF).

In conclusion, induced-IVM mimics some of the characteristics of oocyte maturation *in vivo* and substantially improves oocyte developmental outcomes in two disparate mammalian species. The outcomes of the research presented in this thesis have provided a new perspective to our understanding of the mechanisms regulating oocyte maturation and the acquisition of developmental competence. The novel IVM system will provide new options for a wide range of reproductive biotechnologies including livestock breeding and conservation applications. Application of induced-IVM to human infertility will bring substantial cost and health benefits by simplifying ART protocols.

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 Firas Albuz and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

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*** List of Publications**

1. Maxime Sasseville, Firas K Albuz, Nancy Côté N, Christine Guillemette, Robert B Gilchrist and Francois J Richard. (2009). Characterization of Novel Phosphodiesterases in the Bovine Ovarian Follicle. *Biology of Reproduction.*81: 415-425.

2. Firas K Albuz, Maxime Sasseville, Michelle Lane, David T Armstrong, Jeremy G Thompson and Robert B Gilchrist. (2009). Induced oocyte IVM substantially improves embryo yield and pregnancy outcomes. *Nature Biotechnology*. Manuscript No. NBT-TR21146A (submitted 01/06/2009).

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Glossary/Abbreviations

AC	adenylate cyclase
AM	acetoxy-methyl ester
5‟-AMP	adenosine 5‟-monophosphate
AMPK	AMP-activated protein kinase
ATP	adenosine triphosphate
BSA	bovine serum albumin
B-TCM	bicarbonate-buffered tissue culture medium
CAM	calcein acetoxy-methyl ester
cAMP	cyclic adenosine monophosphate
cAMP-PKA	cAMP-dependant protein kinase A
cGMP	cyclic guanosine monophosphate
CM	cilostamide
COC	cumulus-oocyte complex
dbcAMP	dibutyryl cyclic adenosine monophosphate
DMSO	dimethyl-sulphoxide
DO	cumulus-oocyte complex derived oocyte
ET	embryo transfer
FAF	fatty acid-free
FF-MAS	follicular fluid meiosis activating sterol
FSK	forskolin
FSH	follicle stimulating hormone
GC	granulosa cell
GJC	gap junctional communication
GV	germinal vesicle
GVBD	germinal vesicle breakdown
hCG	human chorionic gonadotrophin
H-TCM	hepes-buffered tissue culture medium
iAC	invasive adenylate cyclase
IBMX	3-isobutyl-1-methylxanthine
ICM	inner cell mass
IVF	*in vitro* fertilization
IVM	*in vitro* maturation
IVP	*in vitro* production (of embryos)
LH	luteinising hormone
M	meiosis phase of the cell cycle
MGC	mural granulosa cell
MI	metaphase I
MII	metaphase II
MR	milrinone
PDE	phosphodiesterase
PDE3	phosphodiesterase subtype 3
PDE4	phosphodiesterase subtype 4
PDE8	phosphodiesterase subtype 8
PVA	polyvinyl alcohol
rhFSH	recombinant human FSH
RIA	radioimmunoassay
TE	trophoectoderm

Publications and Conference Proceedings

Scientific Publications

1. Maxime Sasseville, **Firas K Albuz**, Nancy Côté N, Christine Guillemette, Robert B Gilchrist and Francois J Richard. (2009). Characterization of Novel Phosphodiesterases in the Bovine Ovarian Follicle. *Biology of Reproduction.*81: 415-425.
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2. **Firas K Albuz,** Maxime Sasseville, Michelle Lane, David T Armstrong, Jeremy G Thompson and Robert B Gilchrist. (2009). Induced oocyte IVM substantially improves embryo yield and pregnancy outcomes. *Nature Biotechnology*. Manuscript No. NBT-TR21146A (submitted 01/06/2009).
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Other Journal Contributions

1. **Firas K Albuz**, Maxime Sasseville, David T Armstrong, Jeremy G Thompson and Robert B Gilchrist. (2008). Synergistic effects of cAMP modulating agents in pre-IVM and in IVM on bovine cumulus and oocyte functions. *Reproduction, Fertility and Development*; 20 (Suppl); Abstract 260.

2. Maxime Sasseville, **Firas K Albuz**, Francois J Richard, Robert B Gilchrist. (2008). Evidence for a novel cAMP-phosphodiesterase expressed in the bovine ovarian follicle. *Reproduction, Fertility and Development*; 20 (Suppl); Abstract 254.

3. **Firas K Albuz**, Maxime Sasseville, David T Armstrong, Michelle Lane, Jeremy G Thompson and Robert B Gilchrist. (2009). Induced oocyte *in vitro* maturation (IVM) substantially improves embryo yield and pregnancy outcomes. *Reproduction, Fertility and Development*; 21 (Suppl); Abstract 131.

Conference Proceedings

1. International

1. **Firas K Albuz**, Maxime Sasseville, David T Armstrong, Jeremy G Thompson and Robert B Gilchrist. (2009). Substantial improvements in embryo yield using a novel system of induced IVM by exploiting cAMP modulators in pre-IVM and IVM. Proceedings of the twenty-fifth annual conference of the European Society of Human Reproduction and Embryology, Amsterdam, The Netherlands. Abstract 167. (Oral presentation).

2. Robert B Gilchrist, **Firas K Albuz** and Jeremy G Thompson. (2010). A new approach to IVM and embryo IVP: Induced-IVM substantially improves embryo yield and pregnancy outcomes. Proceedings of the thirty-sixth annual conference of International Embryo Transfer Society Conference, Cordoba, Argentina.

2. National

1. **Firas K Albuz**, Maxime Sasseville, David T Armstrong, Jeremy G Thompson and Robert B Gilchrist. (2008). Synergistic effects of cAMP modulating agents in pre-IVM and in IVM on bovine cumulus and oocyte functions. Proceedings of the thirty-ninth annual conference of The Society for Reproductive Biology, Melbourne, Australia. (Abstract 260)

2. Maxime Sasseville, **Firas K Albuz**, Francois J Richard, Robert B Gilchrist. (2008.) Evidences for a novel cAMP-phosphodiesterase expressed in the bovine ovarian follicle. Proceedings of the thirty-ninth annual conference of The Society for Reproductive Biology, Melbourne, Australia. (Abstract 254)

3. **Firas K Albuz,** Maxime Sasseville, Michelle Lane, David T Armstrong, Jeremy G Thompson and Robert B Gilchrist. (2009). Induced oocyte *in vitro* maturation (IVM) substantially improves embryo yield and pregnancy outcomes. Proceedings of the fortieth annual conference of The Society for Reproductive Biology, Adelaide, Australia. (Abstract 131)

3. Local

1. **Firas K Albuz**. (2008). Improving the efficiency of oocyte maturation *in vitro*. The 2008 AusBiotech-GSK Student Excellence Awards National Lauch: Research to Revenue, Adelaide, Australia.

2. **Firas K Albuz,** Maxime Sasseville, Michelle Lane, David T Armstrong, Jeremy G Thompson and Robert B Gilchrist. (2009). Regulation of cAMP during oocyte maturation substantially improves developmental outcomes. Proceedings of the Australian Society for Medical Research SA conference, Adelaide, Australia. (Abstract RW1.)

3. **Firas K Albuz** (2009). IVF without hormones. The 2009 Women and Children`s Hospital Young Investigator Award (YIA) Semi-finals-Scientific Presentation, Adelaide, Australia.

Provisional Patent

Firas K Albuz, Robert B Gilchrist, Jeremy G Thompson. "Methods for the collection and maturation of oocytes" US Provisional Patent Application No. IP10922/473 (14/05/2009).

Visits to Overseas Laboratories

2009 Prof. Johan Smitz, Follicle Biology lab, Vrije Universitiet Brussel (VUB), Brussels, Belgium.

Invited Guest Seminar - International

2009 Induced oocyte *in vitro* maturation (IVM) substantially improves developmental outcomes. Vrije Universitiet Brussel (VUB), Brussels, Belgium (24/06/2009).

Awards, Scholarship & Prizes

2006-2009	Australian Postgraduate Award Industry (APAI) under the Australian Research Council (ARC) Linkage Project (PhD scholarship)
2008	AusBiotech-GSK Student Excellence Award Finalist
2008	The Society for Reproductive Biology (SRB) Meat and Livestock Australia New Investigator Award Finalist
2008	Society for Reproductive Biology Travel Scholarship
2009	Ross Wishart Memorial Award Finalist
2009	AUGU/RC Heddle Award Finalist
2009	The Society for Reproductive Biology (SRB) Meat and Livestock Australia New Investigator Award Finalist
2009	Research Centre for Reproductive Health Research Scholarship The University of Adelaide
2009	Faculty of Health Science Travelling Fellowship The University of Adelaide
2009	**N**etwork in Genes and Environment in Development Conference Participation Award
2009	The Women & Children`s Hospital Young Investigator Award Semi-Finalist