FACTORS AFFECTING THE DEVELOPMENTAL COMPETENCE OF PIG OOCYTES MATURED *IN VITRO*

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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.

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Publications and conference proceedings

Publications

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

I. Bagg M. A., Nottle M. B, Armstrong D. T., Grupen C. G., 2007. Relationship between follicle size and oocyte developmental competence in prepubertal and adult pigs. Reproduction Fertility and Development, 19 (7), 797-803.

II. Bagg M. A., Grupen C. G., Nottle M. B, Armstrong D. T., 2006. Effect of dibutyryl cAMP on the cAMP content, meiotic progression, cumulus expansion and developmental potential of *in vitro* matured pre-pubertal and adult pig oocytes. Molecular Reproduction and Development, 73 (10): 1326-1332.

III. Bagg M. A., Vassena R., Papasso-Brambilla E., Grupen C. G., Armstrong D. T., Gandolfi F., 2004. Changes in ovarian, follicular, and oocyte morphology immediately after the onset of puberty are not accompanied by an increase in oocyte developmental competence in the pig. Theriogenology, 62; 1003-1011.

Conference Proceedings

International

1. Bagg M. A., Grupen C. G., 2007. Acquisition of oocyte developmental competence in juvenile donors. International Embryo Transfer Society (IETS) Post Conference Tsukuba Meeting for Animal Biotechnology, Tsukuba, Japan
2. Bagg M. A., Grupen C. G., Nottle M., Armstrong D.T., 2005*. Intra-oocyte cAMP content and meiotic progression during IVM of pre-pubertal and adult pig oocytes. Society for the Study of Reproduction (SSR) Conference, Quebec City, Canada.

3. Bagg M. A., Vassena R., Papasso-Brambilla E., Grupen C. G., Armstrong D. T., Gandolfi F., 2003. The onset of puberty in pig immediately changes ovarian morphology but not oocyte *in vitro* developmental competence. IETS Annual Conference, New Zealand
4. Brevini T. A. L., Francisci C., Vassena R., Bagg M. A., Grupen C. G., Armstrong D.T., Gandolfi F., 2003. Follicular Fluid concentration during pig IVM affects oocyte developmental competence and mitochondria distribution. IETS Annual Conference, New Zealand
5. Bagg M. A., Grupen C.G., Nottle M., Gandolfi F., Armstrong D.T., 2003. Nuclear maturation of pre-pubertal versus post-pubertal porcine oocytes. IETS Annual Conference, USA

National

1. Bagg M. A., Grupen C. G., Nottle M., Armstrong D.T., 2005. Effect of donor age and follicle size on oocyte developmental competence in the pig. Society for Reproductive Biology (SRB) Annual Conference, Perth, Western Australia
2. Bagg M. A., Grupen C.G., Gandolfi F., Armstrong D.T., 2003. Kinetics of meiotic maturation differ between pre-pubertal and adult pig oocytes. SRB Annual Conference, Melbourne, Victoria

State

1. Bagg M. A., Grupen C. G., Nottle M., Armstrong D.T., 2005. Follicle size: The key to successful oocyte development. Australian Society For Medical Research (ASMR) Annual SA Conference, Adelaide, South Australia

2. Bagg M. A., Grupen C. G., Nottle M., Armstrong D.T., 2005. Differences in pre-pubertal and adult oocyte developmental competence is correlated with oocyte cAMP content in the pig. ASMR Annual SA Conference, Adelaide, South Australia
3. Bagg M. A., Grupen C. G., Armstrong D.T., 2004. Oocyte Developmental Competence Before Puberty: What is Missing? The Queen Elizabeth Hospital (TQEH) Research Day, Annual Scientific Meeting, Woodville, South Australia
4. 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. ASMR Annual SA Conference, Adelaide, South Australia
5. Bagg M. A., Grupen C.G., Gandolfi F., Armstrong D.T., 2003. Kinetics of meiotic maturation differ between pre-pubertal and adult pig oocytes. ASMR Annual SA Conference, Adelaide, South Australia
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

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* 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.

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Abbreviations

>	larger than
<	smaller than
+	plus
±	plus or minus
=	equals
5’-AMP	adenosine 5’-monophosphate
ana I	anaphase I
AREG	amphiregulin
ATP	adenosine triphosphate
BMP15/GDF9b	bone-morphogenic protein 15
BSA	bovine serum albumin
BTC	betacellulin
B-TCM	bicarbonate buffered-tissue culture medium
cAMP	cyclic adenosine monophosphate
^{o}C	temperature expressed as degrees celcius
CL	corpora lutea present on ovaries
COC	cumulus-oocyte complex
Cx	connexin
D I	diakinesis I
dbcAMP	dibutyryl cyclic adenosine monophosphate
DMAP	6-dimethylaminopurine
DMSO	dimethyl-sulphoxide
DNA	deoxyribonucleic acid
DO	denuded oocyte

E_2	17β-oestradiol
EGF	epidermal growth factor
ER	oestrogen receptor
EREG	epiregulin
FCS	fetal calf serum
FF	follicular fluid
FGF	fibroblast growth factor
fmol	femto moles
FI	fluorescence intensity
FSH	follicle stimulating hormone
FSHR	follicle stimulating hormone receptor
GDF-9	growth differentiation factor-9
GJC	gap junction communication
GV	germinal vesicle
GVBD	germinal vesicle breakdown
h	hour(s)
HB-GF	heparin-binding egf-like growth factor
hCG	human chorionic gonadotropin
H-TCM	hepes-buffered tissue culture medium
iAC	invasive adenylate cyclase
IBMX	3-isobutyl-1-methyxanthine
IGF	insulin growth factor
IGF-BP	insulin growth factor binding protein
IP(3)R	inositol 1,4,5-trisphosphate receptor
IU	international units

IVC	*in vitro* culture
IVF	*in vitro* fertilisation
IVM	*in vitro* maturation
IVP	*in vitro* production
KL/SCF	kit ligand/stem cell factor
LH	luteinising hormone
LHR	luteinising hormone receptor
MAPK	mitogen activated protein kinase
MGC	mural granulosa cell
MI	metaphase I
MII	metaphase II
min	minute(s)
mg	milligram(s)
mIU	milli international units
ml	millilitre(s)
µl	microliter(s)
mM	millimolar concentration
mm	millimetre(s)
µg	microgram(s)
µm	micrometre(s)
µM	micromolar concentration
MPF	maturation promoting factor
MPN	male pronucleus
mRNA	messenger ribonucleic acid
L	litre(s)

NCL	ovaries with no corpora lutea
NCSU	North Carolina State University
nmol	nanomoles
ng	nanogram(s)
P_4	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