The Rational Development of Improved In Vitro Maturation of Bovine Oocytes

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A thesis submitted to the University of Adelaide in total fulfilment of the requirements for the degree of Doctor of Philosophy

November 2004

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Abstract

In vitro embryo production has vastly improved over the past decade through the study of the in vivo environment and the metabolic requirements of embryos. In contrast, in vitro oocyte maturation (IVM) culture conditions have remained relatively unchanged and are suboptimal. The aim of this thesis was to create improved systems for bovine IVM by studying the metabolic profiles and requirements of intact cumulus oocyte complexes (COCs) during IVM and determining the ion and energy substrate composition of bovine follicular fluid (FF).

Glucose, pyruvate and oxygen consumption of bovine COCs increased 2-fold over the 24 h IVM period, with glucose being the preferred energy substrate. While initially the majority of glucose consumed by COCs is metabolised via glycolysis (L-lactate production), a considerable proportion of glucose is used as a substrate for extracellular matrix (ECM) synthesis towards the end of IVM. Glucosamine (an intermediate substrate of hyaluronic acid) supplementation of IVM media lead to decreased glucose consumption and incorporation into ECM during FSH-stimulated expansion.

Biochemical analyses of bovine FF demonstrated that the concentration of some ions and energy substrates varied with follicle size. Although follicular glucose concentrations increased with follicle size, levels were ~2-fold lower than that found in Tissue Culture Medium (TCM199), the most commonly employed medium for bovine IVM. Synthetic Follicular Fluid Medium (SFFM) was created, based on the FF data and also contained glucosamine. Two different glucose concentrations were examined, 2.3 mM glucose to represent physiological concentrations and 5.6 mM glucose, the same concentration as is in TCM199. Culturing COCs in different glucose concentrations manipulated the completion of nuclear maturation and this was dependant on concentration, gonadotrophin supplementation and the timing of media changes, demonstrating the importance of this substrate to meiotic competence.

Although glucosamine had no effect on oocyte nuclear maturation, supplementation during IVM led to a dose-dependent decrease in blastocyst rates. The detrimental effects of glucosamine manifested during early cleavage and were associated with a 0.6-fold decrease in protein synthesis levels within the oocyte compared to oocytes cultured in media with no glucosamine, suggesting a detrimental effect on developmental competence. Interestingly oocytes cultured in media containing glucosamine and EGF had significantly higher protein synthesis compared to the control group.

The biochemical profiles of COCs during IVM and FF were determined and used to create new media that allowed manipulation of oocyte nuclear maturation but compromised cytoplasmic maturation. Further research is required to optimise SFFM and to investigate the detrimental effects of glucosamine on developmental competence.

Declaration

This thesis contains no material that 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 consent to this copy of my thesis, when deposited in the University of Adelaide library, being available for loan and photocopy.

November 2004

Melanie McDowall

Acknowledgements

I would like to express my most sincere gratitude to my two supervisors, Drs Jeremy Thompson and Rob Gilchrist for the support during the past three years, for being there when I was stumped, to share the excitement of getting positive results and especially for learning a new language, namely “Melisms”. The highs and lows of the past three years have been incredible and I am grateful to you for giving me the opportunity to experience them.

My PhD project could not have occurred without the support of Cook Australia, in particular Gus Taddeo, Jason Spittle and Kim Giliam. Thank you for having faith in my project and work.

An enormous thanks goes out to past and present staff and students of the Research Centre for Reproductive Health (Reproductive Medicine Unit), the Department of Obstetrics and Gynaecology and fellow members of the Research Embryology Group. In particular, thanks to Dr Karen Kind for help during the first half of my project, Dr Pablo Cetica for assistance with the COC metabolism profiles and Rachael Collett for too many things to name so I won’t even try. Keep up the “quotes of the week”. Exceptional thanks go to Bec and Nat, my fellow Plastic Spiffies. There are too many bad and dodgy songs lyrics and sayings from the “classics” list I could quote and add as an appendix but will I’ll refrain myself. Sam, my gym buddy and one of the most beautiful people I know. Don’t ever change. Fellow students over the past 3 years: Alex, Deanne, Debbie, Lottie, Kylie, Theresa, Cadence and everyone else at TQEH. All your friendship and support has been invaluable to my sanity.

Thank you to my ever supporting parents and my incredible network of family and friends for having faith in my ability to undertake a PhD, for putting up with my funky hours, weeks or months without contact and for not looking too bored when I start talking about my oocytes! Mum and Dad, thank you for putting up with me during the past 7 years of uni, also for convincing me that science was my calling,

not hairdressing or dolphin training! My two best and craziest friends in the world, Rae and Liv; thanks for the coffee, the songs, Buffy and the mispronunciation of oocyte (oooooocyte). To the extensive "Mel support" crew, Darren, Kathy, Doug, Melissa, Simon, Vindaloo, Jerry, all other members of the Port Moorowie crew, Bec B, Tony, Ali and everyone else. Yay, I'm finally finished and can finally catch up with all you guys.

Lastly a big goosh goes out to my beautiful husband and soul mate, Sam for everything and anything. I love you like crazy. Paper is the traditional gift for the first year anniversary and since there is a lot of it here, I dedicate this thesis to you.

This project was financially supported by the Australia Research Council (SPIRT, C00107702) and Cook Australia Pty Ltd.

Abbreviations

Akt	Protein kinase B pathway
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BMP-15	Bone morphogenic protein
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
COC	Cumulus oocyte complex
DO	Denuded oocyte
ECM	Extra cellular matrix
EGF	Epidermal growth factor
FCS	Fetal calf serum
FF	Follicular fluid
FF-MAS	Follicular fluid meiosis activating sterol
FGF	Fibroblast growth factor
FSH	Follicle stimulating hormone
GDF-9	Growth differentiation factor 9
Glc	Glucosamine
GFAT	Glutamine:Fructose-6-phosphate transferase
GLUT	Glucose transporter
GSH	Glutathione
GV	Germinal vesicle
GVBD	Germinal vesicle breakdown
HA	Hyaluronidase treatment
hCG	Human chorionic gonadotrophin
IVC	In vitro embryo culture
IVF	In vitro fertilization
IVM	In vitro maturation
IVP	In vitro embryo production
LDH	Lactate dehydrogenase
LH	Luteinizing hormone

MI	Metaphase I
MII	Metaphase II
MPF	Maturation promoting factor
MTF	Mouse oviductal fluid
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
OOX	Oocytectomised complex
PI3K	Phosphatidylinositol pathway
PPP	Pentose phosphate pathway
PRPP	Phosphoribosylpyrophosphate
PVA	Polyvinyl alcohol
PVP	Polyvinyl pyrolidone
ROS	Reactive oxygen species
SFFM	Synthetic Follicular Fluid Medium
SOF	Synthetic oviductal fluid
TCA cycle	Tricarboxylic acid cycle
TCM199	Tissue culture medium 199
T-MAS	Testicular meiosis activating sterol
TZP	Transzonal cytoplasmic processes

Publications

Sutton-McDowall ML, Gilchrist RB and Thompson JG 2004 The effect of hexoses and gonadotrophin supplementation on bovine oocyte nuclear maturation during in vitro maturation in a synthetic follicular fluid medium *Reproduction, Fertility and Development* submitted.

Sutton-McDowall ML, Gilchrist RB and Thompson JG 2004 Cumulus-expansion and glucose utilisation by bovine cumulus-oocyte complexes during in vitro maturation: the influence of glucosamine and follicle stimulating hormone *Reproduction* **128** 313-319.

Sutton ML, Cetica PD, Beconi MT, Kind KL, Gilchrist RB and Thompson JG 2003 The influence of oocyte-secreted factors and culture conditions on the metabolic activity of bovine cumulus cell complexes *Reproduction* **126** 27-34.

Sutton ML, Gilchrist RB and Thompson JG 2003 Effects of in vivo and in vitro environments on metabolism of the cumulus-oocyte complex and its influence on oocyte developmental capacity *Human Reproduction Update* **9** 35-48.

Provisional Patents

Sutton-McDowall ML, Gilchrist RB and Thompson JG 2003 Media for oocyte maturation, Provisional Patent (DN-1429).

Conference Proceedings

Sutton-McDowall, ML, Gilchrist, RB and Thompson, JG 2005 Glucosamine supplementation during in vitro maturation leads to perturbed developmental capacity of bovine cumulus oocyte complexes *International Embryo Transfer Society* Copenhagen, Denmark accepted.

Sutton-McDowall, ML, Gilchrist, RB and Thompson, JG 2004 Regulation of bovine oocyte meiotic and developmental capacity by glucose and glucosamine

Society for Reproductive Biology, Reproduction, Fertility and Development Abstract 204.

Sutton-McDowall, ML, Gilchrist, RB and Thompson, JG 2004 The effects of glucose and glucosamine on cumulus expansion and nuclear maturation of bovine cumulus oocyte complexes *International Embryo Transfer Society Portland, Oregon, USA, Reproduction, Fertility and Development* **16** Abstract 317.

Sutton-McDowall, **ML** and Thompson, JG 2003 The importance of cellular communication during bovine oocyte maturation, *Australian Embryo Transfer Society, Gold Coast, Queensland*.

Sutton, ML, Cetica, PD, Gilchrist, RB and Thompson, JG 2003 The metabolic profiles of bovine cumulus-oocyte complexes: effects of oocyte-secreted factors and stimulation of cumulus expansion *International Embryo Transfer Society, Auckland, New Zealand Theriogenology,* **59** 500.