Insulin-Like Growth Factor –II and its Role in Blastocyst Development, Implantation and Placentation

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

December 2007

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ABSTRACT

Impaired implantation and placental development have been implicated in several disorders of pregnancy such as unexplained miscarriage, preeclampsia, and intrauterine growth retardation. Insulin-Like Growth Factor (IGF)-II has previously been shown to promote blastocyst development and placental growth and function. We were interested in how IGF-II interacts with other factors throughout blastocyst development, implantation and placentation in the mouse to improve pregnancy outcome.

In vitro embryo culture increases the risk of pregnancy complications associated with poor placentation. Recent research has focussed on optimising the culture conditions to more resemble that of the *in vivo* environment. IGF-II, Urokinase Plasminogen Activator (uPA) and Plasminogen individually have all been shown to be important for embryo development. However, it is likely that a combination of factors is required to counteract the negative effects of *in vitro* culture. Here we show that IGF-II, uPA and Plasminogen, in combination, significantly improve mouse blastocyst hatching rates and implantation rates on day 8 and doubles the number of mothers that are pregnant after embryo transfer.

Following implantation, IGF-II is suggested to play a role in promoting placental development and function. We demonstrate that IGF-II is co-localised with both IGF receptors throughout early pregnancy in trophoblasts and in the developing blood vessels and adjacent stromal cells in the mesometrial decidua. This suggests that IGF-II may play a role in both decidual angiogenesis and placentation. We suggest that perhaps murine trophoblasts secrete molecules such as IGF-II to promote

angiogenesis in the decidua early in pregnancy to compensate for their shallow invasion and allow for adequate trophoblast remodelling later in pregnancy.

The first trimester human placenta experiences a low oxygen environment. The Hypoxia-Inducible Factors (HIFs) mediate the response to low oxygen, inducing genes such as IGF-II. Currently, the role of oxygen in mouse placentation, the mechanisms by which HIFs promote placentation or their interaction with IGF-II in the placenta is unknown. Here, we demonstrate that the early mouse implantation site is exposed to low oxygen levels similar to those seen in humans and expresses HIF-1α protein. We were interested then in the interaction between IGF-II, oxygen and HIFs in trophoblasts *in vitro*. Prolonged exposure to low oxygen reduced trophoblast outgrowth, and increased *Tpbp* mRNA levels, suggesting commitment to the spongiotrophoblast lineage. Interestingly, we found that antisense (as) *Hif-1α* may mediate the response to prolonged hypoxia in murine trophoblasts. Importantly, *Hif-1α* and *Hif-2α* were differentially regulated by oxygen and IGF-II in cultured trophoblast cells suggesting a novel interaction between IGF-II and oxygen.

In conclusion, it appears that IGF-II is a central growth factor which interacts with other molecules to regulate a wide variety of process in early pregnancy to promote blastocyst development, implantation and placentation. The results outlined in this thesis demonstrate a novel interaction between IGF-II, uPA and Plasminogen in promoting blastocyst development and implantation which may be used to improve pregnancy outcome following ART. In addition, we have also identified a novel interaction between IGF-II, oxygen and the HIF system which may regulate trophoblast function. This has important implications not only for placental research, but also for cancer research.

DECLARATION

This work is original and has not been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge, this thesis does not contain material previously written or published by another, except where due reference in the text has been given.

I give consent to the University of Adelaide to make this thesis available for loan and photocopying after it has been accepted for the degree.

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December 2007

ACKNOWLEDGEMENTS

First and foremost I would like to extend an enormous thankyou to my supervisor Associate Professor Claire Roberts. I can't believe it has been 5 years since we first met and you told me all about this wonderful organ, the placenta. It has been an honour to work with someone so dedicated and so passionate about science, you have been an inspiration. Thank you for encouraging me to grow both as a scientist, and as an individual. I would also like to thankyou for believing in me, for pushing me to be the best that I could be and for staying optimistic when I couldn't see the light at the end of the tunnel. Most importantly, I would like to say how grateful I am for your understanding, for letting me cry on your shoulder (one too may times I think) and for being a friend. It has been both a pleasure and an honour.

Secondly, I would like to say a huge thank you to my co-supervisor Dr Karen Kind for your insight and advice. Your input into this project has always been highly regarded and I really appreciated all of the feedback you have given me over the years. It was nice to know that you were always there when I needed you. In particular I would like to thankyou for letting me pick your brains so often, whether it was about real time PCR or what the heck my results meant!

In addition I would like to acknowledge all of the past and present members of the Placental Development Group that I have had the pleasure of working with. I have gained some wonderful friends and it wouldn't have been the same without you. I would also like to acknowledge Amanda Seferruzzi-Perri, Rachael Nowak, Jamie Zhang, Steven Thompson and Gary Heinemann for their technical assistance with the embryo transfer experiments.

To all of the wonderful staff and students in the Research Centre for Reproductive Health at the University of Adelaide, thank you for making my time in the department so enjoyable. In particular I would like to thank Kylie Dunning, Rachael Nowak and Amanda Sferruzzi-Perri, my PhD buddies, for your understanding, love and support. There is no way I could have made it to the end without you girls!

I would like to acknowledge the financial support of the University of Adelaide, the Faculty of Health Sciences, the Research Centre for Reproductive Health (RCRH) and the Network in Genes and Environment in Development (NGED) for my postgraduate scholarship and for national and international travel grants. In addition, the work outlined in Chapter 2 was financially supported by Medicult.

Lastly, I would like to thank all the other people in my life that I couldn't live without. To my family, Mum & Phil, Dad & Angela, Jay and Joel, I would like to thankyou from the bottom of heart for your unconditional love and support. In particular I would like to extend an enormous thanks to my mum for being there through everything. Thankyou for knowing when to push me and when to let things go and for picking me up when I didn't think I could go on. Thank you to Sadie Peck for always being a wonderful friend and to the Kahl family, for letting me into their home and their hearts. Last but not least, I would like to thank Richard for loving me, encouraging me and supporting me. I can't begin to describe how grateful I am to have you in my life, I love you very much.

PUBLICATIONS ARISING FROM THIS THESIS

1. **Pringle KG**, Kind KL, Thompson JG, Roberts CT. *Complex Interactions Between Hypoxia Inducible Factors, Insulin-like Growth Factor-II and Oxygen in Early Murine Trophoblasts.* Placenta. 2007; Nov-Dec; 28(11-12): 1147-57.

2. **Pringle KG**, Roberts CT. *New light on Early Post-Implantation Pregnancy in the Mouse: Roles for Insulin-Like Growth Factor-II (IGF-II)?* Placenta. 2007 Apr; 28(4): 286-97.

3. **Pringle KG**, Kind KL, Thompson JG, Roberts CT. Control of Placental Development by Oxygen and Hypoxia Inducible Factors. In preparation.

4. **Pringle KG,** Kind KL, Thompson JG, Roberts CT. IGF-II, in combination with uPA and Plasminogen, improves mouse blastocyst development and implantation. In preparation.

ADDITIONAL PUBLICATIONS

5. Sferruzzi-Perri AN, Owens JA, **Pringle KG**, Robinson JS, Roberts CT. *Maternal Insulin-Like Growth Factors-I and -II Act via Different Pathways to Promote Fetal Growth.* Endocrinology. 2006 Jul; 147(7): 3344-55.

ABSTRACTS ARISING FROM THIS THESIS

2007

1. **Pringle KG**, Kind KL, Thompson JG and Roberts CT. *Effect of IGF-II, uPA and Plasminogen in Combination on Blastocyst Development.* Society for Reproductive Biology Annual Scientific Meeting, Christchurch, New Zealand.
2. ***Pringle KG,*** Kind KL, Thompson JG and Roberts CT. *Oxygen, IGF-II and their Interactions in Early Murine Trophoblasts.* 13th meeting of the International Federation of Placenta Associations (IFPA), Kingston, Ontario, Canada.
3. **Pringle KG**, Kind KL, Thompson JG, Roberts CT. *Oxygen, IGF-II and their Interactions in Murine Trophoblasts.* Network in Genes and Environment in Development (NGED) Forum. Palm Cove, Queensland, Australia.

2006

4. **Pringle KG**, Kind KL, Thompson JG and Roberts CT. *Oxygen, Insulin-Like Growth Factor-II (IGF-II) and Their Interactions in Murine Trophoblasts in Vitro.* Abstract 208. Society for Reproductive Biology Annual Scientific Meeting, Gold Coast, Queensland, Australia.
5. Sferruzzi-Perri AN, Owens JA, **Pringle KG**, Robinson JS, Roberts CT. *Maternal Insulin-Like Growth Factor-I and –II Act Via Different Pathways to Increase Fetal Growth Near Term.* Abstract 221. Society for Reproductive Biology Annual Scientific Meeting, Gold Coast, Queensland, Australia.
6. **Pringle KG**, Kind KL, Thompson JG and Roberts CT. *Interactions between the Hypoxia Inducible Factors and Insulin-Like Growth Factor-II (IGFII) in Cultured Murine Trophoblasts.* Australian Society for Medical Research (ASMR) SA Division Annual Scientific Meeting, Adelaide.

7. Sferruzzi-Perri AN, Owens JA, **Pringle KG**, Robinson JS, Roberts CT. *Exogenous insulin-like growth factors in early pregnancy act via different mechanisms to promote fetal growth.* 11th meeting of the International Federation of Placenta Associations (IFPA), Glasgow, Scotland.
8. **Pringle KG** and Roberts CT. *IGF-II and IGF2R expression in early murine implantation sites: a role in both placentation and decidual angiogenesis?* 32nd Annual Meeting of the Fetal and Neonatal Physiological Society, Jeffrey Robinson Symposium, Glenelg, South Australia, Australia.
9. Sferruzzi-Perri AN, Owens JA, **Pringle KG**, Robinson JS, Roberts CT. *Maternal IGF-II treatment in Early pregnancy improves placental structural and functional development and fetal growth near term*. 32nd Annual Meeting of the Fetal and Neonatal Physiological Society, Jeffrey Robinson Symposium, Glenelg, South Australia, Australia.
10. **Pringle KG** & Roberts CT. *Localisation of Insulin-Like Growth Factor-II (IGF-II) and its Receptor in Early Murine Pregnancy: A Role in Placentation and Angiogenesis in the Decidua?* Abstract 210. Society for Reproductive Biology Annual Scientific Meeting, Perth, Western Australia, Australia.
11. **Pringle KG**, Kind KL, Thompson JG and Roberts CT. *Effect of oxygen and insulin-like growth factor-II on ectoplacental cone outgrowth and trophoblast gene expression.* Abstract A162. 9th Annual Conference of the Perinatal Society of Australia and New Zealand (PSANZ), Adelaide, South Australia, Australia.
12. **Pringle KG**, Kind KL, Thompson JG & Roberts CT. *Role of oxygen and IGF-II on early trophoblast outgrowth and gene expression in the mouse.* Abstract A1. 19th National Workshop on Fetal and Neonatal Physiology, Adelaide.

13. **Pringle KG** & Roberts CT. *Localisation of IGF-II and IGF2R in Murine Pregnancy: A Role in Both Placentation and Decidual Angiogenesis?* Abstract 02. Australian Society for Medical Research (ASMR) SA Division Annual Scientific Meeting, Adelaide.
14. Sferruzzi-Perri AN, Owens JA, **Pringle KG**, Robinson JS and Roberts CT. *Maternal IGF-II treatment in early pregnancy impacts on placental structure to promote fetal growth near term.* Abstract R2. Australian Society for Medical Research (ASMR) SA Division Annual Scientific Meeting, Adelaide.

2004

15. **Pringle KG**, Kind KL and Roberts CT*. Oxygen, Insulin-Like Growth Factor-II (IGF-II) and Hypoxia Inducible Factor-1 alpha (HIF-1α) in the murine placenta.* Abstract A134. 8th Annual Conference of the Perinatal Society of Australia and New Zealand (PSANZ), Sydney.
16. **Pringle KG**, Kind KL and Roberts CT. *Localisation of hypoxia, Insulin-Like Growth Factor-II (IGF-II) and Hypoxia Inducible Factors (HIFs) in early murine implantation sites.* Abstract A10. 18th National Workshop on Fetal and Neonatal Physiology.
17. **Pringle KG**, Kind KL and Roberts CT. *Oxygen, IGF-II and HIF-1α in early murine placentation.* Abstract O4. Australian Society for Medical Research (ASMR) SA Division Annual Scientific Meeting, Adelaide.

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ABBREVIATIONS

asHIF-1α	antisense hypoxia inducible factor -1 alpha
ALS	acid labile subunit
Ang-II	angiotensin - II
ANOVA	analysis of variance
ARNT	aryl hydrocarbon receptor nuclear translocator
ART	assisted reproductive technology
ATP	adenosine triphosphate
bHLH	basic helix-loop-helix
BSA	bovine serum albumin
CAD	C-terminal activation domain
CBP	CAP binding protein
cDNA	complementary DNA
CI-M6PR	cation-independent mannose-6 phosphate receptor
CTB	cytotrophoblast
DAB	diaminobenzadine
DNA	deoxyribonucleic acid
eCG	equine chorionic gonadotrophin
ECM	extracellular matrix
EGF	epidermal growth factor
EPAS-1	endothelial PAS domain protein 1
EPC	ectoplacental cone
EPO	erythropoietin
EVT	extravillous cytotrophoblast
ExE	extraembryonic ectoderm

FIH	factor inhibiting HIF
GLUT-1	glucose transporter – 1
H&E	haematoxylin and eosin
HBSS	hank's balanced salt solution
hCG	human chorionic gonadotrophin
HIF	hypoxia inducible factor
HPH	HIF prolyl hydroxylase
HRE	hypoxia response element
HRP	horseradish peroxidase
IGF	insulin-like growth factor
IGF1R	type 1 insulin-like growth factor receptor
IGF2R	type 2 insulin-like growth factor receptor
IGFBP	insulin-like growth factor binding protein
IGFBPrP	insulin-like growth factor binding protein related protein
ICM	inner cell mass
IL-1β	interleukin -1 beta
i.p	intraperitoneal
IPAS	inhibitory PAS domain protein
IR	insulin receptor
IUGR	intrauterine growth restriction
IVF	*in vitro* fertilisation
IVS	intervillous space
mAb	monoclonal antibody
mRNA	messenger ribonucleic acid
M6PR	mannose-6 phosphate receptor
MAPK	mitogen activated protein kinase

MLAp	mesometrial lymphoid aggregate of pregnancy
MMP	matrix metalloproteinase
ODD	oxygen dependent degradation
NAD	N-terminal activation domain
NTC	non-template control
pAb	polyclonal antibody
PBS	phosphate buffered saline
PDZ	primary decidual zone
PHD	prolyl-4 hydroxylase
P_4	progesterone
PAI	plasminogen activator inhibitor
PAS	Per-Arnt-Sim
PC4	proprotein convertase 4
PCOS	polycystic ovary syndrome
PCR	polymerase chain reaction
PDGF	platelet - derived growth factor
PGE_2	prostaglandin E_2
PH	proline-4 hydroxylase related protein
PHD	prolyl -4 hydroxylase
PI3K	phosphatidylinositol-3 kinase
PL-1	placental lactogen-1
PLC	phospholipase C
Plg	plasminogen gene
RNA	ribonucleic acid
RT	reverse transcription
SEM	standard error of the mean

SGA	small for gestational age
STB	syncytiotrophoblast
TE	trophectoderm
TGC	trophoblast giant cell
TGF	transforming growth factor
TIMP	tissue inhibitor of metalloproteinase
TNF	tumour necrosis factor
uPA	urokinase plasminogen activator
uPAR	urokinase plasminogen activator receptor
UTR	untranslated region
VEGF	vascular endothelial growth factor
VHL	von Hippel Lindau
VDU2	VHL-interacting deubiquitinating enzyme 2