

IDENTIFICATION OF MOLECULAR MARKERS OF PREGNANCY SUCCESS FOR ASSISTED REPRODUCTION

Kathryn Michelle Gebhardt

Robinson Institute
School of Paediatrics and Reproductive Health
Research Centre for Reproductive Health
Discipline of Obstetrics and Gynaecology
University of Adelaide, Adelaide
Australia

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Abstract

Current infertility treatments are confounded by an inability to identify oocytes and embryos with the highest developmental potential to generate and sustain a pregnancy resulting in a live birth, reducing the efficiency of treatment cycles and resulting in low pregnancy success rates. Embryos have varying capacity to form a successful pregnancy and embryo developmental potential is particularly reliant on nuclear and cytoplasmic qualities of the oocyte from which it is derived. A biochemical marker of oocyte and therefore embryo developmental potential would improve pregnancy success rates following assisted reproductive technologies by optimising oocyte and embryo selection techniques. The communication between an oocyte and its surrounding cumulus cells is essential for growth, maturation and metabolic activity, and strengthens the rationale to utilise cumulus cells to assess oocyte quality and predict treatment outcomes and health parameters for women undergoing assisted reproduction.

The potential for cumulus cell gene expression to predict clinical embryo grade and pregnancy success was investigated in cumulus masses from single human oocytes which were fertilised and cultured individually. To make direct correlations between cumulus cell gene expression and treatment outcomes patients underwent single embryo culture and transfer. Gene expression was analysed in cumulus cells from independent oocytes that yielded a successful term pregnancy compared to those for which treatment failed and pregnancy was not established. Patient matched cumulus cell pairs were utilised to investigate a potential correlation between cumulus gene expression and clinical embryo grade. Cumulus cell gene expression was assessed using both a microarray platform for non-biased genome wide gene expression analyses and real-time RT-PCR assays focused on genes with known important functions related to oocyte maturation.

Real time RT-PCR analyses identified cumulus expressed genes which significantly correlated with pregnancy success following single embryo transfer. Specifically, cumulus cell *PTGS2*, *VCAN* and *GAS5* mRNA expression significantly ($p < 0.02$) correlated with establishment of a pregnancy resulting in a live birth, while *PTX3* mRNA expression showed a trend towards significance ($p = 0.066$). Additionally, cumulus cell levels of *VCAN*, *GREM1* and *PFKP* showed a significant correlation with birth weight in the patients who achieved pregnancy, indicating their role as potential predictors of health

outcomes for babies born from assisted reproduction. No significant differences were seen for other genes analysed in relation to pregnancy outcome or when gene expression was correlated with clinical embryo grade. The use of a microarray platform led to the identification of new genes, never before identified in the COC as markers of human oocyte quality and pregnancy success. The characterisation of *GAS5* and *PEPSINOGEN* transcripts in both human and murine follicular cells furthered the rationale for their potential as markers of oocyte quality and provided an understanding of the pattern of expression and hormonal regulation within human and murine ovarian cells. The expression of the *GAS5* transcript was confirmed by real time RT-PCR analysis and shown to be significantly correlated with live birth following initial identification by microarray experiments.

The findings demonstrate that expression of *VCAN*, *PTGS2*, *GAS5* and *PTX3* represent molecular markers of oocyte quality. The molecular markers identified in this study provide a unique tool to assess the relative potential of individual oocytes to achieve successful pregnancy from a pool of oocytes generated by one patient. In conjunction with embryo selection techniques of visual assessment and developmental milestones in culture the present biomarkers provide information to differentiate between embryos with similar appearance as viable or non-viable, to improve ART efficiency while decreasing multiple gestations and even extend to predict birth weight and hence general health expectancy for babies conceived following assisted reproduction.

Declaration

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Abbreviations

aMEM	Minimum Essential Medium alpha
Adamts1	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motifs
AHR	Aryl hydrocarbon receptor
AIHW	Australian Institute of Health and Welfare
ALDOA	Aldolase
ANOVA	Analysis of variance
AR	Androgen receptor
AREG	Amphiregulin
ART	Assisted reproductive technology
ATP	Adenosine triphosphate
BCL2L11	BCL2-like 11 apoptosis facilitator
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
BMP	Bone morphogenetic protein
bp	Base pairs
BSA	Bovine serum albumin
CAMK4	Calcium/Calmodulin-dependent protein kinase 4
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CCND2	Cyclin D2
CDC42	Cell division cycle 42
cDNA	Complementary DNA
CEI	Cumulus expansion index
CL	Corpus luteum
cm	Centimetre
COC	Cumulus oocyte complex
CPB	Complement binding protein
COX2	Cyclooxygenase 2
CREB	cAMP regulatory element-binding protein
CS	Chondroitin sulphate
CT	Threshold cycle

CTNND1	Catenin delta-1
CX43	Connexin 43
CXCR4	Chemokine (C-X-C motif) receptor 4
CYP19A1	Cytochrome P450 aromatase
DET	Double embryo transfer
DEPC	Diethyl pyrocarbonate
DHCR7	7-dehydrocholesterol reductase
DMEM	Dulbecco's modified eagle medium
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide
DVL3	Dishevelled dish homolog 3
E2	Oestradiol
E. Coli	Escherichia coli
eCG	Equine chorionic gonadotropin
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid $[\text{CH}_2\text{N}(\text{CH}_2\text{CO}_2\text{H})_2]_2$
Egf	Epidermal growth factor
Egf-L	Egf-like peptide
EgfR	Egf receptor
ENOA	Alpha enolase
ErbB2	Erythroblastic leukemia viral oncogene homolog 2
ERK	Extracellular signal-regulated kinase
FCS	Fetal calf serum
FDX1	Ferredoxin 1
FGF	Fibroblast growth factor
FIG α	Factor in the germline alpha
FSH	Follicle stimulating hormone
FSH-R	Follicle stimulating hormone receptor
g	Grams
GAG	Glycosaminoglycan
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GAS5	Growth-arrest-specific transcript 5
GC	Granulosa cell
GDF	Growth differentiation factor
GLUT	Glucose transporter

GnRH	Gonadotrophin releasing hormone
GPX3	Glutathione peroxidase 3
GREM1	Gremlin 1
GV	Germinal vesicle
GVBD	Germinal vesicle break down
h	Hour
H ₂ O	Water
HA	Hyaluronan
HAS2	Hyaluronan synthase 2
HC	Heavy chain
hCG	Human chorionic gonadotropin
HS	Heparin sulphate
HSD3 β 1	3-beta-hydroxysteroid dehydrogenase
HSPB1	Heatshock 27 kDa protein 1
I α I	Inter- α trypsin inhibitor
ICSI	Intra cytoplasmic sperm injection
INSL3	Insulin-like 3
i.p.	Intraperitoneal
IPA	Ingenuity pathways analysis
IU	International units
IUI	Intra uterine insemination
IVF	In vitro fertilisation
IVM	In vitro maturation
kDa	Kilodalton
KO	Knock out
L	Litre
LB	Luria broth
LDHA	Lactate dehydrogenase A-chain
LEFTY2	Left-right determination factor A
LGR8	InsI3 receptor
LH	Luteinising hormone
LHCGR	Luteinising hormone/choriogonadotropin receptor
LH-R	Luteinising hormone receptor
Lhcgr	Luteinising hormone/choriogonadotropin receptor
M	Mass

MAPK	Mitogen-activated protein kinase
mGC	Mural granulosa cells
MgCl ₂	Magnesium chloride
MI	Metaphase I
MII	Metaphase II
Min	Minute
MIR202	MicroRNA 202
mL	Millilitre
mM	Millimolar
MMP	Matrix metalloproteinase
mRNA	Messenger RNA
n	DNA/plasmid size (Base pairs)
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NaOH	Sodium hydroxide
NFIB	Nuclear factor I/B
NPSU	National Perinatal Statistics Unit
nt	Nucleotide
°C	Degrees Celsius
OHSS	Ovarian hyperstimulation syndrome
OI	Ovulation induction
OPU	Oocyte pick up
OSF	Oocyte secreted factor
<i>p</i>	Probability
PB	Polar body
PBS	Phosphate Buffered Saline
PCA	Principal components analysis
PCK1	Phosphoenolpyruvate carboxykinase 1
PCO	Polycystic ovary
PCOS	Polycystic ovarian syndrome
PCR	Polymerase chain reaction
PDRG1	p53 and DNA damage regulated 1
PG	Prostaglandins
PGA	Pepsinogen
PGC	Progastricsin

PGD	Preimplantation genetic diagnosis
PGK1	Phosphoglycerate kinase 1
PFK L	Phosphofructokinase Liver
PFKM	Phosphofructokinase Muscle
PFKP	Phosphofructokinase Platelet
PKA	Protein kinase A
PKM2/PK	Pyruvate Kinase Muscle Variant 2
PR	Progesterone receptor
PRKO	Progesterone receptor knockout
Ptger2	Prostaglandin E receptor 2, subtype EP2
PTGS2	Prostaglandin-endoperoxide synthase 2
PTX3	Pentraxin 3
qPCR	Quantitative polymerase chain reaction
RGS2	Regulator of G-protein signalling 2
RHAMM	Receptor for HA-mediated motility
RhoA	Ras homolog gene family, member A
RMA	Robust multichip averaging
RNA	Ribonucleic acid
RNaseP	Ribonuclease P
RPA2	Replication protein A2 32 kDa
RPL19	Ribosomal protein L19
Rpm	Revolutions per minute
RT	Reverse transcription
RT-PCR	Reverse transcription polymerase chain reaction
SCD1	Stearoyl-coenzyme A desaturase 1
SCD5	Stearoyl-coenzyme A desaturase 5
S.E.M	Standard error of the mean
SERPINE2	Serine proteinase inhibitor clade E member 2
SET	Single embryo transfer
snoRNA/SNORD	Small nucleolar RNA
STAR	Steroid acute regulatory protein
STS	Steroid sulfatase
T	Testosterone
Taq	Thermus aquaticus
TBE	Tris/borate/EDTA

TCA	Tricarboxylic acid
TGF β	Transforming growth factor β
TIAM1	T-cell lymphoma invasion and metastasis 1
TNFAIP6	Tumor necrosis factor alpha-induced protein 6
TPI	Triose phosphate isomerase
TRIM27	Tripartite motif-containing 27
TRIM28	Tripartite motif-containing 28
Tris	Tris(hydroxymethyl)aminomethane (HOCH ₂) ₃ CNH ₂
UV	Ultraviolet
V	Volts
VCAN	Versican
w/v	Weight/volume percentage solution
ZP	Zona pellucida