Seminal plasma cytokines as determinants of ovulation, embryo development and pregnancy success in the pig

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“Somewhere, something incredible is waiting to be known”

Carl Sagan 1934 - 1996

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ABSTRACT

Determinants of litter size in the pig are ovulation rate, fertilisation rate and embryo and fetal mortality. In practice, litter size is normally about half the ovulation rate with 40% or more of potential piglets being lost before day 30 of pregnancy. Successful embryo development depends on optimal timing of events beginning with ovulation, fertilisation and preparation of the uterine environment for the attachment of the developing embryo. In the pig these processes are tightly controlled and are highly sensitive to disruption. The determinants of optimal early embryo development remain to be fully elucidated but evidence provided in mouse and human studies indicate that constituents of seminal plasma may provide a beneficial 'priming' stimulus acting at natural mating to synchronise and enhance early reproductive events. The cytokine transforming growth factor beta (TGFβ) is present in large quantities in mouse and human seminal plasma and is a principal active constituent in mediating seminal fluid signalling in the female reproductive tract.

Experiments described in this thesis were designed to investigate whether boar seminal plasma can exert changes in the female reproductive tract during the pre-attachment period in the pig that are comparable to those described in mouse and human. Studies in this thesis demonstrate that seminal plasma causes a transient inflammatory response in the uterus characterised by induction of cytokine gene expression and immune cell changes that occur during the critical period in which the pig embryo is most vulnerable to demise. Seminal factors were also observed to enhance ovarian function, promoting synthesis of progesterone and influenced the rate of embryo development. The effect of these early changes due to seminal plasma was investigated in a large-scale field trial. However, this failed to demonstrate an effect of frozen-thawed seminal plasma on reproductive outcome in gilts. Moreover, the presumed active constituent of seminal plasma, TGFβ, was identified at high levels in boar semen but did not correlate with boar fertility.

The information from these experiments provide a comprehensive understanding of mechanisms underlying the potentially beneficial actions of seminal plasma in early pregnancy. Ongoing studies will assist in the strategic design of (1) novel 'surrogate seminal plasma' or 'semen extender' products incorporating active constituents of seminal plasma, and (2) assays for measuring cytokine / immunoactive constituents of seminal plasma that are predictive of boar fertility.

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 to Sean O'Leary 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.

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PUBLICATIONS ARISING FROM THESE STUDIES

Invited seminars (Australia)

1999 Seminal plasma induces a local inflammatory response in the reproductive tract of gilts. Australian Pig Science Association Biannual Meeting. Adelaide, South Australia

2000 Seminal factors influences serum progesterone levels during early pregnancy in the pig. 11th International conference of Endocrinology (Satellite symposia) Adelaide, South Australia

Intrauterine seminal plasma induces endometrial inflammatory response in gilts. Fertility Society of Australia and Australian Society for Reproductive Biologists. Canberra, Australia

Presence of transforming growth factor beta in boar seminal plasma. Australian Society for Medical Research. Adelaide, Australia

2001 Seminal plasma treatment stimulates progesterone synthesis during early pregnancy in the pig. Endocrine Society of Australia and Society for Reproductive Biology Annual Scientific Meeting. QLD, Australia.

Intrauterine seminal plasma increases ovarian steroidogenesis during early pregnancy in the pig. Australian Pig Science Association Biannual Meeting. Adelaide, South Australia

Invited Seminars (International)

2001 *Seminal plasma cytokines as determinants of ovulation, embryo development and pregnancy success in the pig.* Division of Animal Physiology, School of Biological Sciences (Prof. Morag Hunter) , University of Nottingham, 18th June. Loghborough, UK.

Seminal plasma cytokines, implications for pre-natal mortality in pigs. Department of Obstetrics, Gynaecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, (Assoc. Prof. Deborah Anderson) 24th June, Boston, USA.

Intrauterine seminal plasma increases ovarian steroidogenesis in the pig. Society for Study of Reproduction, 34th Annual meeting. Ottawa, Canada.

Seminal plasma cytokines as determinants of ovulation, embryo development and pregnancy success in the pig. Department of Animal Science, Ontario Veterinary College (Prof. Anne Croy) 7th July, Guelph, Canada.

Seminal plasma influences on ovarian function, implications for fertility enhancement in pigs. Department of Agriculture, University of Alberta (Prof. George Foxcroft) 11th June, Edmonton, Canada.

Seminal plasma cytokines as determinants of ovulation, embryo development and pregnancy success in the pig. Department of Animal Science, University of Missouri (Prof. Billy Day) 23rd July, Columbia, USA.

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ABBREVIATIONS

Ab	Antibody
AI	Artificial insemination
BMP	Bone morphogenic protein
Bp	Base pairs
BPE	Bovine pituitary extract
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
cDNA	Complimentary DNA
CL	Corpora lutea or corpus luteum
Ct	Cycle threshold
cv	Coefficient of variation
DAB	Diaminobenzidine tetrachloride
DMEM	Dulbecco's modified minimal essential medium
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
EU	Equivalent units
FACS	Fluorescence-activated cell scanning
FCS	Fetal calf serum
FSH	Follicle stimulating hormone

GM-CSF	Granulocyte-macrophage colony-stimulating factor
GIFT	Gamete intrafallopian transfer
HBSS	Hank's balanced salt solution
hCG	Human chorionic gonadotrophin
HLA	Human leukocyte antigen
h	Hour
HRP	Horseradish peroxidase
ID	Identification
IDO	Indoleamine 2,3-dioxygenase
IFNγ	Interferon gamma
IL	Interleukin
IU	International units
IUGR	Intrauterine growth retardation
IVF	In vitro fertilisation
Kb	Kilobase pairs
kDa	Kilo-dalton
L	Litre
LAL	Limulus amebocyte lysate assay
LAP	Latency associated protein
LCA	Leukocyte common antigen
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

Min	Minute
MIP	Macrophage inflammatory protein
Mls	millilitres
MMP	Matrix metalloproteinase
MQ	Milli-Q
mRNA	Messenger RNA
NHS	Normal human serum
NK	Natural killer
^{o}C	Degrees celcius
OCT	Optimal Cutting Temperature Compound
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PGE	Prostaglandin E
PGF	Prostaglandin F
PSP	Pig seminal protein
RNA	Ribonucleic acid
RNAse	Ribonuclease
rpm	Revolutions per minute
RT	Room temperature
RT-PCR	Reverse transcriptase polymerase chain reaction
SABOR	South Australian Boar
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SEM	Standard error of the mean
SLA	Swine leukocyte antigen
TGF	Transforming growth factor

TLR	Toll-like receptor
TNF	Tumour necrosis factor
v/v	Volume per volume
VIA	Video image analysis
w/v	Weight per volume