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

Deanne Kate Feil

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

Thesis submitted to The University of Adelaide in fulfillment of the requirements for admission to the degree Doctor of Philosophy

January 2010

THE UNIVERSITY OF ADELAIDE
AUSTRALIA

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

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.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Deanne Kate Feil

January 2010

Acknowledgements

I would firstly like to thank my supervisors Dr Michelle Lane, Associate Professor Jeremy Thompson and Dr Darryl Russell. Jeremy you founded my interest in reproductive medicine for which I owe you a great deal. You and Darryl have admirable scientific thoroughness, dedication to your commitments, attention to detail, and skills and areas of expertise that I greatly valued in the completion of my studies and this thesis. Thank you both for your advice and support in study, work and life. Michelle you gave me the opportunity to begin a career in clinical embryology, which developed into a passion. From that foundation you inspired me to undertake research in that field. Thank you for supporting my career and offering me further opportunities that were invaluable experiences.

I would like to gratefully acknowledge the Australian National Health and Medical Research Council for their financial support in the form of a Dora Lush Biomedical Research Scholarship, which allowed me to explore and contribute to an area of research able to help couples worldwide come closer to their dream of a healthy child.

Thank you to the nursing and embryology staff at Repromed. You were a pleasure to work with and allowed me to recruit and manage my research patients and their embryos as I required, despite the occasional reduction in your efficiencies.

Without the patients I had the pleasure of working with at Repromed, none of this study would have been possible. Thank you for your generous willingness to be involved in research, sharing your journey with me and allowing me to study your embryos and meet some of your success stories down the track. It is the ultimate reward in this field. If only all embryologists had that opportunity!

Through every aspect of this PhD and the person that I have become because of it, I must thank the special people in my world that have encouraged and supported me. I am extremely lucky to be surrounded by and have encountered some amazing people on this journey. In particular I must mention my parents and sister, who have always supported my aspirations. In particular Kirsty, you have been an absolute rock without always understanding what I do and why, but accepting it. Thank you Matt, for your computer knowledge and time, to maintain my sanity. To Miranda, Chris, Lisa, Zanna, Acushla, Don, Ashlee and Abigail, for your ultimate belief in me through the many challenges and rewards, I cherish your friendships. To Kathryn, Mel, Laura, Annie, Megan and many others at the University, thank you for your encouragement, assistance and positivity.

To my partner in love and life, my husband Mark, thank you for your patience and love and for nurturing my ambition during this period. I know you have made huge sacrifices for me to do what I wanted to do. You encouraged my first interests in research and reproductive medicine and I haven't looked back since. I love you more than I could ever say.

Publications arising from this thesis

1. **FEIL D,** HENSHAW RC & LANE M. (2008) Day 4 embryo selection is equal to Day 5 using a new embryo scoring system validated in single embryo transfers. *Human Reproduction 23 (7) p1505-10*.

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

1. **FEIL D,** HAMILTON H, RUSSELL D & LANE M. (2008) Human cumulus cell REDOX state is predictive of subsequent embryo viability in single embryo transfers. Obex Research Day. Auckland, New Zealand.

2. **FEIL D**, HENSHAW RC & LANE M. (2007) Day 4 embryo transfer is a successful alternative to blastocyst transfer. 23rd Annual Meeting of the European Society of Human Reproduction and Embryology, Lyon, France. *Abs O-020.*

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.

4. **FEIL D,** THOMPSON JG & LANE M. (2006) Placental size can be influenced by pre-implantation culture conditions. Healthy Development Adelaide Research Symposium. Adelaide, Australia.

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.

Table of Contents

List of Figures

List of Tables

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