

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

Thesis submitted by

ISMAYA DipEd BSc Ir MSc

December 2003

**for the degree of Doctor of Philosophy in the
Australian Institute of Tropical Veterinary and Animal Science,
James Cook University,
Australia**

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institute of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Ismaya

December 2003

STATEMENT OF ACCESS TO THESIS

I, the undersigned, the author of this thesis, understand that the James Cook University will make it available for use within the University Library and, by microfilm or other photographic means, allow access to users in other approved libraries. All users consulting this thesis will have to sign the following statement:

In consulting this thesis I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author; and to make proper written acknowledgement for any assistance which I have obtained from it.

Beyond this, I do not wish to place any restriction on access to this thesis

Ismaya

December 2003

ELECTRONIC COPY

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to the James Cook University Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

Signature

Date

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 there 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 heterogeneous 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.

TABLE OF CONTENTS

	Page No.
Abstract	ii
Table of Contents	iv
List of Tables	vii
List of Figures	x
List of Abbreviations	xv
Acknowledgements	xvii
CHAPTER 1 GENERAL INTRODUCTION	1
1.1 Background	1
1.2 Working Hypothesis of this thesis	2
1.3 Aims and Objectives of this thesis	2
CHAPTER 2 REVIEW OF THE LITERATURE	4
2.1 Female Reproductive Tract	4
2.1.1 Vagina	4
2.1.2 Cervix	5
2.1.3 Uterus	7
2.1.4 Oviduct	7
2.2 Spermatozoa Reservoirs and the Life Span of Spermatozoa in the Female Reproductive Tract	8
2.2.1 Spermatozoa reservoir	8
2.2.2 Life-span of spermatozoa within the female reproductive tract	10
2.3 Transport of Spermatozoa in the Female Reproductive Tract	11
2.3.1 Transport of spermatozoa into and through the cervix	12
2.3.2 Transport of spermatozoa in the uterus and oviduct	13
2.4 Contractions of the Female Reproductive Tract and Spermatozoa Transport	14
2.5 Influence of Seminal Plasma and Hormones on Transport of Spermatozoa	16
2.5.1 Influence of seminal plasma	16
2.5.2 Hormonal influences	17
2.6 Distribution of Spermatozoa in the Female Reproductive Tract	18
2.7 Retrograde Loss of Spermatozoa	24
2.8 The Post-insemination Inflammatory Response and Loss of Spermatozoa by Phagocytosis	25
2.9 Interaction of Spermatozoa with Oviduct Epithelium	26
2.10 Capacitation	27
2.10.1 <i>In vivo</i> capacitation and the acrosome reaction	28
2.10.2 <i>In vitro</i> capacitation	30
2.11 Morphological and Motility Characteristics of Spermatozoa	31
2.12 Hyperactivation of Spermatozoa	33
2.13 Summary and Conclusion	35

CHAPTER 3	MATERIALS AND METHODS	36
3.1	Animals	36
	3.1.1 Rams	36
	3.1.2 Ewes	36
	3.1.3 Wethers	37
3.2	Ethics Approval	37
3.3	Preparation of Media	37
3.4	Collection of Ram Semen	37
3.5	Semen Analysis and Estimation of Spermatozoa Concentration	39
3.6	Determination of Motility and Velocity Parameters of Spermatozoa in Ram Semen	39
	3.6.1 CASA settings and definitions	39
3.7	Oestrus Synchronization and Mating	41
3.8	Collection of Samples from the Reproductive Tract of Ewes	41
	3.8.1 Description of the procedures for analysis of the samples	44
	3.8.2 Determination of motility characteristics of spermatozoa in CASA	44
	3.8.3 Preparation of samples for capacitation	45
	3.8.4 Determination of number of spermatozoa in samples	45
	3.8.5 Measurement of head and tail length of spermatozoa	45
3.9	Chlortetracycline assay for Capacitation	45
3.10	Statistical Analyses	50
CHAPTER 4	<i>IN VITRO</i> MOTILITY, LONGEVITY AND CAPACITATION STATUS OF MERINO RAM SPERMATOZOA	51
4.1	Introduction	51
4.2	Materials and Methods	53
	4.2.1 Animals	53
	4.2.2 Sperm preparation and analysis	53
	4.2.3 Statistical analyses	54
4.3	Results	54
	4.3.1 Characteristics of Merino ram semen	54
	4.3.2 Effect of incubation time on the motility and morphology characteristics of ram spermatozoa in TALP medium	55
	4.3.3 Distribution of ram spermatozoa into velocity and movement groups after incubation in TALP medium	64
	4.3.4 Distribution of spermatozoa into velocity and movement groups	64
	4.3.5 Comparison between TALP and HSOF medium on motility and velocity characteristics of ram spermatozoa <i>in vitro</i>	71
	4.3.6 Capacitation of spermatozoa after <i>in vitro</i> culture in HSOF medium	71
4.4	Discussion	76

CHAPTER 5	THE INFLUENCE OF CLIMATIC FACTORS AND THE VARIATION IN SEMEN PARAMETERS BETWEEN AND WITHIN MERINO RAMS AS DETERMINED BY COMPUTER-AIDED SEMEN ANALYSIS	78
5.1	Introduction	78
5.2	Materials and Methods	79
5.2.1	Climatic conditions	79
5.2.2	Animals	79
5.2.3	Semen analysis	79
5.2.4	Statistical analyses	79
5.3	Results	80
5.3.1	Effect of ram and month on the semen characteristics	80
5.3.2	Effect of month on the motility characteristics of spermatozoa	83
5.3.3	Effect of ram on the motility and morphology characteristics of spermatozoa	86
5.3.4	Correlation (r) between climatic conditions and semen characteristics of rams	86
5.4	Discussion	90
CHAPTER 6	TEMPORAL CHANGES IN MOTILITY CHARACTERISTICS, RECOVERY AND DIMENSIONS OF SPERMATOZOA AT VARIOUS SITES IN THE REPRODUCTIVE TRACT OF MERINO EWES	93
6.1	Introduction	93
6.2	Materials and Methods	94
6.2.1	Animals	94
6.2.2	Reaction time, mating and frequency of mating	95
6.2.3	Determination of motility characteristics and number of spermatozoa in the female reproductive tract	95
6.2.4	Measurement of the size of spermatozoa	95
6.2.5	Statistical analyses	96
6.3	Results	96
6.3.1	Recovery of spermatozoa from the reproductive tract of ewes	96
6.3.2	Effect of the side of the pre-ovulatory follicle or corpus haemorrhagicum on the number of spermatozoa recovered from the uterine horns and oviducts	97
6.3.3	Motility of spermatozoa recovered from the reproductive tract of ewes	97
6.3.4	Velocity and morphological features of spermatozoa recovered from the reproductive tract of ewes	102
6.3.5	Velocity of spermatozoa in the ipsilateral and contralateral anterior uterus and oviducts	107

6.3.6	The dimensions of spermatozoa in Merino ram semen	107
6.3.7	The dimensions of spermatozoa in the reproductive tract of ewes after natural mating	110
6.4	Discussion	115
CHAPTER 7	CAPACITATION STATUS OF SPERMATOZOA AT VARIOUS SITES IN THE REPRODUCTIVE TRACT OF MERINO EWES AFTER NATURAL MATING	120
7.1	Introduction	120
7.2	Materials and Methods	121
7.2.1	Animals	121
7.2.2	Identification of capacitation status	122
7.2.3	Statistical analysis	122
7.3	Results	123
7.3.1	<i>In vivo</i> capacitation status in the reproductive tract of ewes	123
7.4	Discussion	126
CHAPTER 8	GENERAL DISCUSSION	129
8.1	Scope of the Research Work	129
8.2	Future Research Directions	132
8.3	Conclusions	134
REFERENCES		135

LIST OF TABLES

	Page No.	
Table 2.1	The life-span of gametes in the female reproductive tract, representing the period during which they are able to achieve normal fertilization and subsequent cleavage (from Hunter, 1988).	10
Table 2.2	Distribution of spermatozoa in the female reproductive tract of farm animals.	20
Table 2.3	The percentage of motile spermatozoa in the reproductive tract of farm animals.	24
Table 2.4	Definitions of sperm kinematics measures (Davis and Siemers, 1995).	32
Table 3.1	Composition of modified Tyrode's albumin-lactate-pyruvate medium (TALP) and Hepes-synthetic oviduct fluid (HSOF) medium.	38
Table 4.1	The mean (\pm SEM) semen volume, motility of spermatozoa, semen colour and longevity of spermatozoa of the four rams in Group I.	55
Table 4.2	Effect of incubation time in TALP medium at 39 °C on the motility and morphological characteristics of ram spermatozoa (mean \pm SEM).	58
Table 4.3	Effect of dilution in either TALP or HSOF medium on the motility and velocity characteristics of ram spermatozoa <i>in vitro</i> (mean \pm SEM).	71
Table 5.1	Mean daily climatic data for the period June 2002 – July 2003.	80
Table 5.2	Mean (\pm SEM) values of the semen characteristics for each ram during the period of study.	81
Table 5.3	The mean (\pm SEM) values for semen characteristics of the six rams for each month during the study.	82
Table 5.4	Coefficient of variation (%) between and within rams in semen characteristics.	82
Table 5.5	Effect of ram (mean \pm SEM) on the elongation and head area of spermatozoa.	86

Table 5.6	The percentage of motile (M), progressively motile (P) and rapidly motile (R) spermatozoa of each ram and their coefficient of variation (CV).	88
Table 5.7	Correlation (r) between climatic conditions and semen characteristics of rams.	90
Table 5.8	Correlation (r) between climatic conditions and motility characteristics of ram spermatozoa.	90
Table 6.1	The number of ewes from which samples of spermatozoa were collected from different sites of the reproductive tract.	94
Table 6.2	The performance of Merino rams during the one hour mating period (mean \pm SEM).	95
Table 6.3	The relationship between the recovery (mean \pm SEM) of spermatozoa from the oviducts and uterus and the side of the pre-ovulatory follicle or corpus haemorrhagicum.	100
Table 6.4	Mean (\pm SEM) elongation and head area of spermatozoa at different sites of the reproductive tract of ewes.	103
Table 6.5	The mean (\pm SEM) dimensions of Merino ram spermatozoa.	107
Table 6.6	The mean (\pm SEM) dimensions of ram spermatozoa derived from ewes 6 hours after mating.	111
Table 6.7	The mean (\pm SEM) dimensions of ram spermatozoa derived from ewes 24 hours after mating.	113
Table 7.1	The number of ewes from which samples of spermatozoa were collected to determine the capacitation status.	121
Table 7.2	The mean (\pm SEM) number and range of spermatozoa examined for capacitation status at different sites in the reproductive tract of ewes at 3, 6 and 24 hours after mating.	122
Table 7.3	The percentage of capacitated spermatozoa in the uterine horn and oviducts ipsilateral and contralateral to the ovary bearing the preovulatory follicle 6 hours after mating.	124

LIST OF FIGURES

		Page No.
Figure 2.1	Schematic diagram of spermatozoon kinematic measures by CASA. (modified from Davis and Siemers, 1995).	33
Figure 3.1	Photographs to illustrate an androgenized wether wearing a marker harness (white arrow) and apron (black arrow) used in the detection of oestrus in ewes. The ewe in figure A is not in oestrus and the ewe in figure B is in oestrus.	42
Figure 3.2	Chlortetracycline fluorescence staining patterns for uncapacitated spermatozoa. A bright band of yellow fluorescence was present on the head and on the mid-piece (arrows) of the spermatozoon.	47
Figure 3.3	Chlortetracycline fluorescence staining patterns for capacitated acrosome-intact spermatozoa. The post acrosomal region was non-fluorescent (arrow).	48
Figure 3.4	Chlortetracycline fluorescence staining patterns for capacitated acrosome-reacted spermatozoa. A bright band of fluorescence was present only on the mid-piece (arrow) and the head of the spermatozoa was non-fluorescent.	49
Figure 4.1	Effects of incubation time and rams (R1, R3, R5, R6) on the motility of spermatozoa in undiluted semen (Figure A) at room temperature (23 °C) and semen diluted (Figure B) in HSOF medium at 39 °C. The results are the mean (\pm SEM) of three replicates for each ram.	56
Figure 4.2	Relationship between incubation time and dilution rate of semen (1:25, 1:20, 1:15, 1:10) in HSOF medium on the motility of spermatozoa. The results are the mean (\pm SEM) of three replicates for each ram (R1, R3, R5, R6).	57
Figure 4.3	Effect of incubation time in TALP medium at 39 °C on the percentage of motile (Figure A), progressively motile (Figure B) and rapidly motile (Figure C) spermatozoa from rams (R13, R12, R16, R9) (mean \pm SEM). Different letters above bars indicate significant differences ($P \leq 0.05$) within each incubation time.	60

- Figure 4.4** Effect of incubation in TALP medium at 39 °C on the average path velocity (Figure A), straight-line velocity (Figure B) and curvilinear velocity (Figure C) of spermatozoa from rams (R13, R12, R16, R9) (mean ± SEM). Different letters above bars indicate significant differences ($P \leq 0.05$) within each incubation time. 61
- Figure 4.5** Effect of incubation in TALP medium at 39 °C on the amplitude of lateral head displacement (Figure A), beat cross frequency (Figure B) and straightness (Figure C) of spermatozoa from rams (R13,R12,R16,R9) (mean ± SEM). Different letters above bars indicate significant differences ($P \leq 0.05$) within each incubation time. 62
- Figure 4.6** Effect of incubation in TALP medium on linearity (Figure A), elongation (Figure B) and head area (Figure C) of spermatozoa from rams (R13, R12, R16, R9) (mean ± SEM). Different letters above bars indicate significant differences ($P \leq 0.05$) within each incubation time. 63
- Figure 4.7** Distribution of spermatozoa (mean ± SEM) into velocity groups for average path velocity (Figure A) and straight-line velocity (Figure B) after incubation in TALP medium for 0, 30, 60, 90, and 120 minutes. Different letters above bars indicate significant differences ($P \leq 0.05$) within each velocity group. 66
- Figure 4.8** Distribution of spermatozoa (mean ± SEM) into velocity groups for curvilinear velocity (Figure A) and linearity (Figure B) after incubation in TALP medium for 0, 30, 60, 90 and 120 minutes. Different letters above bars indicate significant differences ($P \leq 0.05$) within each group. 67
- Figure 4.9** Distribution of spermatozoa (mean ± SEM) into groups for amplitude of lateral head displacement (Figure A) and beat cross frequency (Figure B) after incubation in TALP medium for 0, 30, 60, 90 and 120 minutes. Different letters above bars indicate significant differences ($P \leq 0.05$) within each group. 68
- Figure 4.10** Distribution of spermatozoa (mean ± SEM) into velocity groups for the average path velocity (VAP), straight-line velocity (VSL) and curvilinear velocity (VCL) (Figure A) and straightness (STR), linearity (LIN) and elongation (ELO) (Figure B) for rams (R9, R12, R13, R16). These were three semen collections for each ram and the semen was diluted in TALP medium at 39 °C 69

- Figure 4.11** Distribution of spermatozoa (mean \pm SEM) into groups for amplitude of lateral head displacement (ALH) (Figure A) and beat cross frequency (BCF) (Figure B) for rams (R9, R12, R13, R16). There were three semen collections from each ram and the semen was diluted in TALP medium at 39 °C. 70
- Figure 4.12** Relationship between uncapacitated (UC), capacitated acrosome-intact (CAI) and capacitated acrosome-reacted (CAR) ram spermatozoa during *in vitro* culture in HSOF medium. The results are the mean (\pm SEM) for four rams (R1, R3, R5, R6) with three replicates for each ram. 73
- Figure 4.13** Influence of *in vitro* incubation time and dilution rate (1:25, 1:20, 1:15, 1:10) of semen on the percentage of spermatozoa that had undergone capacitation but were acrosome-intact (Figure A) and that had undergone the acrosome reaction (Figure B). The results are the mean (\pm SEM) of three replicates for each ram (R1, R3, R5, R6). 74
- Figure 4.14** The mean (\pm SEM) percentage of capacitated acrosome-intact (Figure A) and capacitated acrosome-reacted (Figure B) spermatozoa from four rams (R1, R3, R5, R6) during *in vitro* culture in HSOF medium. *Indicates a significant different ($P \leq 0.05$) between R5 and the other rams. 75
- Figure 5.1** The effect of month on the percentage of motile and progressive, rapid, medium and slowly motile ram spermatozoa. The results are the mean (\pm SEM) of the data from six rams. 84
- Figure 5.2** Effect of month on the average path velocity (VAP), straight-line velocity (STR) and curvilinear velocity (VCL) of ram spermatozoa. The results are the mean (\pm SEM) of the data from six rams. 84
- Figure 5.3** Effect of month on the straightness (STR), linearity (LIN) and elongation (ELO) of ram spermatozoa. The results are the mean (\pm SEM) of the data from six rams. 85
- Figure 5.4** Effect of month on the amplitude of lateral head displacement (ALH), beat cross frequency (BCF) and head area (AREA) of ram spermatozoa. The results are the mean (\pm SEM) of six rams. 85
- Figure 5.5** Effect of ram on the percentage of motile and progressive, rapid, medium and slowly motile spermatozoa (Figure A), average path velocity (VAP), straight-line velocity (VSL) and curvilinear velocity (VCL) (Figure B), straightness (STR), linearity (LIN) and elongation (ELO)(Figure C) of spermatozoa. The results are the mean (\pm SEM) of 14 replicates for each ram. Different letters above bars indicate significant differences within each group. 89

- Figure 6.1** The mean (\pm SEM) concentration of spermatozoa from the anterior vagina (Figure A) of ewes at 1, 3, 6 and 24 hours after mating and number of spermatozoa recovered from the cervix, uterus and oviduct (Figure B) of ewes at 3, 6 and 24 hours after mating. Different letters above bars indicate significant differences ($P \leq 0.05$) between the time after mating (Figure A) and within that part of the female reproductive tract of ewes (Figure B). AV = anterior vagina, PC, MC, AC = posterior, mid, anterior cervix; BU = body of uterus; MUR, AUR = mid, anterior uterus-right; MUL, AUL = mid, anterior uterus-left; RI, LI = right, left isthmus; RA, LA = right, left ampulla. 99
- Figure 6.2** The mean (\pm SEM) percentage of motile (Figure A), progressively motile (Figure B) and rapidly motile (Figure C) spermatozoa in the anterior vagina, cervix, uterus and oviducts of ewes at 1, 3, 6 and 24 hours after mating. Different letters above bars indicate significant differences ($P \leq 0.05$) within that part of the female reproductive tract. AV = anterior vagina, PC, MC, AC = posterior, mid, anterior cervix; BU = body of uterus; MUR, AUR = mid, anterior uterus -right; MUL, AUL = mid, anterior uterus-left; RI, LI = right, left isthmus; RA, LA = right, left ampulla. 101
- Figure 6.3** The mean (\pm SEM) average path velocity (Figure A), straight-line velocity (Figure B) and curvilinear velocity (Figure C) of spermatozoa in the anterior vagina, cervix, uterus and oviducts of ewes at 1, 3, 6 and 24 hours after mating. Different letters above bars indicate significant differences ($P \leq 0.05$) within that part of the female reproductive tract. AV = anterior vagina, PC, MC, AC = posterior, mid, anterior cervix; BU = body of uterus; MUR, AUR = mid, anterior uterus-right; MUL, AUL = mid, anterior uterus-left; RI, LI = right, left isthmus; RA, LA = right, left ampulla. 104
- Figure 6.4** The mean (\pm SEM) beat cross frequency (Figure A), amplitude of lateral head displacement (Figure B) and straightness (Figure C) of spermatozoa in the anterior vagina, cervix, uterus and oviducts of ewes at 1, 3, 6 and 24 hours after mating. Different letters above bars indicate significant differences ($P \leq 0.05$) within that part of the female reproductive tract. AV = anterior vagina, PC, MC, AC = posterior, mid, anterior cervix; BU = body of uterus; MUR, AUR = mid, anterior uterus-right; MUL, AUL = mid, anterior uterus-left; RI, LI = right, left isthmus; RA, LA = right, left ampulla. 105

- Figure 6.5** The mean (\pm SEM) linearity (Figure A), elongation (Figure B) and head area (Figure C) of spermatozoa in the anterior vagina, cervix, uterus and oviducts of ewes at 1, 3, 6 and 24 hours after mating. Different letters above bars indicate significant differences ($P \leq 0.05$) within that part of the female reproductive tract. AV = anterior vagina, PC, MC, AC = posterior, mid, anterior cervix; BU = body of uterus; MUR, AUR = mid, anterior uterus-right; MUL, AUL = mid, anterior uterus-left; RI, LI = right, left isthmus; RA, LA = right, left ampulla. 106
- Figure 6.6** The mean (\pm SEM) average path velocity (Figure A), straight-line velocity (Figure B) and curvilinear velocity (Figure C) of spermatozoa in the ipsilateral and contralateral uterus and oviducts of five ewes six hours after mating. Different letters above bars indicate significant differences ($P \leq 0.05$) within that part of the female reproductive tract. MU = mid-uterus; AU = anterior uterus; IST = isthmus; AMP = ampulla; IPS = ipsilateral; CON = contralateral. 108
- Figure 6.7** The mean (\pm SEM) average path velocity (Figure A), straight-line velocity (Figure B) and curvilinear velocity (Figure C) of spermatozoa in the ipsilateral and contralateral uterus and oviducts of five ewes 24 hours after mating. Different letters above bars indicate significant differences ($P \leq 0.05$) within that part of the female reproductive tract. MU = mid-uterus; AU = anterior uterus; IST = isthmus; AMP = ampulla; IPS = ipsilateral; CON = contralateral. 109
- Figure 7.1** The mean (\pm SEM) percentage of uncapacitated (Figure A), capacitated acrosome-intact (Figure B) and capacitated acrosome-reacted (Figure C) spermatozoa in the anterior vagina, cervix, uterus and oviducts of ewes at 3, 6 and 24 hours after mating. Different letters above bars indicate significant differences ($P \leq 0.05$) within that part of the reproductive tract. Capacitation status in the oviducts was only determined at 6 hours and 24 hours after mating. AV = anterior vagina; PC, MC, AC = posterior, mid, anterior cervix; BU = body of uterus; MUR, AUR = mid, anterior uterus-right; MUL, AUL = mid, anterior uterus-left; RI, LI = right, left isthmus; RA, LA = right, left ampulla. 125

LIST OF ABBREVIATIONS

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-hydroxyethylpiperazine-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 _{2α}	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

ACKNOWLEDGMENTS

I would like to take this opportunity to express my deep gratitude and sincere appreciation to Professor Phillip Summers, my supervisor, for his invaluable guidance, continuous supervision, constructive criticism, encouragement, understanding and helpful advice throughout the course of this study. His assistance with the preparation of this thesis is also much appreciated. My gratitude and appreciation are also extended to Associate Professor Lee Fitzpatrick, my co-supervisor, for his advice.

I am grateful to the Head, School of Biomedical Sciences and the Director Australian Institute of Tropical Veterinary and Animal Science for all facilities provided during the course of this work. I am also grateful to the Office Manager, Lorraine Henderson and her staff for their assistance and Denis Hankanson from the Bureau of Meteorology in Townsville for access to meteorological data.

Sincere thanks are due to Laurie Reilly for his assistance and technical advice. Thanks are also extended to Scott Blyth and Christopher Coleman for their assistance in management of animals, collection of samples and instructions on the use of the computer-aided semen analyzer.

Sincere thanks are also due to the Government of the Republic of Indonesia and the Gadjah Mada University, Yogyakarta, for granting me study leave. Moreover, sincere thanks are due to the World Bank via the Quality of Undergraduate Education Project (QUEP), Department of Animal Production, Faculty of Animal Science, Gadjah Mada University, for financial support. Special thanks are due to Professor Wartomo, Executive Director of QUEP and his staff.

Lastly, I would like to dedicate this thesis to my parents, my wife Supartini, my son Ikhsan Wakhida and my daughter Nadia Tattakuna. Their understanding and patience has enabled me to complete this work.