INFLUENCE OF THE FEMALE REPRODUCTIVE TRACT ON THE MOTILITY AND MORPHOLOGICAL CHARACTERISTICS OF RAM SPERMATOZOA

Thesis submitted by

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DECLARATION

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ABSTRACT

In mammals, millions of spermatozoa are deposited in the posterior female reproductive tract but only a few hundred reach the oviducts and from these only one will fertilise an oocyte in a mono-ovulatory species. Investigating why so few spermatozoa reach the oviduct and what was so special about these spermatozoa was the central theme to the studies reported in this thesis. The studies were conducted in the facilities of the Biomedical and Tropical Veterinary Science precinct, James Cook University and used Merino sheep as the model.

In initial studies, semen was collected from rams by electroejaculation and it was demonstrated that not only did ram spermatozoa in undiluted semen have a limited life span but that were differences between rams. Some rams had spermatozoa that survived for less than six hours whereas in others, spermatozoa survived for 30 hours. These differences between rams were not present in semen diluted in Tyroide's-albumin-lactate-pyruvate (TALP) medium. Nearly all spermatozoa in freshly ejaculated semen were uncapacitated but after 12 hours incubation in Hepes-synthetic oviduct fluid (HSOF), 70% were capacitated.

Baseline information on the detailed motility and movement characteristics was determined with a computer-aided semen analyzer (CASA). The results demonstrated that there is a heterogenous population of spermatozoa in a semen sample and that some rams had spermatozoa that had a significantly larger head area than others. This result was supported in later studies by manual measurements of the width and length of heads of spermatozoa.

Semen was collected each month for 13 months from a group of six rams. A range of measurements of the semen was made including volume, colour and velocity and movement characteristics of spermatozoa as determined by CASA. These data were correlated with meteorological data. The quality of semen was significantly influenced by the mean daily maximum temperature and hours of bright sunshine with the months January to March being the times when ram semen was of poorest quality.

Samples of spermatozoa were collected from a range of sites in the reproductive tract of naturally-mated ewes at 3, 6 and 24 hours after mating. The spermatozoa were examined for detailed velocity and movement characteristics, capacitation status and dimensions of spermatozoa. A surprising result was the low and variable percentage (range 2%-22%) of motile spermatozoa in the uterus particularly at 3 and 6 hours after mating declining to a mean of 2.7% 24 hours after mating. No difference in the velocity and movement characteristics of spermatozoa between the anterior and posterior reproductive tract could be identified.

However, two important and interesting results were found. There was evidence that the ovary bearing the pre-ovulatory follicle or corpus haemorrhagicum influenced the distribution of spermatozoa as significantly more spermatozoa were found in the mid and anterior ipsilateral uterine horn and oviducts than the contralateral side 24 hours after mating. The same occurred 6 hours after mating except there was only a non significant trend for the uterine horns. The second important finding was that the spermatozoa in the oviducts had a significantly smaller head than elsewhere in the reproductive tract.

Analysis of the capacitation status of spermatozoa demonstrated that spermatozoa can undergo capacitation and the acrosome reaction in all sites of the reproductive tract and by 24 hours after mating most spermatozoa were capacitated and acrosome-reacted.

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AC	anterior cervix
ALH	amplitude of lateral head displacement
AMP	ampulla
ANOVA	analysis of variance
AR	acrosome reaction
AV	anterior vagina
AUL	anterior uterus-left
AUR	anterior uterus-right
BCF	beat cross frequency
BSA	bovine serum albumin
BU	body of uterus
C	Celsius
CAI	capacitated acrosome-intact
cAMP	cyclic adenosine monophosphate
CAR	capacitated acrosome-reacted
CASA	computer-aided semen analysis
CIDR	controlled internal drug release
CON	contralateral
CTC	chlortetracycline
G	gauge
g	gram
h	hour
Hepes	N-2-hydroxyethilpiperazine-N'-2ethanesulphonic acid
HSOF	hepes-buffered synthetic oviduct fluid
Hz	Hertz
IPS	ipsilateral
IU	international unit
IVOS	integrated visual optical system
LIN	linearity
LVS	low VAP cut off
LVV	low VSL cut off
NM	natural mating
MC	midcervix
mg	milligram
mM	millimol
MPA	medroxy progesterone acetate
MVV	medium VAP cut off
MUL	miduterus-left
MUR	miduterus-right
O	oestrus
ODB	oestradiol benzoate
P	progesterone
PBS	phosphate buffered saline
PGE	prostaglandin E
$PGF_{2\alpha}$	prostaglandin F2 alpha
pH	potential hydrogen
PMSG	pregnant mare serum gonadotrophin

s	synchronized
SEM	standard error of the mean
So	threshold straightness
STR	straightness
TALP	Tyrode's albumin-lactate-pyruvate
µg	microgram
µl	microlitre
µm	micrometer
µmsq	micrometer square
UTJ	utero-tubal junction
us	unsynchronized
VAP	average path velocity
VCL	curvilinear velocity
VSL	straight-line velocity
v/v	volume/volume

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