

**BOAR SPERMATOZOA DEVELOP ABILITY TO BIND
TO OVIDUCT EPITHELIUM DURING PASSAGE
THROUGH THE EPIDIDYMIS**

**Thesis submitted by
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DECLARATION OF ETHICS

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics, Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (Animal Ethics approval number A1007).

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STATEMENT ON THE CONTRIBUTION OF OTHERS

Professor Phillip Summers supervised the research reported in this thesis, carried out the surgical castrations of the boars, provided advice and assistance with the preparation of the thesis and was a co-author on all papers resulting from this thesis.

A stipend was provided by the Australian Agency for International Development (AusAID) for the duration of the research candidature. Project costs were met from IRA and Reproduction Service accounts held by Professor Summers.

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Date

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*The LORD bless thee, and keep thee: The LORD make his face shine upon thee, and be gracious unto thee: The LORD lift up his countenance upon thee, and give thee peace.
Numbers 6: 24-26 (Holy Bible, KJV)*

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ABSTRACT

The aim of this study was to investigate the relationship of maturation of spermatozoa in the epididymis and the ability to bind to oviduct epithelium. It was hypothesized that testicular spermatozoa need to pass through the regions of the epididymis in order to acquire the ability to bind to the oviduct.

Spermatozoa were collected from the rete testis and the caput, corpus and cauda epididymides from 10-14 month-old Large White or Large White x Landrace boars. Boars were first unilaterally castrated and then slaughtered four to five weeks later to obtain the second testicle, epididymis and seminal vesicles. Oviducts were obtained from slaughtered pre-pubertal gilts and explants from the isthmus and ampulla prepared. Spermatozoa were suspended in modified Androhep medium, added to oviduct explants and incubated at 39⁰ C in a humidified atmosphere containing 5% CO₂ in air for 15 minutes. The number of spermatozoa attached to 1.25 mm² of explant was counted after fixation and staining of explants.

The possibility of using oviducts from slaughtered cows rather than porcine oviducts was examined using ejaculated spermatozoa. Significantly more ejaculated spermatozoa bound to the isthmus of gilts than cows hence, porcine oviducts were used in the succeeding experiments. There was a sequential increase in the number of spermatozoa that bound to the oviductal epithelium from the rete testis to the cauda epididymidis (2 ±0.30, 4.36 ±0.53, 9.3 ±1.60 and 15±1.22 for rete testis, caput corpus and caudal spermatozoa on isthmic explants, respectively). Significantly more (*P* ≤0.05) spermatozoa, either ejaculated or epididymal, bound to isthmus than ampulla explants (26.33±2.27 and 13.55±1.42 ejaculated spermatozoa on isthmic and ampullary explants, respectively).

Incubation in medium containing albumin and asialofetuin which are known to contain mannose and lactose respectively inhibited the binding of epididymal spermatozoa to oviduct explants (3.42±0.56 caudal spermatozoa on isthmic explants in medium with albumin and 14.75±2.02 caudal spermatozoa on isthmic explants in modified Androhep medium).

The number of spermatozoa from the caput and corpus that bound to oviduct explants significantly ($P \leq 0.05$) increased after incubation in caudal fluid for 30 minutes (7.52 ± 1.10 and 12.78 ± 1.64 corpus spermatozoa on isthmic explants for modified Androhep and caudal fluid, respectively). This result suggests that caudal fluid has distinct features that directly or indirectly influence the attachment of spermatozoa to oviduct epithelium. The motility of spermatozoa also decreased after incubation in caudal fluid for 30 minutes. This result is not surprising because *in vivo*, spermatozoa remain in a quiescent state during storage in the cauda epididymidis.

Exposure of epididymal spermatozoa to seminal plasma for 30 minutes significantly reduced the number of spermatozoa that bound to oviduct explants while exposure for one minute significantly increased the number of bound caput spermatozoa. Incubation in seminal plasma also caused capacitation of spermatozoa and this is the likely reason for the reduction in the number of bound spermatozoa. There is also the possibility that some components in the seminal plasma may form a coating over the plasma membrane of spermatozoa sufficient to inhibit the expression of pre-existing binding molecules. On the other hand, the seminal plasma may also be a source of binding molecules for immature caput spermatozoa. There was an increase in the motility of spermatozoa after exposure to seminal plasma.

In conclusion, this study found that as spermatozoa pass down the epididymis, there is an increase in the number of spermatozoa that bind to oviduct explants. This result was interpreted to mean that the maturation of spermatozoa in the epididymis involves the acquisition of the ability to bind to oviduct epithelium.

TABLE OF CONTENTS

	Page No.
Statement of Access	ii
Statement of Sources	ii
Declaration of Ethics	iii
Statement of the Contribution of Others	iv
Acknowledgement	v
Abstract	vi
Table of Contents	viii
List of Tables	xii
List of Figures	xiii
List of Abbreviations	xvi
CHAPTER 1	1
INTRODUCTION.....	1
1.1 Introduction.....	1
1.2 Working Hypothesis.....	4
1.3 Objectives.....	4
CHAPTER 2	6
REVIEW OF LITERATURE	6
2.1 Introduction.....	6
2.2 A General Perspective to the Role of the Epididymis.....	6
2.3 The Fertilising Ability of Epididymal Spermatozoa.....	7
2.4 Comparative Morphology of Spermatozoa from the Caput, Corpus and Cauda Epididymidis	9
2.5 Histological Features of the Boar Epididymis	10
2.6 Ultrastructural Characteristics of the Boar Epididymis	12
2.7 Physiological Correlations	14
2.8 The Epididymal Plasma	15
2.8.1 Epididymal secretory proteins.....	16
2.8.2 Epididymal secretory proteins of the boar and related compounds ..	17
2.8.3 Secretory enzymes in the epididymal fluid of the boar.....	21

2.9	Maturation of Boar Spermatozoa in the Epididymis.....	22
2.9.1	Modifications of the DNA-protein complex	23
2.9.2	Modifications of the plasma membrane.....	23
2.9.3	Concentration of spermatozoa during the epididymal transit	27
2.10	Protection of spermatozoa during storage in the cauda epididymidis.....	27
2.11	The Oviduct.....	28
2.11.1	Structural anatomy and histology of the oviduct	28
2.11.2	Blood supply to the oviduct	30
2.12	The Oviduct Luminal Fluid.....	30
2.13	Binding of Spermatozoa to the Oviduct.....	33
2.13.1	The role of the sperm reservoir	34
2.14	Mechanisms Involved in the Formation of the Sperm Reservoir.....	37
2.14.1	Physical aspects.....	38
2.14.2	Carbohydrate recognition.....	39
2.15	Features of the Binding of Spermatozoa to Oviductal Explants	41
2.16	Sperm-to-Oviduct Binding in Relation to the Region of the Oviduct, the Stage of the Oestrous Cycle and the Reproductive Status of the Animal .	42
2.17	Sperm-to-Oviduct Binding between Epididymal and Ejaculated Spermatozoa and between Capacitated and Uncapacitated Spermatozoa	43
2.18	The Release of Spermatozoa from the Sperm Reservoir	43
2.19	Sperm-Oviduct Binding and the Role of the Seminal Plasma	44
2.20	Conclusion.....	46
CHAPTER 3		47
MATERIALS AND METHODS		47
3.1	Boars	47
3.2	Preparation of oviductal explants for the binding assay	47
3.3	Preparation of spermatozoa and determination of motility characteristics	49
3.3.1	CASA settings and definitions	49
3.4	Co-incubation of spermatozoa and explants	51
3.5	Fixation and counting bound spermatozoa.....	51
3.6	Comparison of the binding capacity of ejaculated boar spermatozoa to porcine and bovine oviducts.....	52
3.7	The binding of boar epididymal spermatozoa to porcine oviducts	52

3.7.1	Comparison of the binding capacity of epididymal boar spermatozoa to oviducts of sows and gilts	53
3.8	The binding of epididymal spermatozoa after dilution with either albumin, asialufetuin and modified Androhep solutions.....	54
3.9	The binding of epididymal spermatozoa after incubation in caudal fluid.	55
3.10	The binding of epididymal spermatozoa to oviductal epithelium after incubation with seminal plasma	55
3.10.1	Determination of the percentage of live and dead spermatozoa	57
3.10.2	Capacitation status of spermatozoa	57
3.11	Data presentation and analyses	58
CHAPTER 4		59
BINDING OF BOAR EPIDIDYMAL SPERMATOZOA TO OVIDUCTAL EPITHELIUM.....		59
4.1	Introduction	59
4.2	Binding of ejaculated boar spermatozoa to porcine and bovine oviductal explants.....	61
4.3	Motility of epididymal spermatozoa	62
4.4	Binding of boar epididymal spermatozoa to the oviductal epithelium of sows and gilts	64
4.5	Developmental influence on the binding ability of boar spermatozoa from the rete testis and the caput, corpus and cauda epididymidis to porcine oviducts	64
4.6	Comparison of the binding capacity of epididymal spermatozoa between boars	67
4.7	Carbohydrate-binding molecules recognised by epididymal spermatozoa.....	67
CHAPTER 5		77
EXPOSURE TO CAUDAL EPIDIDYMAL FLUID INCREASES THE BINDING ABILITY OF SPERMATOZOA FROM THE CORPUS AND THE CAPUT EPIDIDYMIDIS.....		77
5.1	Introduction	77
5.2	Motility of epididymal spermatozoa after incubation in caudal fluid.....	79

5.3	Binding of epididymal spermatozoa after incubation in caudal fluid	80
CHAPTER 6		85
INFLUENCE OF SEMINAL PLASMA ON THE BINDING ABILITY OF EPIDIDYMAL SPERMATOZOA.....		85
6.1	Introduction	85
6.2	Motility characteristics of epididymal spermatozoa before and after incubation with seminal plasma	87
6.3	Capacitation status of spermatozoa before and after incubation in modified Androhep medium or seminal plasma.....	90
6.4	The viability of spermatozoa before and after incubation in modified Androhep medium or seminal plasma.....	92
6.5	The influence of seminal plasma on the binding capacity of epididymal spermatozoa to oviductal epithelium.....	95
CHAPTER 7		101
GENERAL DISCUSSION.....		101
7.1	Scope of research project.....	101
7.2	Future research directions.....	106
7.3	Conclusion.....	107
REFERENCES.....		109

LIST OF TABLES

	Page No.
Table 3.1 Composition of Modified Tyrode's Solution	48
Table 3.2 Composition of the Modified Androhep Solution	51
Table 3.3 Composition of Gill's Haematoxylin stain	52
Table 4.1 Motility characteristics of epididymal spermatozoa immediately after collection	63
Table 5.1 Motility characteristics (mean \pm SEM) of epididymal spermatozoa after incubation in either modified Androhep medium or caudal fluid.	80
Table 6.1 Motility characteristics (mean \pm SEM) of spermatozoa before and after incubation for 30 minutes in either modified Androhep medium or seminal plasma.	90
Table 6.2 Motility characteristics (mean \pm SEM) of spermatozoa before and after incubation for one minute in either modified Androhep medium or seminal plasma.	92

LIST OF FIGURES

		Page No.
Figure 4.1	Binding of ejaculated porcine spermatozoa to porcine and bovine oviductal and tracheal explants.	62
Figure 4.2	The mean (+ SEM) percentage of motile spermatozoa from the rete testis and epididymis.	63
Figure 4.3	Binding of epididymal spermatozoa to the isthmus.	64
Figure 4.4	Binding of epididymal spermatozoa to the ampulla.	65
Figure 4.5	Binding of boar spermatozoa from the rete testis and epididymis to the isthmus and ampulla.	66
Figure 4.6	Comparison of the binding of ejaculated and caudal spermatozoa to isthmic and ampullary explants.	66
Figure 4.7	Comparison between boars in the binding of spermatozoa from the rete testis and epididymis to isthmic explants.	68
Figure 4.8	Comparison between boars in the binding of spermatozoa from the rete testis and epididymis to ampullary explants.	68
Figure 4.9	Comparison between the left and the right testicles of boars in the binding capacity of spermatozoa to isthmic explants.	69
Figure 4.10	Comparison between the left and the right testicles of boars in the binding capacity of spermatozoa to ampullary explants.	69
Figure 4.11	The binding of epididymal spermatozoa to isthmic explants after incubation of spermatozoa in medium containing either albumin, asialufetuin or modified Androhep medium.	70
Figure 4.12	The binding of epididymal spermatozoa to ampullary explants after incubation of spermatozoa in medium containing albumin, asialufetuin or modified Androhep medium.	70
Figure 5.1	The reduction in the motility of spermatozoa from the caput and the corpus after incubation in caudal fluid.	79

Figure 5.2	The influence of incubation of epididymal spermatozoa in caudal fluid on the binding of spermatozoa to isthmic explants.	81
Figure 5.3	The influence of incubation of epididymal spermatozoa in caudal fluid on the binding of spermatozoa to ampullary explants.	81
Figure 6.1	The mean (+ SEM) percentage of motile spermatozoa from the rete testis and the epididymis of boars immediately after collection into either modified Androhep medium or seminal plasma.	88
Figure 6.2	The mean (+ SEM) percentage of motile spermatozoa from the rete testis and the epididymis of boars after incubation for 30 minutes in either modified Androhep medium or seminal plasma.	89
Figure 6.3	The mean (+ SEM) percentage of motile spermatozoa from the rete testis and the epididymis of boars before and after incubation for one minute in either modified Androhep medium or the seminal plasma.	91
Figure 6.4	The capacitation status of spermatozoa before incubation in modified Androhep medium (control) or after incubation for 30 minutes in either modified Androhep medium or seminal plasma.	93
Figure 6.5	The capacitation status of spermatozoa after incubation for one minute in either modified Androhep medium (control) or seminal plasma.	93
Figure 6.6	The percentage (+SEM) of live spermatozoa before incubation in modified Androhep medium or after incubation for 30 minutes in either modified Androhep medium or seminal plasma.	94
Figure 6.7	The percentage (+SEM) of live spermatozoa after incubation for one minute in either modified Androhep medium or seminal plasma.	94
Figure 6.8	Effect of incubation for 30 minutes in either modified Androhep medium or seminal plasma on the binding of spermatozoa from the rete testis and epididymis to isthmic explants.	95
Figure 6.9	Effect of incubation for 30 minutes in either modified Androhep medium or seminal plasma on the binding of spermatozoa from the rete testis and epididymis to ampullary explants.	96
Figure 6.10	Effect of incubation for one minute in either modified Androhep medium or seminal plasma on the binding of spermatozoa from the rete testis and epididymis to isthmic explants.	96

Figure 6.11 Effect of incubation for one minute in either modified Androhep or seminal plasma on the binding of spermatozoa from the rete testis and epididymis to ampullary explants. **97**

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
ATP	adenosine triphosphate
BSP	bovine seminal plasma protein
cAMP	cyclic Adenosine Monophosphate
cAMP-PKA	cAMP-protein kinase A
CTC	chlortetracycline
CRISP	cysteine-rich secretory protein
CTP	cholesterol transferase protein
DNA	deoxyribonucleic acid
EAP	oestrous-associated glycoprotein
E-RABP	epididymal retinoic acid-binding protein
FITC-sZP	fluorescein-conjugated solubilized zona pellucida
GPX	glutathione peroxidase
<i>g</i>	<i>g</i> -force/ <i>g</i> -load/gravitational acceleration
HBP s	heparin-binding proteins
HE	human epididymal protein
HPLC	high performance liquid chromatography
HTM-IVOS	Hamilton-Thorne Machine-integrated visual optical system
kDa	kilodalton
K⁺/Na⁺ ratio	potassium/sodium ratio
MAN2B	135 kDa alpha-D-mannosidase
MMP s	matrix metalloproteinases
mRNA	messenger ribonucleic acid
OECM	oviduct epithelial cell monolayer
OGP	oestrogen-dependent protein
PAGE	polyacrylamide gel electrophoresis
PGDS	prostaglandin D2 synthase
pI	isoelectric point
pOGP	porcine oestrogen-dependent protein
SDS	sodium dodecyl sulfate
TALP	Tyrode's albumin lactate phosphate