Actions of seminal fluid signalling factors in the female reproductive tract and on pregnancy outcome

Danielle Jannette Glynn

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

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We used to think that if we knew one, we knew two, because one and one are two. We are finding that we must learn a great deal more about "and."

~Arthur Stanley Eddington

Some people walk in the rain, others just get wet.

~Roger Miller

The important thing is not to stop questioning. Curiosity has its own reason for existing. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvellous structure of reality.

~Albert Einstein

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Abstract

The cytokine environment of early pregnancy is known to be a key determinant of the development of the pre-implantation embryo, and its subsequent implantation and growth. Factors in male seminal fluid have been identified as regulators of the expression of cytokines in the female tract of mice, humans and other mammalian species, with insemination eliciting a cascade of molecular and cellular events, reminiscent of a classic inflammatory response. In humans, perturbations in seminal fluid signalling have been proposed to predispose to pathologies of pregnancy including implantation failure, recurrent miscarriage and pre-eclampsia. Seminal transforming growth factor-beta (TGFβ) is identified as one key molecule present in seminal fluid responsible for inducing the female post-mating cytokine response in mice. Research in humans however, has shown the seminal TGFβ content of fertile versus infertile couples to be similar, while the content of other known seminal constituents such as interferon-gamma (IFNγ), correlate with reproductive success. This project aimed to investigate the nature of active factors present in seminal fluid in mice, and their interactions in regulating the uterine cytokine environment during early pregnancy, utilising a variety of in vitro and in vivo experimental strategies. Further, the effect of perturbation in the peri-conception cytokine environment on short and long term pregnancy and postnatal outcomes was investigated.

Evaluation of uterine fluids from estrous and mated mice showed a marked upregulation of a number of cytokines following mating, including granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-6 (IL-6) and the chemokine KC (rodent IL-8 homologue). Increased production of factors such as GM-CSF and subsequent generation of a receptive uterine environment is thought to be crucial for optimal embryo development and placentation. It has previously been shown that seminal factors such as TGFβ contribute to the uterine post-mating inflammatory response, however other moieties present in seminal fluid, for instance cytokines induced in response to infection such as IFNγ or products from the mucosal microflora, may also play a regulatory role. Using uterine epithelial cells cultured in vitro, it was shown that a variety of immune modulators including the cytokines TGFβ and IFNγ, as well as bacterial products, gram negative lipopolysaccharide (LPS) and gram positive lipoteichoic acid (LTA), can alter basal cytokine production. IFNγ, a pro-inflammatory cytokine secreted by activated natural killer cells and T-cells, is known to interfere with TGFβ signalling in other contexts. Independently TGFβ, LPS and LTA stimulate GM-CSF production while differentially regulating IL-6 and KC production. Conversely IFNγ inhibits GM-CSF production, without effecting IL-6 or KC. Pair wise combinations of TGFβ, LPS and LTA resulted in additive stimulation of GM-CSF, while addition of

IFNγ to cultures in conjunction with any of these molecules downregulated GM-CSF and KC stimulation. These in vitro studies indicate factor-specific interactions between seminal fluid constituents and highlight the complex nature of seminal fluid signalling. Consequently we propose that the relative ratio of seminal signalling factors is likely to be more important than the absolute concentration of various regulators, in determining the optimal female reproductive tract response.

Using the mouse as an in vivo model, I have in addition demonstrated that LPS and LTA instilled into an estrous uterus can elicit cytokine production comparable to that observed following insemination. Further, these studies have shown that IFNγ instilled into the uterus of a recently mated mouse can reduce the post-copulatory GM-CSF and KC surge. However administration of IFNγ had no effect on near term pregnancy outcomes including fetal or placental weights, fetal crown-rump length, or implantation or resorption rates. The 'developmental origins of adult disease hypothesis' proposes the idea that the early uterine environment encountered by the conceptus contributes toward the risk of metabolic disorders in adulthood, hence a long term study of progeny conceived after IFNγ administration was also undertaken. Neo-natal outcomes, such as birth weight, litter size and gestation length were unaltered, as was growth trajectory to 22 weeks of age. Adult metabolic markers, glucose tolerance, organ weight, muscle weight, adiposity and systolic blood pressure were not affected by the perturbation of peri-conceptual cytokine parameters.

This work has examined the potential regulatory role of a number of seminal fluid signalling agents in directing the post-mating cytokine response, and has furthermore shown the relatively resilient nature of the early cytokine environment to subtle perturbation. Delineating the identity and roles of seminal fluid factors in early pregnancy brings us closer to an understanding of the key physiological events of early pregnancy and assists in identifying potential risk factors for human pregnancy pathologies.

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.

Danielle Jannette Glynn

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Publications arising from these and related studies

1. SA Robertson, JJ Bromfield, DJ Glynn, DJ Sharkey, MJ Jasper.
   Actions of seminal plasma cytokines in priming female reproductive tract receptivity for embryo implantation. In Gil Mor (Ed) Immunology of Implantation 2005, Landes Bioscience, Georgetown TX.

2. DJ Glynn and SA Robertson (in preparation)
   Role of LPS and LTA in regulation of the post-mating inflammatory response in mice.

3. DJ Glynn and SA Robertson (in preparation)
   Inhibitory effect of IFNγ on seminal fluid signalling in mice.

4. DJ Glynn and SA Robertson (in preparation)
   Perturbation of early cytokine environment influences fetal programming in mice.

Patent

1. Treatment and diagnosis of a reproductive disorder by measuring or inhibiting Interferon gamma. International publication number IP0240US. Published 20th September 2002.

2. Treatment and diagnosis of a reproductive disorder by measuring or inhibiting Interferon gamma. International publication number IP0240AU. Published 19th September 2003.

3. Treatment and diagnosis of a reproductive disorder by measuring or inhibiting Interferon gamma. International publication number IP0240CA. Published 15th May 2006

Abstracts and presentations arising from these studies

Presenting author underlined

2003

- DJ Glynn and SA Robertson
  "SEMINAL FACTORS AND UTERINE EPITHELIAL RESPONSIVENESS TO TGFβ"
  Australian Society for Medical Research (South Australian Division) Annual Meeting.

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2004

- DJ Glynn, DJ Sharkey and SA Robertson
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  Invited speaker Perinatal Research Centre University of Alberta, Edmonton, Canada.

- DJ Glynn and SA Robertson

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  Department Seminar – RCRH, Adelaide University Adelaide Australia.

2005

- DJ Glynn and SA Robertson

  "LPS INTRODUCED AT MATING INDUCES KC PRODUCTION IN THE MURINE UTERUS DURING EARLY PREGNANCY"

  36th Annual Conference of the Society for Reproductive Biology, Perth, Australia. (Abs. 287)

- DJ Glynn and SA Robertson

  "THE IMPACT OF FACTORS INTRODUCED AT INSEMINATION ON THE FEMALE IMMUNE RESPONSE AND FETAL OUTCOMES"

  Department Seminar – RCRH, Adelaide University Adelaide Australia.

2006

- DJ Glynn and SA Robertson

  "THE IMPACT OF IFNγ AT INSEMINATION ON THE FEMALE IMMUNE RESPONSE AND REPRODUCTIVE OUTCOMES"

  Department Seminar – RCRH, Adelaide University Adelaide Australia.

Abbreviations

A	Adenine
Ab	Antibody
BMP	Bone morphogenic protein
Bp	Base pairs
BSA	Bovine serum albumin
C	Cytosine
cAMP	Cyclic adenosine monophosphate
cDNA	Complimentary DNA
Ct	Cycle threshold
DAB	Diaminobenzidine tetrachloride
DNA	Deoxyribonucleic acid
DNAse	Deoxyribonuclease
DPBS	Dulbecco's PBS
DTH	Delayed-type hypersensitivity
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacteic acid
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbancy assay
FCS	Fetal calf serum
FSH	Follicle stimulating hormone
G	Guanine
GM-CSF	Granulocyte-macrophage colony-stimulating factor
hCG	Human chorionic gonadotrophin
HLA	Human leukocyte antigen
HRP	Horse radish peroxidase

ICSI	Intra-cytoplasmic sperm injection
IFN	Interferon
IL	Interleukin
IUGR	Intrauterine growth retardation
IVF	In vitro fertilisation
Kb	Kilobase pairs
kDa	Kilo-dalton
LAP	Latency associated protein
LCA	Leukocyte common antigen
LGL	Large granular lymphocytes
LH	Luteinizing hormone
LIF	Leukaemia inhibitory factor
LPS	Lipopolysaccharide
LTBP	Latent transforming growth factor β binding protein
mAb	Monoclonal antibody
MCP	Monocyte chemotactic protein
MHC	Major histocompatibility complex
MIP	Macrophage inflammatory protein
MMP	Matrix metalloproteinase
MQ	Milli-Q
mRNA	Messenger RNA
NK	Natural killer
^{o}C	Degrees celsius
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PGE	Prostaglandin
PSA	Prostate specific antigen

RNA	Ribonucleic acid
RNAse	Ribonuclease
rpm	Revolutions per minute
RT-PCR	Reverse transcriptase polymerase chain reaction
SDS	Sodium dodecyl sulphate
T	Thymine
TGF	Transforming growth factor
TIMP	Tissue inhibitor of metalloproteinase
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TSP-1	Thrombospondin-1
U	Uracil
v/v	Volume per volume
VIA	Video image analysis
w/v	Weight per volume
WHO	World Health Organisation