THE ROLE OF THE HEXOSAMINE BIOSYNTHESIS PATHWAY AND β-O-LINKED GLYCOSYLATION IN DETERMINING OOCYTE DEVELOPMENTAL COMPETENCE

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Abstract

Maternal diabetes and conditions such as obesity in which blood glucose levels are elevated are associated with reduced fertility and poor pregnancy outcomes. Many studies have examined the effects of hyperglycaemia on the early embryo and fetus; however, it is becoming increasingly evident that the peri-conceptual environment surrounding the oocyte has a significant impact on developmental competence and the long-term health of offspring.

In this thesis, I aimed to investigate the role of the hexosamine biosynthesis pathway (HBP) in oocyte developmental competence. The HBP is a glucose-metabolising pathway which can also be up-regulated by glucosamine, a potent hyperglycaemic mimetic which enters the HBP downstream of the rate-limiting enzyme. The HBP produces uridine diphosphate-N-acetylglucosamine, which can be used for the β-O-linked glycosylation (O-GlcNAcylation) of proteins, regulating their function in a similar manner to phosphorylation.

Firstly I established the effect of hyper- and hypo-glycaemic conditions during in vitro maturation (IVM) of mouse cumulus-oocyte complexes (COCs) on a range of measures associated with oocyte developmental competence, including cumulus expansion, meiotic maturation, cleavage and blastocyst development rates. A low (1 mM) glucose concentration achieved optimal oocyte competence, and glucose supplementation during only the first hour of IVM was necessary and sufficient to support oocyte maturation and embryo development to the blastocyst stage. Glucosamine was able to substitute for glucose during this first hour.

In the absence of glucose throughout IVM, glucosamine was not able to increase developmental competence, and at higher concentrations (2.5 and 5 mM) had a detrimental effect on these outcomes. These experiments underscored the importance of the other glucose metabolic pathways, during COC maturation, and supported the concept that excess flux through the HBP has detrimental consequences.

Using Western blots and immunohistochemistry, it was shown that both glucosamine and high glucose levels induced an increase in total O-GlcNAcylation in COCs, which was reduced in the presence of an inhibitor of the β-O-linked glycosyltransferase enzyme. Several specific proteins were identified using mass spectrometry as potential targets of O-GlcNAcylation in COCs, including heat-shock protein 90 (HSP90, both α and β isoforms). While glucosamine treatment of COCs significantly decreased blastocyst development rate, inhibiting HSP90 with 17-allylamino-17-demethoxygeldanamycin during IVM in the presence of glucosamine recovered blastocyst rates to control levels. This effect was not due to an increase in overall HSP90 levels, since inhibiting HSP90 in control COCs did not affect blastocyst rate. These results suggest O-GlcNacylated HSP90 has an aberrant function in the COC.

This study is the first to examine in detail O-GlcNAcylation levels in the COC, and their correlation to oocyte developmental competence. HSP90 was identified as a potential target of O-GlcNAcylation in the COC, and subsequently shown to mediate oocyte developmental competence. This research is significant because of the increasing numbers of women wishing to become pregnant who have high blood glucose levels due to diabetes, obesity or poor diet. I have generated critically needed knowledge towards understanding how these lifestyle factors affect fertility and identifying possible avenues for new therapies.

Declaration

I certify that 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 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.

In addition, I certify that no part of this work will, in the 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.
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Laura Alice Frank

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Dedication

I dedicate this thesis to my grandfather, Professor William Herdman Elliott.

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Abbreviations

17AAG	17-allylamino-17-demethoxygeldanamycin
ADP	adenosine diphosphate
ANOVA	analysis of variance
APS	ammonium persulphate
AR	aldose reductase
ATP	adenosine triphosphate
BADGP	benzyl-2-acetamido-2-deoxy-α-D-galactopyranoside
BMI	body mass index
BMP15	bone morphogenetic protein 15
BSA	bovine serum albumin
cAMP	cyclic AMP (adenosine monophosphate)
CEI	cumulus expansion index
COC	cumulus-oocyte complex
DAPI	4',6-diamidino-2-phenylindole
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DOHaD	Developmental Origins of Health and Disease
DON	6-diazo-5-oxo-L-norleucine
eCG	equine chorionic gonadotrophin
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid

ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
FCS	fetal calf serum
FOX01a	forkhead box protein 01a
FSH	follicle-stimulating hormone
G6PDH	glucose-6-phosphate dehydrogenase
GDF9	growth differentiation factor 9
GFPT	glutamine:fructose-6-phosphate amidotransferase
GlcN	glucosamine
GlcNAc	N-acetylglucosamine
GnRH	gonadotrophin-releasing hormone
GRP	glucose-regulated protein
GV	germinal vesicle
GVBD	germinal vesicle breakdown
H_2O	water
HbA1c	glycosylated haemoglobin
HBP	hexosamine biosynthesis pathway
HSP90	heat-shock protein 90
ICM	inner cell mass
ICSI	intracytoplasmic sperm injection
IRS	insulin receptor substrate
IVF	in vitro fertilisation
IVM	in vitro maturation

JNK	c-Jun N-terminal kinase
kg	kilogram
KILLER	tumor necrosis factor–related apoptosis-inducing ligand receptor
L	litre
LB	Laemmli buffer
LH	luteinising hormone
LSD	least-significant difference
mg	milligram
MII	metaphase II
mL	millilitre
mM	millimolar
mRNA	messenger RNA (ribonucleic acid)
NAD+/NADH	nicotinamide adenine dinucleotide
NADP+/NADPH	nicotinamide adenine dinucleotide phosphate
O-GlcNAc	β-O-linked N-acetylglucosamine
O-GlcNAcase	β-N-acetylglucosaminidase
O-GlcNAcylated	β-O-linked glycosylated
O-GlcNAcylation	β-O-linked glycosylation
OGT	O-linked β-N-acetylglucosaminyltransferase
PBS	phosphate buffered saline
PFK	phosphofructokinase
PI	propidium iodide
PI 3-K	phosphoinositide 3-kinase

PPP	pentose phosphate pathway
PUGNAc	O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-N-phenylcarbamate
PVDF	polyvinylidene
REDOX	reduction-oxidation
RIPA	radioimmunoprecipitation
RNA	ribonucleic acid
RT	room temperature
SDH	sorbitol dehydrogenase
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	standard error of the mean
SLC2Ax	solute carrier family 2 (facilitated glucose transporter), member x
SP1	specificity protein 1
SV40	simian vacuolating virus 40
TCA	tricarboxylic acid
Thr	threonine
TEMED	*N,N,N',N'*-tetramethylethylenediamine
TRAIL	tumor necrosis factor–related apoptosis-inducing ligand
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labelling
UDP-GlcNAc	uridine diphosphate-N-acetylglucosamine
UV	ultraviolet