The physiological significance of insemination in programming pregnancy outcome

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Abstract

The cellular and molecular environment of the uterus during the pre- and peri-implantation period of early pregnancy is critical for implantation success and optimal fetal and placental development. Perturbations to this environment not only have consequences for the success of pregnancy and neonatal health and viability, but can also drive adverse health outcomes in the offspring after birth, particularly the development of metabolic disorders such as obesity, hypertension and insulin resistance.

The influence of seminal plasma on the cytokine and immune uterine environment has been previously well characterised in mice, however the effects of disruption in uterine seminal plasma exposure for pregnancy outcome have not been investigated. The studies described in this thesis employed the use of surgical seminal vesicle ablation in males and embryo transfer experiments to investigate the physiological significance of uterine seminal plasma exposure on programming fetal and neonatal outcomes, and growth and metabolic status in adult offspring.

We demonstrate that in the absence of seminal plasma, oocyte fertilisation and embryo implantation are reduced, showing that seminal plasma acts primarily to facilitate fertilisation, possibly by promoting sperm transport and survival in the reproductive tract. In addition we show that pregnancies initiated in the absence of seminal plasma give rise to offspring which display accelerated growth after birth and increased adiposity in adulthood, compared to those developed in a tract exposed to seminal plasma at the time of conception. Offspring conceived in the absence of seminal plasma also displayed alterations in serum leptin and adiponectin content, similar to those known to be associated with obesity in the mouse.

Using embryo transfer experiments, we showed that some, but not all aspects of the perturbed postnatal development are recapitulated when embryos fertilised in the presence of what semen are transferred to a recipient tract which has not been exposed to seminal plasma. More severe perturbations were seen in 2-cell transfer than in blastocyst transfer experiment. Additionally, there was a significant effect of the embryo transfer procedure, irrespective of seminal plasma exposure, on fetal and postnatal development that confounded interpretation of these experiments.
In addition, we investigated the potential mechanisms by which the influence of seminal plasma is exerted. Mediators of pre-implantation embryo development, implantation and the modulation of the maternal immune response to pregnancy were all assessed for regulation by seminal plasma using QRT-PCR. It was demonstrated that seminal plasma exposure induces the up-regulation of key embryotrophic factors, LIF, GM-CSF and IL-6, in the oviduct following insemination. Factors important in tissue remodelling required for implantation and angiogenesis, MMP-2, MMP-3 and VEGF-C, were also shown to be increased at the time of implantation after seminal plasma exposure. Additionally the generation of T-regulatory cells in uterine tissues, demonstrated by the up-regulation of the transcription factor FOXp3 was shown to be dependent on semen exposure. The influence of seminal plasma on embryonic development, implantation and modulation of the maternal immune response to pregnancy may therefore be mechanisms which contribute to the adverse outcomes seen in pregnancies initiated in the absence of seminal plasma.

Together these experiments show a role for seminal plasma signalling at the time of insemination in influencing the pre-implantation embryo to program later fetal and neonatal development, thereby impacting on the metabolic health of offspring. We conclude that seminal plasma is not simply a transport medium for sperm, but acts also as a key regulator of a female tract environment providing optimal support for the developing embryo.
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 further grant my consent to the University of Adelaide to make this thesis available for loan and photocopying once accepted for the degree.

John James Bromfield

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2006


• **JJ Bromfield** and SA Robertson. “Seminal plasma regulates MMP-2, MMP-3 and VEGF-C mRNA expression in the peri-implantation mouse uterus”. Society for Reproductive Biology. 37th Annual Conference, Gold Coast Australia.

Table of contents

Chapter 1  Literature Review ................................................................. 1

1.1  INTRODUCTION .................................................................................. 2

1.2  ESTABLISHMENT OF PREGNANCY .................................................. 3

1.2.1  Insemination and fertilisation .......................................................... 4

1.2.2  Pre-implantation embryo development ........................................... 6

1.2.3  Endometrial receptivity and embryo implantation ............................ 8

1.2.3.1  Embryo attachment ..................................................................... 9

1.2.3.2  Decidualisation ........................................................................... 10

1.2.3.3  Trophoblast invasion and endometrial remodelling ..................... 11

1.2.4  Placental development ..................................................................... 13

1.2.5  Immunology of pregnancy ............................................................... 16

1.2.5.1  Hormones as modulators of immune tolerance ............................ 16
Chapter 2   Materials and Methods ......................................................... 37

2.1   ANIMALS AND SURGERIES ................................................................. 38
  2.1.1 General Procedures ........................................................................... 38
  2.1.2 Vasectomies and Seminal Vesicle Removal ...................................... 38

2.2   EMBRYO TRANSFER ............................................................................... 39
  2.2.1 Ovarian Hyper-Stimulation ............................................................... 39
  2.2.2 Embryo Collection and Culture .......................................................... 39
  2.2.3 Uterine Blastocyst Transfers .............................................................. 40
  2.2.4 Oviductal 2-cell Embryo Transfers .................................................... 40

2.3   ASSESSMENT OF THE FERTILISATION CAPABILITY OF MALES .......... 40

2.4   ASSESSMENT OF PREGNANCY AT DAY 18 ........................................... 41

2.5   PLACENTAL MORPHOMETRY .............................................................. 41

2.6   POST NATAL ASSESSMENT OF PROGENY ........................................ 41

2.7   DIFFERENTIAL PROTEIN ARRAY ....................................................... 42
  2.7.1 Sample Preparation ............................................................................ 42
  2.7.2 2D Electrophoresis ............................................................................ 43

2.8   REAL TIME RT-PCR ............................................................................ 43
  2.8.1 RNA Extraction ................................................................................ 43
  2.8.2 Reverse transcription ....................................................................... 44
Chapter 3  Effects of Seminal Vesicle Ablation on Pregnancy Outcome After Natural Mating .......................54

3.1  INTRODUCTION.................................................................................................................55
3.2  EFFECT OF SEMINAL PLASMA EXPOSURE AT DAY 18 OF PREGNANCY .....................57
3.3  EFFECT OF SEMINAL PLASMA ON DAY 18 PLACENTAL HISTOLOGY .......................58
3.4  FERTILITY OF SEMINAL VESICLE DEFICIENT MALES...............................................59
3.5  SIGNIFICANCE OF SEMINAL PLASMA EXPOSURE ON POSTNATAL GROWTH AND BODY COMPOSITION ..............................................................................60
3.6  IMPACT OF SEMINAL PLASMA EXPOSURE ON ENDOCRINE MARKERS OF METABOLISM IN PROGENY ............................................................................66
Chapter 4  Effects of Seminal Vesicle Ablation on Pregnancy Outcome After Embryo Transfer

4.1  INTRODUCTION......................................................................................................................... 72

4.2  EFFECT OF SEMINAL PLASMA EXPOSURE ON PREGNANCY PARAMETERS RESULTING FROM EMBRYO TRANSFER........................................................................... 74

4.3  EFFECT OF SEMINAL PLASMA EXPOSURE ON FETAL AND PLACENTAL DEVELOPMENT ................................................................................................................................. 75

4.4  EFFECT OF EMBRYO TRANSFER ON FETAL AND PLACENTAL DEVELOPMENT ................................................................................................................................. 76

4.5  EFFECT OF SEMINAL PLASMA EXPOSURE ON POSTNATAL GROWTH AND DEVELOPMENT ................................................................................................................................. 78

4.6  EFFECTS OF EMBRYO TRANSFER ON POSTNATAL GROWTH AND DEVELOPMENT ................................................................................................................................. 80

4.7  EFFECT OF SEMINAL PLASMA EXPOSURE ON BODY COMPOSITION OF PROGENY ................................................................................................................................. 82

4.8  EFFECT OF EMBRYO TRANSFER ON BODY COMPOSITION OF PROGENY .......... 87

4.9  EFFECTS OF SEMINAL PLASMA EXPOSURE ON ENDOCRINE MARKERS OF METABOLISM IN PROGENY ......................................................................................................... 90

4.10 EFFECT OF EMBRYO TRANSFER ON ENDOCRINE MARKERS OF METABOLISM IN PROGENY ................................................................................................................................. 91

4.11 DETERMINATION OF THE RELATIVE CONTRIBUTION SEMINAL PLASMA AND EMBRYO TRANSFER HAVE IN PROGRAMMING PREGNANCY OUTCOME ......................... 93

4.12 DISCUSSION ................................................................................................................................. 95
Chapter 5  Molecular Mechanisms of Seminal Plasma Actions in the Female Reproductive Tract

5.1 INTRODUCTION

5.2 EFFECT OF SEMINAL PLASMA EXPOSURE ON MRNA EXPRESSION OF EMBRYOTROPHIC MOLECULES IN THE OVIDUCT

5.3 EFFECT OF SEMINAL PLASMA ON THE UTERINE PROTEOME AT THE TIME OF IMPLANTATION

5.4 EFFECT OF SEMINAL PLASMA EXPOSURE ON ENDOTHELIAL CELL DENSITY AT THE TIME OF IMPLANTATION

5.5 EFFECTS OF SEMINAL PLASMA EXPOSURE ON EXPRESSION OF ENDOTHELIAL CELL REGULATORS

5.6 EFFECTS OF SEMINAL PLASMA EXPOSURE ON MRNA EXPRESSION OF MOLECULES IMPORTANT IN TISSUE REMODELING

5.7 EFFECTS OF SEMINAL PLASMA EXPOSURE ON MRNA EXPRESSION OF FOXP3

5.8 DISCUSSION

Chapter 6  General Discussion and Conclusions

6.1 DISCUSSION AND CONCLUSION

Chapter 7  Bibliography

List of figures

Figure 1.1 Schematic illustration of early embryo invasion.

Figure 1.2 Schematic illustration of a transverse section through a term placenta.
Figure 1.3  Schematic illustration of lymphocyte activation following insemination. .............................. 30

Figure 1.4  Schematic illustration describing the hypothesised mechanisms of semen during early pregnancy. ........................................................................................................... 35

Figure 2.1  Gel electrophoresis of RT-PCR products using designed sequence specific oligonucleotide primers. ........................................................................................................ 49

Figure 3.1  Effect of seminal plasma exposure on postnatal weight gain in progeny. ................................ 62

Figure 3.2  Effect of seminal plasma exposure on postnatal growth trajectory of progeny. .............................................................. 63

Figure 4.1  Effect of seminal plasma exposure on growth of progeny derived from embryo transfer. .......................................................................................................................... 79

Figure 4.2  Effect of embryo transfer on postnatal growth. .................................................................................. 81

Figure 5.1  Effect of seminal plasma on the uterine proteome at the time of implantation. ................................................................. 109

Figure 5.2  Effect of seminal plasma exposure on uterine endothelial cell density on day 4 after mating ................................................................. 112

Figure 5.3  Effect of seminal plasma exposure on endothelial cell density in the endometrium 4 days after mating .............................................................................................. 113

Figure 5.4  Expression of FOXp3 mRNA in the uterus after exposure to sperm ........................................... 124

Figure 6.1  Schematic illustration of semen action in the female tract during early pregnancy ....... 136
List of tables

Table 2.1  PCR primers developed for real time RT-PCR analysis supplied by Sigma-Proligo. ................................................................. 46 – 47

Table 3.1  Effect of seminal plasma exposure on fecundity and pregnancy parameters at day 18................................................................. 57

Table 3.2  Effect of seminal plasma exposure on placental morphology at day 18 ................................................................. 58

Table 3.3  Effects of seminal vesicle removal on fertilisation potential of mice ................................................................. 59

Table 3.4  Effect of seminal plasma exposure on body composition of male progeny at 14 weeks of age................................................................. 64

Table 3.5  Effect of seminal plasma exposure on body composition of female progeny at 14 weeks of age ................................................................. 65

Table 3.6  Effect of seminal plasma exposure on metabolic markers of nutrition in blood plasma of male and female progeny. ................................................................. 66

Table 3.7  Effect of seminal plasma exposure on metabolic markers of nutrition in blood plasma from progeny regardless of gender ................................................................. 67

Table 4.1  Effect of seminal vesicle removal on pregnancy parameters in mice undergoing embryo transfer. ................................................................. 75

Table 4.2  Effect of seminal plasma exposure on fetal and placental development after embryo transfer. ................................................................. 77

Table 4.3  Effect of seminal plasma exposure on fetal and placental development ................................................................. 77

Table 4.4  Effect of embryo transfer on fetal and placental development ................................................................. 77

Table 4.5  Effect of seminal plasma exposure on body composition of female progeny derived from embryo transfer ................................................................. 83

Table 4.6  Effect of seminal plasma exposure on body composition of male progeny derived from embryo transfer ................................................................. 84
Table 4.7 Effect of seminal plasma exposure on body composition of female progeny from combined embryo transfer and natural mating data................................. 85

Table 4.8 Effect of seminal plasma exposure on body composition of male progeny from combined embryo transfer and natural mating data.................................................. 86

Table 4.9 Effect of embryo transfer on body composition of female adult progeny.................................................. 88

Table 4.10 Effect of embryo transfer on body composition of male adult progeny.................................................. 89

Table 4.11 Effect of seminal plasma exposure on metabolic markers of nutrition in blood plasma of progeny derived from embryo transfer.......................................................... 90

Table 4.12 Effect of seminal plasma exposure on metabolic markers of nutrition in blood plasma of progeny.............................................................................................. 91

Table 4.13 Effect of embryo transfer on metabolic markers of nutrition in blood plasma of progeny:.............................................................................................. 92

Table 4.14 Regression analysis showing the relative contribution of seminal plasma and embryo transfer as determinants of pregnancy outcome.................................................. 94

Table 5.1 Expression of mRNAs encoding embryotrophic molecules in the oviduct after exposure to seminal constituents. ........................................................................... 107

Table 5.2 Expression of mRNAs encoding embryotrophic molecules in the oviduct after exposure to seminal plasma. ................................................................................... 108

Table 5.3 Identity of differential expressed proteins determined by differential protein array.................................................................................................................. 110

Table 5.4 Expression of mRNAs encoding endothelial cell-regulating factors in the uterus after exposure to seminal constituents.............................................................. 118

Table 5.5 Expression of mRNAs encoding endothelial cell-regulating factors in the uterus after exposure to seminal plasma................................................................. 119

Table 5.6 Expression of mRNAs encoding tissue remodelling factors in the uterus after exposure to seminal constituents. ................................................................. 122

Table 5.7 Expression of mRNAs encoding regulators of tissue remodelling in the uterus after exposure to seminal plasma................................................................. 123
Table 5.8  Expression of FOXp3 mRNA in the uterus after exposure to seminal constituents...

124
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>Ab</td>
<td>Antibody</td>
</tr>
<tr>
<td>ADCC</td>
<td>Antibody dependent cell cytotoxicity</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen presenting cell</td>
</tr>
<tr>
<td>ART</td>
<td>Assisted reproductive technique</td>
</tr>
<tr>
<td>BDKR</td>
<td>Bradykinin receptor</td>
</tr>
<tr>
<td>BFGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>Bp</td>
<td>Base pairs</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>C</td>
<td>Cytosine</td>
</tr>
<tr>
<td>oC</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>CDNA</td>
<td>Complimentary DNA</td>
</tr>
<tr>
<td>CG</td>
<td>Chorionic gonadotrophin</td>
</tr>
<tr>
<td>CL</td>
<td>Corpus luteum</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>Ct</td>
<td>Cycle threshold</td>
</tr>
<tr>
<td>DAB</td>
<td>Diaminobenzidine tetrachloride</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic cell</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNAse</td>
<td>Deoxyribonuclease</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>FASL</td>
<td>FAS ligand</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>G</td>
<td>Guanine</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte-macrophage colony-stimulating factor</td>
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<tr>
<td>HB-EGF</td>
<td>Heparin binding-epidermal growth factor-like growth factor</td>
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<tr>
<td>hCG</td>
<td>Human chorionic gonadotrophin</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic/pituitary/adrenal</td>
</tr>
<tr>
<td>HRP</td>
<td>Horse radish peroxidase</td>
</tr>
<tr>
<td>ICM</td>
<td>Inner cell mass</td>
</tr>
<tr>
<td>ICSI</td>
<td>Intra-cytoplasmic sperm injection</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>IGFBP</td>
<td>Insulin-like growth factor binding protein</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>ISPN</td>
<td>Implantation serine protease</td>
</tr>
<tr>
<td>IUGR</td>
<td>Intrauterine growth retardation</td>
</tr>
<tr>
<td>IVF</td>
<td><em>In vitro</em> fertilisation</td>
</tr>
<tr>
<td>LAK</td>
<td>Lymphokine activated killer</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>LIF</td>
<td>Leukaemia inhibitory factor</td>
</tr>
<tr>
<td>LN</td>
<td>Lymph node</td>
</tr>
<tr>
<td>mAb</td>
<td>Monoclonal antibody</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>MIP</td>
<td>Macrophage inflammatory protein</td>
</tr>
<tr>
<td>MLN</td>
<td>Mesenteric lymph node</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
</tbody>
</table>
MQ  Milli-Q
mRNA  Messenger RNA
MUC  Mucin
NK  Natural killer
NRP  Neuropilin
PALN  Para-aortic lymph node
PBS  Phosphate buffered saline
PCR  Polymerase chain reaction
PGE  Prostaglandin
PGF  Placental growth factor
RNA  Ribonucleic acid
RNAse  Ribonuclease
rpm  Revolutions per minute
RT-PCR  Reverse transcriptase polymerase chain reaction
SDS  Sodium dodecyl sulphate
T  Thymine
TCR  T cell receptor
TGF  Transforming growth factor
Th  T helper
TIMP  Tissue inhibitor of metalloproteinase
TNF  Tumour necrosis factor
Tr  T represser
U  Uracil
uNK  Uterine natural killer
VEGF  Vascular endothelial growth factor
VEGF-R  Vascular endothelial growth factor receptor
VIA  Video image analysis