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


October 2009

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

Scientific Publications

   [Impact Factor: 3.6]

   [Impact Factor: 22.3]

Other Journal Contributions


Conference Proceedings

1. **International**


2. **National**


3. Local


Provisional Patent


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  Network in Genes and Environment in Development Conference Participation Award

2009  The Women & Children’s Hospital Young Investigator Award Semi-Finalist