

DETERMINATION OF NON-INVASIVE VIABILITY MARKERS FOR HUMAN EMBRYOS IN IN VITRO FERTILIZATION

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Abstract

The major challenge currently facing human in vitro fertilization (IVF) programs is the prevention of multiple gestation pregnancies. The replacement of multiple embryos to fertility patients is common practice to increase pregnancy rates. However this introduces a significant risk of a resultant multiple pregnancy (25-50%), with associated increased complications such as perinatal morbidity, mortality and long-term disabilities, as well as significant maternal complications. The transfer of a single embryo during IVF treatment is the only acceptable way to overcome the issues related to multiple gestation pregnancies. To encourage the replacement of only one embryo, pregnancy rates must be able to be maintained. There is therefore a great need for clinical laboratories to use markers that can identify highly viable embryos.

Current laboratory embryo selection techniques involve selecting an embryo based on its appearance. However it is well established that this is only weakly linked to pregnancy success. Therefore other less subjective quantitative markers are required to select embryos that are the most viable within a patient's cohort. Any quantitative techniques which provide additional information to combine with current morphological assessment protocols must be rapid, simple, non-invasive and highly discriminating. This thesis describes the investigations of candidate non-invasive viability markers to assess the competence of the oocyte/embryo.

In order to track investigations of individual oocytes and embryos, single embryo culture is a prerequisite. Mouse and human embryo development and implantation were studied as a prelude to investigations of biomarkers of human embryo viability. No differences were observed in human embryo development or viability after single or group culture. Mouse embryos cultured singly were found to have a reduced cell number, however this did not affect implantation or fetal viability. Interestingly, mouse placental weights were reduced. This and a lack of power in the human studies suggest that single embryo culture in the human needs further long-term examination.

Following preliminary investigations of single embryo culture, morphological, biochemical and metabolic studies were undertaken as potential biomarkers of oocyte/embryo viability in the human. Firstly, a new morphological scoring system was described for day 4 human embryos, a stage of embryo development that has previously been overlooked. This morphological scoring system was easy to implement in the clinic and able to improve implantation rates over morphological scoring of cleavage stage embryos. It has subsequently been adopted in several clinics. Secondly, the reduction-oxidation (REDOX) state of cumulus cells surrounding oocytes retrieved for IVF was measured and retrospectively found to correlate with subsequent embryo viability. Finally, a direct measure of metabolism was also investigated in cumulus-oocyte complexes as well as cleavage stage embryos with determination of

metabolic turnover of glucose, lactate, pyruvate and alanine, with pyruvate uptake by the early embryo and pyruvate: alanine turnover of the cumulus-oocyte complex being related to viability.

Overall, these experiments showed that some metabolic parameters were correlated with subsequent viability. Biochemical markers in combination with current morphological measures show promise in selecting the most viable embryo for replacement. Further validations of these markers in randomized controlled trials are the next step in the introduction of these technologies to improve success rates of single embryo transfers in fertility treatment.

Declaration

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Publications arising from this thesis

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2. **FEIL D, HAMILTON H, RUSSELL D & LANE M.** Cumulus cell REDOX levels correlate with subsequent embryo viability following single embryo transfer. *In preparation.*
3. **FEIL D, MITCHELL M, THOMPSON JG & LANE M.** Single embryo culture equals group embryo culture with implantation and development in an optimised culture system. *In preparation.*

Abstracts arising from this thesis

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3. HAMILTON H, **FEIL D**, BARRY M, RUSSELL D & LANE M. (2006) Cumulus cell REDOX state is predictive of subsequent embryo quality. Annual Meeting of the Fertility Society of Australia. Sydney, Australia.
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5. **FEIL D** & LANE M. (2006) Retrospective analysis of the implementation of a single embryo transfer policy in an IVF program. Australian Society for Medical Research Scientific Meeting, Adelaide, Australia.

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Abbreviations

°C	degrees celsius
ADP	adenosine diphosphate
ANOVA	analysis of variance
AREG	amphiregulin
ART	assisted reproductive technologies
ATP	adenosine triphosphate
β-hCG	beta-human chorionic gonadotrophin
BCL2L11	BCL2-like 11
BDNF	brain-derived neurotrophic factor
BMP-15	bone morphogenic protein-15
Ca-125	cancer antigen 125
CEA	carcinoembryonic antigen
CO ₂	carbon dioxide
COC	cumulus-oocyte complex
Complex I	NADH dehydrogenase
Complex II	succinate dehydrogenase
Complex III	cytochrome bc1 complex
Complex IV	cytochrome c oxidase
CX43	gap junctional alpha-1
Cyt C	cytochrome c
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
e-	electron
EGF	epidermal growth factor
FSH	follicle stimulating hormone
g	gram

g	gravity
G	gauge
G6PDH	glucose-6-phosphate dehydrogenase
GDF-9	growth differentiation factor-9
GIFT	gamete intrafallopian tube transfer
GPT	glutamate-pyruvate transaminase
GREM1	gremlin 1
h	hours
H ⁺	proton
HAS2	hyaluronan synthase 2
hCG	human chorionic gonadotrophin
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HSA	human serum albumin
ICM	inner cell mass
ICSI	intracytoplasmic sperm injection
IFFS	International Federation of Fertility Societies
IGF-1	insulin-like growth factor 1
IGF-2	insulin-like growth factor 2
IGFBP-1	insulin-like growth factor 1 binding proteins
IGFBP-2	insulin-like growth factor 2 binding proteins
IMM	inner mitochondrial membrane
IMS	intermembrane space
IU	international units
IVF	in vitro fertilization
L	litre
LDH	lactate dehydrogenase
LSD	least significant difference
LH	luteinizing hormone

n	nano
N ₂	nitrogen
NADH/NAD ⁺	nicotinamide adenine dinucleotide
NADPH/NADP ⁺	nicotinamide adenine dinucleotide phosphate
NFIB	nuclear factor 1B gene
NIR	near infra-red
nM	nanomolar
μL	microlitre
μg	microgram
M	molar
m	mol
mg	milligram
mm	millimetre
mM	millimolar
mL	millilitre
mmHg	millimetres of mercury
MOPS	3-(N-morpholino)propanesulfonic acid
NPBs	nucleolar precursor bodies
NHMRC	National Health and Medical Research Council
O ₂	oxygen
O ₂ ⁻	superoxide
OCP	oral contraceptive pill
OPU	oocyte pick up
p	probability
p	pico
PCK1	phosphoenolpyruvate carboxykinase 1
PCOS	polycystic ovary syndrome
PGD	pre-implantation genetic diagnosis

PI	propidium iodide
PN	pronucleus
PTGS2	prostaglandin-endoperoxide synthase 2
PTX3	pentraxin-3
PVP	polyvinylpyrrolidone
Q	ubiquinone
r	Pearson's correlation coefficient
R ²	coefficient of determination for linear regression
ρ	rho, Spearman's correlation coefficient
REDOX	reduction-oxidation
ROS	reactive oxygen species
SCD1	delta-9 desaturase 1
SCD5	delta-9 desaturase 5
SEM	standard error of the mean
SET	single embryo transfer
SOD	superoxide dismutase
SPSS	Statistical Package for Social Sciences
STAR	steroidogenic acute regulatory protein
TAC	total antioxidant capacity
TdT	terminal deoxynucleotidyl transferase
TE	trophectoderm
TGFβ1	transforming growth factor beta 1
TIFF	tagged image file format
TNF-α	tumour necrosis factor alpha
TNFαIP6	tumour necrosis factor alpha inducible protein 6
TUNEL	terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling
UV	ultra violet
VEGF	vascular endothelial growth factor