FACTORS AFFECTING THE DEVELOPMENTAL COMPETENCE OF PIG OOCYTES MATURED IN VITRO

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Table of Contents

ABSTRACT ........................................................................................................................................... 1
DECLARATION ....................................................................................................................................... 4
ACKNOWLEDGEMENTS ...................................................................................................................... 5
PUBLICATIONS AND CONFERENCE PROCEEDINGS .................................................................. 8
AWARDS ............................................................................................................................................... 11
ABBREVIATIONS .............................................................................................................................. 12

1 LITERATURE REVIEW .................................................................................................................. 17

1.1 INTRODUCTION ........................................................................................................................ 17

1.2 THE OVARIAN FOLLICLE .......................................................................................................... 20
   1.2.1 Follicle growth and development in the pig ........................................................................ 20
   1.2.2 Antral follicle components ................................................................................................. 22
   1.2.3 Follicle cell communication ............................................................................................... 23
   1.2.4 Gonadotrophic control ....................................................................................................... 24
   1.2.5 Steroids in oocyte maturation ............................................................................................ 27
   1.2.6 Paracrine control ............................................................................................................... 31

1.3 OOCYTE MEIOTIC RESUMPTION ........................................................................................ 33
   1.3.1 Follicle cells & meiotic resumption ................................................................................... 34
   1.3.2 Follicular fluid & meiotic resumption ............................................................................... 35
   1.3.3 Gap Junction Communication & meiotic resumption ....................................................... 35
   1.3.4 cAMP ............................................................................................................................... 37
      1.3.4.1 Follicle sources of cAMP ............................................................................................... 37
      1.3.4.2 Intra-oocyte cAMP measurements ............................................................................. 38
      1.3.4.3 Treatments to increase cAMP ..................................................................................... 39
      1.3.4.4 cAMP paradox ............................................................................................................ 40
      1.3.4.5 MPF/MAPK ............................................................................................................... 41

1.4 IN VITRO OOCYTE MATURATION ............................................................................................ 43
1.4.1 Nuclear maturation ................................................................. 46
1.4.2 Cytoplasmic maturation ....................................................... 47

1.5 DEVELOPMENTAL COMPETENCE ....................................... 51

1.6 FACTORS EFFECTING MATURATION AND DEVELOPMENTAL COMPETENCE IN THE PIG ................. 53
1.6.1 Donor age ........................................................................... 53
1.6.2 Follicle and oocyte size ...................................................... 58

1.7 SUMMARY ............................................................................. 63

1.8 THESIS HYPOTHESIS AND AIMS ....................................... 63
1.8.1 General hypothesis ............................................................. 63
1.8.2 Specific hypotheses ............................................................ 64

2 METHODS AND MATERIALS .................................................. 67

2.1 CHEMICALS AND MEDIA .................................................... 67
2.2 In vitro oocyte maturation ....................................................... 68
2.3 MEASUREMENTS ................................................................. 69
2.4 CUMULUS CELL REMOVAL .................................................. 71
2.5 EMBRYO PRODUCTION ........................................................ 71
2.5.1 Parthenogenetic activation ................................................ 71
2.5.2 Fertilisation ....................................................................... 72
2.5.3 Embryo culture ............................................................... 72
2.6 STEROID CONTENT ASSESSMENT .................................... 73
2.7 INTRA-OOCYTE CAMP ASSAY .......................................... 73
2.8 ASSESSMENT OF CUMULUS EXPANSION ............................ 74
2.9 STAINING ............................................................................ 75
2.9.1 Orcein staining of oocytes ................................................ 75
2.9.2 Hoechst staining of blastocysts ........................................ 76
2.10 GJC ASSAY ........................................................................ 76
2.11 STATISTICAL ANALYSIS ..................................................... 78

3 EFFECT OF THE ONSET OF PUBERTY ON OVARIAN MORPHOLOGY AND OOCYTE
DEVELOPMENTAL COMPETENCE ........................................................................... 80
3.1 INTRODUCTION ........................................................................................................................ 80

3.2 EXPERIMENTAL DESIGN .............................................................................................................. 82

3.2.1 Experiment 1: Ovarian and oocyte morphology at the onset of puberty ............................... 82

3.2.2 Experiment 2: Oocyte developmental competence at the onset of puberty ......................... 82

3.3 RESULTS ...................................................................................................................................... 83

3.3.1 Ovarian and oocyte morphology at the onset of puberty ................................................... 83

3.3.2 Oocyte developmental competence at the onset of puberty ................................................ 84

3.4 DISCUSSION ............................................................................................................................... 85

4 EFFECT OF DIBUTYRYL CAMP ON IN VITRO MATURED PRE-PUBERTAL AND ADULT OOCYTES .................................................................................................................. 90

4.1 INTRODUCTION ........................................................................................................................ 90

4.2 EXPERIMENTAL DESIGN ........................................................................................................... 93

4.2.1 Experiment 1: Cyclic AMP content during IVM and subsequent parthenote embryo development of pre-pubertal and adult oocytes ................................................................. 93

4.2.2 Experiment 2: Meiotic progression of pre-pubertal and adult oocytes during IVM .......... 93

4.2.3 Experiment 3: Cumulus expansion of pre-pubertal and adult COCs during IVM .......... 93

4.2.4 Experiment 4: Progesterone secretion by pre-pubertal and adult COCs during IVM ...... 94

4.2.5 Experiment 5: Embryo development of pre-pubertal and adult oocytes following IVF ..... 94

4.3 RESULTS ...................................................................................................................................... 94

4.3.1 Cyclic AMP content during IVM and subsequent parthenote embryo development of pre-pubertal and adult oocytes ................................................................. 94

4.3.2 Meiotic progression of pre-pubertal and adult oocytes during IVM ................................. 98

4.3.3 Cumulus expansion of pre-pubertal and adult COCs during IVM ................................. 100

4.3.4 Progesterone secretion by pre-pubertal and adult COCs during IVM ............................. 101

4.3.5 Embryo development of pre-pubertal and adult oocytes following IVF .................... 102

4.4 DISCUSSION ........................................................................................................................... 104

5 EFFECT OF FOLLICLE SIZE ON PRE-PUBERTAL AND ADULT OOCYTE DEVELOPMENTAL COMPETENCE ................................................................................................................. 110

5.1 INTRODUCTION ........................................................................................................................ 110
5.2 EXPERIMENTAL DESIGN ......................................................................................................... 112
  5.2.1 Experiment 1: Follicle size distribution and steroid content analysis .................. 112
  5.2.2 Experiment 2: Follicle size and parthenote embryo development .................. 112
5.3 RESULTS .............................................................................................................................. 113
  5.3.1 Experiment 1: Follicle size distribution and steroid content analysis ........ 113
  5.3.2 Experiment 2: Follicle size and parthenote embryo development .............. 119
5.4 DISCUSSION ......................................................................................................................... 122

6 EFFECT OF FOLLICLE SIZE AND DBCAMP ON THE CAMP CONTENT OF PRE-
PUBERTAL OOCYTES AND COCS .................................................................................................. 127
  6.1 INTRODUCTION ..................................................................................................................... 127
  6.2 EXPERIMENTAL DESIGN ......................................................................................................... 128
    6.2.1 Experiment 1: Oocyte cAMP content ................................................................. 128
    6.2.2 Experiment 2: COC cAMP content ................................................................. 129
    6.2.3 Experiment 3: Cumulus expansion .................................................................. 129
  6.3 RESULTS .............................................................................................................................. 129
    6.3.1 Oocyte cAMP content ...................................................................................... 129
    6.3.2 COC cAMP content ......................................................................................... 132
    6.3.3 Cumulus expansion ......................................................................................... 134
  6.4 DISCUSSION ......................................................................................................................... 136

7 EFFECT OF FOLLICLE SIZE AND DBCAMP ON CUMULUS CELL-OOCYTE GJC 
DURING IVM .................................................................................................................................... 140
  7.1 INTRODUCTION ..................................................................................................................... 140
  7.2 EXPERIMENTAL DESIGN ......................................................................................................... 142
    7.2.1 Experiment 1: Oocyte-cumulus cell GJC assay ........................................... 142
  7.3 RESULTS .............................................................................................................................. 142
  7.4 DISCUSSION ......................................................................................................................... 144

8 GENERAL DISCUSSION ........................................................................................................... 150
  8.1 Effect of the onset of puberty on ovarian morphology and oocyte developmental 
competence ................................................................................................................................. 150
8.2 **EFFECT OF DIBUTYRYL CAMP ON IN VITRO MATURED PRE-PUBERTAL AND ADULT OOCYTES** 151

8.2.1 *Intra-oocyte cAMP content* ................................................................. 151

8.2.2 *Kinetics of meiotic maturation* ............................................................ 152

8.2.3 *Treatment with dibutyryl cAMP* ......................................................... 152

8.2.4 *Cumulus expansion* .............................................................................. 153

8.2.5 *Progesterone secretion* ......................................................................... 154

8.3 **EFFECT OF FOLLICLE SIZE ON OOCYTE DEVELOPMENTAL COMPETENCE** 154

8.3.1 *Follicle size and developmental competence* ....................................... 154

8.3.2 *Dibutyryl cAMP treatment and follicle size* ....................................... 155

8.3.3 *Follicular fluid steroid concentrations* ................................................. 156

8.4 **EFFECT OF FOLLICLE SIZE AND DBCAMP ON THE CAMP CONTENT OF PRE-PUBERTAL OOCYTES AND WHOLE COCS FOLLOWING IVM** ........................................... 157

8.4.1 *Intra-oocyte cAMP* ............................................................................ 157

8.4.2 *Whole COC cAMP* ........................................................................... 158

8.4.3 *Cumulus expansion* ........................................................................... 159

8.5 **EFFECT OF FOLLICLE SIZE AND DBCAMP ON CUMULUS CELL-OOCYTE GJC DURING IVM** 159

8.5.1 *GJC under control IVM conditions* .................................................... 159

8.5.2 *GJC following dbcAMP treatment* .................................................... 161

8.6 **FINAL CONCLUSIONS** ........................................................................ 162

8.7 **FUTURE STUDIES** ................................................................................ 162

**BIBLIOGRAPHY** ......................................................................................... 165

**APPENDIX 1** ................................................................................................. 200

**APPENDIX 2** ................................................................................................. 202

**APPENDIX 3** ................................................................................................. 204
Abstract

Pre-pubertal pig oocytes possess lower developmental competence than those from adult pigs following *in vitro* maturation (IVM). Previous studies have demonstrated that exposure of pre-pubertal oocytes to 1 mM dibutyryl cAMP (dbcAMP), a membrane permeable cyclic adenosine monophosphate (cAMP) analogue, for the first 20 h of IVM improves the rate of blastocyst development. Developmental competence of *in vitro* matured pig oocytes has been reported to increase with increasing follicle size. In this thesis, experiments were carried out using pre-pubertal and adult pig oocytes to investigate the relationship between donor age, intra-oocyte cAMP level and follicle size in terms of oocyte maturation and developmental competence.

These experiments demonstrated that, while ovarian, follicular and oocyte morphology are immediately altered with the onset of puberty, pre-pubertal oocytes must be exposed to more than the first oestrous cycle to achieve improved developmental competence *in vitro*. Later experiments demonstrated that pre-pubertal oocytes accumulate less cAMP during IVM, undergo more rapid meiotic progression and display reduced rates of blastocyst development compared to *in vitro* matured adult oocytes. Treatment with dbcAMP for 22 h IVM increased the cAMP content of pre-pubertal oocytes, slowed meiotic progression during IVM and improved the rate of blastocyst formation. While the cAMP concentration of pre-pubertal oocytes was increased to levels similar to that of adult oocytes, rates of blastocyst formation remained lower, suggesting that additional factor(s) are required for oocyte maturation.

This thesis also examined the follicle size cohorts that make up the 3-8 mm aspiration range on pig ovaries. The surface of pre-pubertal ovaries contained around double the number of 3 mm follicles compared with adult ovaries. Blastocyst development of pre-
pubertal oocytes increased with increasing follicle size and was highest using oocytes from 5-8 mm follicles, while adult oocytes from all follicle size cohorts displayed similar high rates of blastocyst formation. The interaction between follicle size and cAMP content in pre-pubertal oocytes was examined next. Cumulus-oocyte complexes (COCs) from 3 mm follicles accumulated less intra-oocyte and inter-COC cAMP and displayed reduced cumulus expansion compared with COCs from 5-8 mm follicles. While dbcAMP treatment increased the cAMP content of oocytes from 3 mm follicles, it had no effect on the cAMP content of the whole COC. These findings suggest that inadequate levels of intra-oocyte cAMP during IVM contribute to the low developmental competence of pre-pubertal oocytes from 3 mm follicles, suggesting that cAMP transfer, production or degradation processes are incomplete. Analysis of steroid content from different follicle size cohorts revealed that the progesterone content of pre-pubertal follicular fluid (FF) increased with increasing follicle size, yet overall was lower than that of adults. This suggests that differences may exist in the gonadotropin-stimulated steroidogenic activity of granulosa cells of pre-pubertal COCs from different follicle sizes. Since progesterone secretion did not differ between pre-pubertal and adult COCs, it appears that the downstream pathway from the granulosa cell response rather than the actual quantity of progesterone is important for subsequent maturation processes.

These studies then examined gap junction communication (GJC) within the pre-pubertal COC during IVM to examine whether the positive effects of increasing follicle size and dbcAMP on intra-oocyte cAMP levels relates to improved cAMP transfer between the cumulus cell layer and oocyte. Cumulus cell-oocyte GJC during IVM was maintained for a longer period in pre-pubertal COCs from 3 mm follicles than in those from 5-8 mm follicles. Treatment with dbcAMP had minimal effect on GJC in either COC type,
thus the dbcAMP-induced increase in intra-oocyte cAMP levels appears independent of GJC. Differences in GJC during IVM together with the COCs ability to increase intra-oocyte cAMP levels during IVM, suggests that differences may exist in the quantity of gonadotropin receptors, which are responsible for cAMP production, within the cumulus layer of COCs from 3 mm compared with 5-8 mm follicles.

In conclusion, this thesis has demonstrated that an increase in intra-oocyte cAMP is necessary during maturation for completion and synchronisation of maturation and high developmental competence of the pig oocyte. Comparison of 3, 4 and 5-8 mm follicle sizes in the pre-pubertal pig, as described here, provides an excellent model for further investigation into the role of cAMP and the other factors required for co-ordination of oocyte nuclear and cytoplasmic maturation and subsequent embryo production.
Declaration

This thesis 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 for when due reference has been made in the text.

I give consent to this copy of my thesis being made available in the University of Adelaide Library.

I acknowledge that copyright of published works contained within this thesis (as listed below) resides with the copyright holder/s of those works.

Melanie Anna Bagg, BSc, BHSc (Honours)

October 2007
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spend time with in my life, it is impossible to say enough to do you all justice here! In regards to the last five years or so, to all my friends and family I say: “Thank-you, each and every one of you, for loving at my worst”, The Whitlams, 1999!

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Publications and conference proceedings

Publications

Published manuscripts arising from experiments within this thesis (Appendix 1):


Conference Proceedings

International


National


State


6. **Bagg M. A.,** Vassena R., Grupen C.G., Armstrong D.T., Gandolfi F., 2003. Changes in ovarian morphology immediately after the onset of puberty are not accompanied by an increase in oocyte developmental competence. TQEH Research Day Annual Scientific Meeting, Woodville, South Australia

Note: Presenter underlined

* This conference paper was presented in scientific poster form and supervised by colleagues from the Research Centre for Reproductive Health when the presenting author(s) was unavoidably absent at short notice.
**Awards**

The Queen Elizabeth Hospital Research Foundation

Postgraduate Research Scholarship 2003-06

Australian Society for Medical Research Ross Wishart New Investigator Award 2005

Society for Reproductive Biology Travel Scholarship 2005

Research Centre for Reproductive Health Travel Scholarship 2005

North Western Adelaide Health Service Research Day Prize Finalist 2004

North Western Adelaide Health Service Research Day Poster Prize 2003

Australian Society for Medical Research

Holden Young Investigator Award Finalist 2003

Department of Anatomy of Domestic Animals, University of Milan

Borsa di Studio (Scholarship for Doctorate Study) 2001-02

Reproductive Medicine Unit Postgraduate Scholarship 2001

The University of Adelaide Walter and Dorothy Duncan Trust Grant 2001

The Friends Of the Queen Elizabeth Hospital Travel Grant 2001

The University of Adelaide Research Abroad Scholarship 2001
### Abbreviations

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<thead>
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<th>Abbreviation</th>
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<tbody>
<tr>
<td>&gt;</td>
<td>larger than</td>
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<tr>
<td>&lt;</td>
<td>smaller than</td>
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<td>+</td>
<td>plus</td>
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<tr>
<td>±</td>
<td>plus or minus</td>
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<tr>
<td>=</td>
<td>equals</td>
</tr>
<tr>
<td>5’-AMP</td>
<td>adenosine 5’-monophosphate</td>
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<tr>
<td>ana I</td>
<td>anaphase I</td>
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<tr>
<td>AREG</td>
<td>amphiregulin</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BMP15/GDF9b</td>
<td>bone-morphogenic protein 15</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>BTC</td>
<td>betacellulin</td>
</tr>
<tr>
<td>B-TCM</td>
<td>bicarbonate buffered-tissue culture medium</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>°C</td>
<td>temperature expressed as degrees celcius</td>
</tr>
<tr>
<td>CL</td>
<td>corpora lutea present on ovaries</td>
</tr>
<tr>
<td>COC</td>
<td>cumulus-oocyte complex</td>
</tr>
<tr>
<td>Cx</td>
<td>connexin</td>
</tr>
<tr>
<td>D I</td>
<td>diakinesis I</td>
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<tr>
<td>dbcAMP</td>
<td>dibutyryl cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>DMAP</td>
<td>6-dimethylaminopurine</td>
</tr>
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<td>DMSO</td>
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<td>DNA</td>
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<td>DO</td>
<td>denuded oocyte</td>
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<td>Abbreviation</td>
<td>Description</td>
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</tr>
<tr>
<td>E₂</td>
<td>17β-oestradiol</td>
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<td>EREG</td>
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</tr>
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</tr>
<tr>
<td>GV</td>
<td>germinal vesicle</td>
</tr>
<tr>
<td>GVBD</td>
<td>germinal vesicle breakdown</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>HB-GF</td>
<td>heparin-binding egf-like growth factor</td>
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<tr>
<td>hCG</td>
<td>human chorionic gonadotropin</td>
</tr>
<tr>
<td>H-TCM</td>
<td>hepes-buffered tissue culture medium</td>
</tr>
<tr>
<td>iAC</td>
<td>invasive adenylate cyclase</td>
</tr>
<tr>
<td>IBMX</td>
<td>3-isobutyl-1-methyxanthine</td>
</tr>
<tr>
<td>IGF</td>
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<td>IGF-BP</td>
<td>insulin growth factor binding protein</td>
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<tr>
<td>IP(3)R</td>
<td>inositol 1,4,5-trisphosphate receptor</td>
</tr>
<tr>
<td>IU</td>
<td>international units</td>
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</table>
NCL ovaries with no corpora lutea
NCSU North Carolina State University
nmol nanomoles
ng nanogram(s)
P₄ progesterone
PB-NCSU phosphate buffered North Carolina State University- 23 medium
PBS phosphate buffered saline
PDE phosphodiesterase
PI-3 kinase phosphoinositide 3-kinase
PKA protein kinase A
PKC protein kinase C
PR progesterone receptor
PVA polyvinyl alcohol
rhFSH recombinant human FSH
RIA radioimmunoassay
sec second
SPM sperm pre-incubation medium
TALP tyrode-albumin-lactate-pyruvate
telo I telophase I
TGFα transforming growth factor α
TGFβ transforming growth factor β
VEGF vascular endothelial growth factor
vs. versus