Mitochondrial Sirtuin 3 and Sirtuin 5 in granulosa and cumulus cells and their contribution to the altered follicular environment in women with either reduced ovarian reserve or advanced maternal age.

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Abstract

Women with reduced ovarian reserve or advanced maternal age are known to have poorer IVF outcomes compared to younger women with normal ovarian reserve. While previous studies in humans have correlated metabolite concentrations in follicular fluid to IVF outcome, the impact of maternal age and reduced ovarian reserve have yet to be determined. Oocytes are dependent on mitochondrial metabolism for viability and disruptions to mitochondrial activity can reduce oocyte viability. It has been suggested that oocytes from women with either reduced ovarian reserve or advanced maternal age have a reduction in metabolic function, however, the exact mechanisms behind this reduction remain largely unknown. Interestingly, a family of proteins, the Sirtuins, are able to sense cellular metabolic state and post-translationally alter protein function, thus implicating these proteins in metabolic function. Sirtuin 3 (SIRT3) and Sirtuin 5 (SIRT5) are two proteins that are specifically located to mitochondria, thus may be important in understanding metabolic control in the pre-ovulatory follicle. Thus the aim of this thesis was to determine if a difference exists in the follicular environment in women with reduced ovarian reserve or advanced maternal age and if so does SIRT3 or SIRT5 play role in ovarian follicular cells.

Women (n=111) undergoing routine IVF treatment were recruited to participate in this study. They were allocated to one of three cohorts based on maternal age (young maternal age [≤35]; advanced maternal age ≥40) and ovarian reserve for age, as measured by serum anti-mullerian hormone (AMH) levels. Surplus follicular fluid, granulosa and cumulus cells were collected, de-identified and randomly allocated to experimental protocols. Follicular fluid concentrations of carbohydrates (glucose, lactate and pyruvate), hormones FSH, LH, progesterone, estrogen and AMH), and selected ions were determined. Metabolic analysis of granulosa and cumulus cells was performed. Granulosa and cumulus gene expression of phosphofructokinase platelet (*PFKP*)

and lactate dehydrogenase A (*LDHA*) was determined. *SIRT3* and *SIRT5* gene expression and protein activity was confirmed in granulosa and cumulus cells via qPCR, immunohistochemistry, western blotting and deacetylation/desuccinylation activity. Granulosa and cumulus cell carbamoyl phosphate synthase I (CPS1) protein, a SIRT5 target, was confirmed using immunohistochemistry. Follicular fluid ammonium concentration and granulosa and cumulus cell glutamate dehydrogenase (GDH) activity, a SIRT3 target, were assessed using microfluorometry. Granulosa and cumulus cell acetylated mitochondrial proteins were separated by immunoprecipitation and acetylation of GDH was assessed via western blotting. Data from young women with normal ovarian reserve were compared with those from young women with reduced ovarian reserve and those of advanced maternal age.

Young women with normal ovarian reserve had significantly lower starting FSH doses and fewer previous cycles compared to the remaining two groups. Young women with normal ovarian reserve had significantly more oocytes collected compared to young women with reduced ovarian reserve and the advanced maternal age women. Fertilisation rate was significantly higher in the young women with normal ovarian reserve compared with the advanced maternal age group. Women of young maternal age with normal ovarian reserve had significantly less embryos transferred compared to the advanced maternal age group. The clinical pregnancy rate in young women with normal ovarian reserve was significantly increased compared to both the reduced ovarian reserve and advanced maternal age groups. No differences were found in clinical pregnancy rate between the reduced ovarian reserve and advanced maternal age groups.

Follicular fluid glucose concentrations were significantly decreased, whereas lactate and progesterone concentrations, granulosa and cumulus cell glucose uptake, lactate production, and phosphofructokinase platelet gene expression were significantly increased in women with

reduced ovarian reserve and in women of advanced maternal age. *SIRT3* and *SIRT5* mRNA and active protein were present in granulosa and cumulus cells and co-localized to the mitochondria. Women with reduced ovarian reserve or advanced maternal age had decreased granulosa and cumulus cell *SIRT5* mRNA, protein, desuccinylation activity and an accumulation of follicular-fluid ammonium. CPS1 protein was present in granulosa and cumulus cells. Compared to young women with normal ovarian reserve granulosa cell *SIRT3* mRNA was decreased in young women with reduced ovarian reserve and advanced maternal age whereas cumulus cell *SIRT3* mRNA was decreased in women of advanced maternal age only. Granulosa cell GDH activity was decreased in young women with reduced ovarian reserve and in women of advanced maternal age, whereas cumulus cell GDH activity was reduced in the advanced maternal age group only. Granulosa and cumulus cell acetylated mitochondrial GDH was increased in women of advanced maternal age while young women with reduced ovarian reserve had increased granulosa cell GDH acetylation only.

The data presented within this thesis suggest that in women with either reduced ovarian reserve or advanced maternal age both SIRT3 and SIRT5 may regulate granulosa and cumulus cell GDH and CPS1 activity, therefore altering the microenvironment surrounding the oocyte, as reflected by the altered follicular environment. This perturbed microenvironment may be responsible for impaired oocyte developmental competence, subsequent embryo development and reduced clinical pregnancy rates, also reported in this study. Considering the association between the decline in pregnancy rates in women with reduced ovarian reserve and in women of advanced maternal age and the knowledge of perturbed granulosa and cumulus cell SIRT3 and SIRT5 function this may lead to novel therapies to improve mitochondrial metabolism in the oocyte and follicular cells in women undergoing IVF treatment.

Declaration

The content presented within this thesis contains no material that has previously been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief this thesis contains no material previously published or written by any other person, except where reference is made in the text. I certify that no part of this work will in future be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree. All experiments outlined in this thesis were performed myself and any contributions and assistance received from others is acknowledged.

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Leanne Pacella-Ince

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"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less."

-**Marie Curie-**

Glossary/Abbreviations

ACECS2	Acetyl-CoA Sythetase 2
Acetyl CoA	Acetyl Coenzyme A
aCGH	Array Comparative Genomic Hybridisation
AMH	Anti-Mullerian Hormone
AQP	Aquaporin
ART	Assisted Reproductive Technologies
ATP	Adenosine Triphosphate
BCL-2	β-Cell Lymphoma 2
BMP-15	Bone Morphogenetic Protein 15
cAMP	Cyclic Adenosine Monoposphate
CDK1	Cumulus Expansion Enabling Factor
CEEF	Cyclin-Dependent Kinase 1
COC	Cumulus Oocyte Factor
CPS1	Carbamoyl Phosphate Synthase 1
CytC	Cytochrome C
DNA	Deoxyribonucleic Acid
ETC	Electron Transport Chain
FAD	Flavin Adenine Dinucleotide
FADH2	Reduced Flavin Dinucleotide

FSH	Follicle Stimulating Hormone
GAPDH	Glyceraldehyde 3-phosphate Dehydrogenase
GDF-9	Growth Differentiation Factor 9
GDH	Glutamate Dehydrogenase
GLUT	Glucose Transporter
GV	Germinal Vesicle
GVBD	Germinal Vesicle Breakdown
hCG	Human Chorionic Gonadotropin
HAT	Histone Acetylase
HDAC	Histone Deacetylase
ICDH	Isocitrate Dehydrogenase
ICSI	Intracytoplasmic Sperm Injection
IMM	Inner Mitochondrial Membrane
IP_3	Inositol Trisphosphate
IVF	In Vitro Fertilisation
LDHA	Lactate Dehydrogenase A
LH	Luteinising Hormone
MAPK	Mitogen-Activated Protein Kinase
MPF	Maturation Promoting Factor
mRNA	Messenger RNA

mtDNA	Mitochondrial DNA
NAD	Nicotinamide Adenine Dinucleotide
NAD^+	Oxidised Nicotinamide Adenine Dinucleotide
NADH	Reduced Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
OMM	Outer Mitochondrial Membrane
OSF	Oocyte Secreted Factors
PCOS	Polycystic Ovarian Syndrome
PFKP	Phosphokinase Platelet
PGER	Progesterone Receptor
PKA	Protein Kinase A
PTGER2	Prostaglandin E Receptor 2
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SIR2	Silent Information Regulator 2
SIRT	Sirtuin
TCA	Tricarboxcylic Acid Cycle
TGF-β	Transforming Growth Factor-β
UPC	Uncoupling Protein
VEGF	Vascular Endothelial Growth Factor

Publications, Conferences, Scholarship and Awards

Scientific Publications

1. **Pacella, L.** Zander-Fox, D.L., Armstrong, D.T. and Lane, M. (2012). Women with reduced ovarian reserve or advanced maternal age have an altered follicular environment. *Fertility and Sterility*, 98; 986-994.

2. **Pacella-Ince, L.**, Zander-Fox, D.L. and Lane, M. (2013). Mitochondrial SIRT5 is present in follicular cells and is altered by reduced ovarian reserve and advanced maternal age. *Reproduction, Fertility and Development*. Published online 27 August 2013.

3. **Pacella-Ince, L.**, Zander-Fox, D.L. and Lane, M. (2014) Mitochondrial SIRT3 and its target glutamate dehydrogenase are altered in follicular cells of women with reduced ovarian reserve or advanced maternal age. *Human Reproduction*, 29 (7), 1490-1499.

Conferences

1. **Pacella, L.**, Zander-Fox D.L., Hussein T., Fullston, T., and Lane M. (2010). SIRT3 in ovarian cells is altered by maternal age and ovarian reserve. Society for reproductive Biology Conference, Sydney, Australia (Oral Presentation)

2. **Pacella, L.**, Zander-Fox D.L., and Lane M. (2011). Follicular fluid glucose and lactate levels are altered by maternal age and ovarian reserve. The World Congress on Reproductive Biology and Society for Reproductive Biology Conferences, Cairns, Australia (Poster Presentation)

3. **Pacella, L.**, Zander-Fox D.L., and Lane M. (2012). Women with reduced ovarian reserve or advanced maternal age have an altered follicular environment. Postgraduate Research Conference, Adelaide, Australia. (Poster Presentation)

Scholarship and Awards

2011 – 2014 *Faculty of Health Science Postgraduate Award (Scholarship)*

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2010 *Society for Reproductive Biology Travel Award*

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2011 *Society for Reproductive Biology Travel Award*

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2012 Adelaide Research and Innovation (ARI) Prize Finalist

Postgraduate Research Conference, Adelaide, Australia

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