



INDUCTION OF MITOGENESIS AND  
CELL-CELL ADHESION BY PORCINE  
SEMINAL PLASMA

By

MICHAEL HADJISAVAS  
B.Sc. (Hons)

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY,  
THE UNIVERSITY OF ADELAIDE

A thesis submitted to the University of Adelaide in  
fulfilment of the requirements for admission to the degree of  
Doctor of Philosophy

August 1992

*Awarded. 1993*

# TABLE OF CONTENTS

Abstract	i
Declaration	iv
Acknowledgments	v
Short Communications and Publications	vi
Tables and Figures	vii
Abbreviations	ix
<b>Chapter 1</b>	
<b>Literature Review</b>	
1.1 General Introduction	1
1.1.1 Embryonic Development during Early Pregnancy	2
1.1.2 Literature Review	3
1.2 Uterine Immune Involvement in Early Pregnancy	5
1.2.1 Uterine Immune Reactivity and Early Pregnancy	5
1.2.2 Immunesurveillance in the Female Reproductive Tract during Early Pregnancy and Following Mating	9
1.2.3 Uterine Cytokine Regulation Following Mating	12
1.2.4 Cytokine Networks in Early Mouse Pregnancy	15
1.2.5 A Role for Macrophages in Early Pregnancy	17
1.2.6 Effect of Uterine Immune Presensitization on Pregnancy	18
1.3 Immunology of Seminal Plasma	22
1.3.1 Immunosuppressive Activities of Seminal Plasma	23
1.3.2 Trophoblast-Lymphocyte Cross Reactive Antigens in Human Seminal Plasma	27
1.4 Cell Adhesion Molecules	30
1.4.1 Integrins	32
1.4.1.1 Specificity of Integrin-Ligand Interactions	34
1.4.1.2 Cell Signalling and Integrins	37
1.4.2.3 Integrin Involvement in Inflammation and Immune Responses	38
1.5 Conclusions	42

## **Chapter 2**

### **Materials and Methods**

2.1	Preparation and Cryopreservation of Porcine Peripheral Blood Lymphocytes	44
2.2	Semen Collection and Preparation of Seminal Plasma	44
2.3	Surgical Removal of Seminal Vesicles	45
2.4	Surgical Vasectomy of Boars	46
2.5	Preparation of Accessory Gland Secretions	47
2.6	Measurement of Lymphocyte Proliferation by Tritiated Thymidine Incorporation	48
2.7	Quantitation of Cell-Cell Adhesion	48
2.8	Purification of Adherent Cell Population from Peripheral Blood Lymphocytes	49
2.9	Purification of B Cells from Peripheral Blood Lymphocytes	49
2.10	Purification of T cells from Peripheral Blood Lymphocytes	50
2.11	Reagents	51
2.12	Chromatography	52
2.13	Gel Electrophoresis and Silver Staining of Proteins	53
2.14	Protein Quantitation	54
2.15	Preparation of 6M Guanidine Hydrochloride	54
2.16	Isolation of Total Porcine Seminal Vesicle RNA	54
2.17	Radiolabelling of DNA Oligomer Probe	56
2.18	Biotinylation of DNA Oligomer Probe	56
2.19	Northern Analysis of Porcine Seminal Vesicle RNA	57

## **Chapter 3**

### **Induction of Cell-Cell Adhesion and Mitogenesis by Porcine Seminal Plasma**

3.1	Introduction	59
3.2	Results	59
3.3	Discussion	67

## **Chapter 4**

### **Characterization of the Induction of Cell-Cell Adhesion by Porcine Seminal Plasma**

4.1 Introduction	70
4.2 Results and Discussion	71

## **Chapter 5**

### **Purification of Cell Adhesion Inducing Proteins from Porcine Seminal Vesicle Fluid**

5.1 Introduction	78
5.2 Results	78
5.3 Discussion	95

## **Chapter 6**

### **General Discussion**

6.1 Regulation of Integrins by Seminal Plasma	97
6.2 Possible Role of Adhesion Inducing Factors in Seminal Plasma	97
6.3 Future Prospects	101

## **Chapter 7**

<b>Bibliography</b>	<b>103</b>
---------------------	------------

## ABSTRACT

The study described in this thesis seeks to evaluate the nature of the interactions occurring between semen and cells of the uterus that occur following mating in pig. This thesis describes a previously unrecognized and novel ability of porcine seminal plasma to induce dose dependent cell-cell adhesion and mitogenesis amongst peripheral blood lymphocytes *in vitro*. The induction of cell-cell adhesion was shown to occur independently of the induction of mitogenesis and was not associated with immunosuppression or cytotoxicity. Seminal plasma induced homotypic cell-cell adhesion amongst T cells and macrophage/monocytes, but not amongst B cells. The response was inhibited by the exogenous fibronectin tetrapeptide, arg-gly-asp-ser (RGDS), inhibitors of intracellular signalling, cytochalasins, heparin and required divalent metal ions. This evidence indicated that the mechanism of cell-cell adhesion was analogous to that occurring between regulated cell surface integrins and molecules of the extracellular matrix and was the result of cellular activation by seminal factors. Integrins are ubiquitous cell surface heterodimeric glycoproteins, consisting of  $\alpha$  and  $\beta$  subunits linked to the cytoskeleton via transmembrane linkages. Furthermore, they appear on all cell types and extend throughout the most of the phylogentic tree.

Assaying the various boar accessory gland secretions on lymphocytes *in vitro*, indicated that the mitogenic and adhesive activity originated in the seminal vesicles. Furthermore, surgical removal of the seminal vesicles resulted in ejaculates devoid of mitogenic and cell-cell adhesive activity. Fractions from chromatographic separations of seminal vesicle proteins produced by cation exchange, hydrophobic interaction, diafiltration, Phenyl-Superose™ and C-18 separations, were assayed for induction of cell-cell adhesion amongst lymphocytes *in vitro*. Reversed phase separations

conducted on Phenyl-Superose, revealed two distinct forms of cell-cell adhesion; one forming a network of inter-connecting strands and the other forming regular circular clumps of adhered cells. Reversed phased chromatography of the former on C-18 silica revealed a single peak of activity containing a protein of 15kDa molecular weight, which represented approximately 0.5-1% of the total seminal vesicle fluid protein content. Purity of the adhesion inducing factor-1 (AIF-1), was estimated at 97% by N-terminal sequencing, from which a 32 amino acid sequence was determined. Screening of the SwissProt protein sequence data base revealed that AIF-1 is a novel protein. A region of the N-terminal amino acid sequence of AIF-1 is homologous to a highly conserved region in two bovine seminal vesicle glycoproteins, of similar molecular weight to AIF-1. This sequence also appeared in the collagen binding domain of type II bovine fibronectin. The bovine seminal vesicle proteins share a high degree of homology with the collagen binding domain of type II bovine fibronectin and together with AIF-1, potentially represent a novel class of bioactive proteins with unique biological activities. Northern analysis of total porcine RNA by a synthetic 51mer DNA oligonucleotide complementary to the predicted mRNA identified an abundant mRNA species of 0.9kb.

The *in vivo* role of seminal plasma proteins, which functionally upregulate cell surface integrins remains uncertain. However, cellular adhesive interactions mediated through integrins, particularly in leukocytes, transduce a variety of intracellular signals important to the regulation of growth, development, differentiation, gene expression and activation state of the cell. Furthermore, expression of functionally upregulated cell surface adhesion receptors is a feature of cellular activation, particularly at sites of inflammation. Hence, the modulation of function and activation of uterine leukocytes and possibly of other cell types within the uterus by seminal plasma following mating is postulated.

Studies in rodents indicate that seminal plasma induces a post-mating uterine inflammatory response, associated with cytokine production by lymphoid and epithelial cells. Uterine and embryo derived cytokines are recognized as playing a role in the regulation of embryonic and uterine development during early pregnancy. In this regard, seminal plasma adhesion inducing proteins such as AIF-1, may augment the post-mating uterine inflammatory response and thus, participate in the establishment of an appropriate uterine milieu important to the establishment of pregnancy.

The mechanisms involved in the regulation of cell adhesive interactions and its cellular consequences, remains one of the most fundamentally important and potentially rewarding aspects of cell biology.