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

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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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Santiago T. Peña, Jr. 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)

I thank God first of all for this rare opportunity he has given me to continue my professional upliftment in this prestigious school, the School of Veterinary and Biomedical Sciences, James Cook University, Townsville, North Queensland, Australia. I thank Him for the great chance to enjoy bountiful blessings he has bestowed upon me in this country and in advancing my knowledge of my professional career along the course of my study.

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To all of you, I tender this humble piece of work.

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 39^0 C in a humidified atmosphere containing 5% CO_2 in air for 15 minutes. The number of spermatozoa attached to 1.25 mm^2 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 ($P \leq 0.05$) 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.

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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
g	g-force/g-load/gravitational acceleration
HBPs	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
MMPs	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