

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

A STUDY OF THE EFFECTS
OF POST-MATING PROGESTERONE SUPPLEMENTATION
ON THE REPRODUCTIVE PERFORMANCE IN THE EWE

A thesis presented in partial fulfilment
of the requirements for the degree
of Master of Agricultural Science
in Animal Science
at Massey University.

PETER ALLAN WALSH

1989



ABSTRACT

The aim of this study was to investigate the effects of post-mating progesterone supplementation, in the form of CIDRs, on the reproductive performance in recently mated ewes. The effect of two nutrition treatments were also examined by feeding two different pasture allowances to these ewes during a two week period immediately following a synchronized mating.

Two hundred and thirty four Border Leicester X Romney first cross ewes, comprised of 130 mixed-age ewes (3-8 years) and 104 two-tooth (maiden) ewes were flushed on increasing pasture allowances prior to joining. These ewes were naturally mated at a synchronized oestrus following a 13 day CIDR treatment period. During the three days following CIDR withdrawal, 88.0% of the ewes were mated. The mixed-age ewes came into oestrus significantly earlier than the two-tooth ewes ($P < 0.001$). There was a significant linear relationship between liveweight and onset of oestrus ($P < 0.05$), with the heavier two-tooth ewes coming on oestrus earlier than the lighter two-tooth ewes. This was not apparent in the mixed-age ewes.

On Day 2 following mating, ewes were randomly divided into either the high or low pasture allowance levels. Pasture allowance levels were monitored using an Ellinbank Pasture Meter (EPM). Levels of feed intake were estimated for a random sample of 20 ewes both before and after mating through the use of intraruminal chromium releasing devices (CRDs). Sward heights (representing quantity), botanical compositions and in vitro digestibilities (representing quality) were recorded for both the flushing and the post-mating period. This information led to the estimation of voluntary herbage intakes during the flushing period (Period I) of 1.2 M, while the intake levels of the ewes following mating (Period II) were calculated to be approximately 1.6 M and 1.0 M for the high and low pasture allowance levels, respectively.

Liveweight changes during Period I indicated that the ewes gained weight. During the differential feeding (Period II) the high fed ewes in Period II tended to continue to gain body weight (4.1% of initial liveweight at mating), while ewes on the low feeding level lost weight over the same period (2.8% of initial liveweight).

Ovulation rates were determined on Days 4 and 5 after mating. The mixed-age ewes had a higher ovulation rate (1.87 ± 0.04) than the two-tooth ewes (1.55 ± 0.06). Both age ($P < 0.001$) and liveweight ($P < 0.01$) of the ewes had significant effects on the ovulation rates.

Post-mating CIDR treatment was randomly administered to half the ewes in each of the two differential pasture allowance levels during Days 8-15 following mating. Forty five animals representing both age groups, feeding levels, ovulation rates and CIDR treatment, chosen at random, were blood sampled over the luteal phase of the oestrous cycle (Days 9 to 14 after mating). The blood samples were collected twice daily both at AM and PM over a six day period. The blood samples were then assayed on a pooled individual basis for progesterone concentration determination.

Analysis of the pooled progesterone data revealed that there were no statistically significant differences between two-tooth versus mixed-age ewes, nor pregnant versus non-pregnant ewes, with respect to peripheral plasma progesterone levels ($P > 0.10$). Progesterone levels however were found to differ significantly ($P < 0.001$) between ewes treated with CIDRs and unsupplemented ewes (1.88 ± 0.10 versus 1.34 ± 0.10 ng/ml), as well as between ewes having a single ovulation and those having twin ovulations (1.49 ± 0.10 versus 1.73 ± 0.09 ng/ml). When the effect of liveweight was corrected for, there was also a significant difference ($P < 0.05$) in pooled progesterone levels between ewes on the high (1.49 ± 0.11 ng/ml) and low pasture allowance levels (1.73 ± 0.09 ng/ml). A significant relationship ($P < 0.001$) was found between ewe liveweight and pooled progesterone concentrations for the two post-mating nutritional levels. The ewes on the high feed level had a constant relationship, while the low fed ewes had a positive relationship between liveweight and pooled progesterone concentrations.

Reproductive performance, as measured by the pregnancy rate, embryo survival rate and lambing percentage, all expressed to the first service, were not significantly affected by either age, feed level or CIDR treatment. The high feeding level (1.6 M) and CIDR treatment were found to slightly improve reproductive performance. The effect of age also appeared to slightly favour the two-tooth ewes

compared to the mixed-age ewes, although this was not found to be statistically significant ($P > 0.10$). There was however a significant effect ($P < 0.05$) of ovulation rate on the resulting pregnancy rate (50.8% for a single CL versus 68.3% for ewes having multiple CLs). The lambing percentage was also significantly affected by the ovulation rate of the ewes ($P < 0.001$), with single ovulating ewes having a lambing percentage of $56.0 \pm 11.1\%$, while those with multiple ovulations had a lambing percentage to the first service of $115.3 \pm 6.7\%$. The mixed-age ewes also had a slightly better percentage of multiple births to the first mating (72.4%) than did the two-tooth ewes (61.6%), although this difference was not statistically significant ($P > 0.10$).

There was a significant interaction between the post-mating feeding level and the CIDR treatment ($P < 0.05$) in the pregnancy and embryo survival rates, as well as for the lambing percentage to the first mating. The post-mating CIDR supplementation had a beneficial effect for the ewes on the 1.0 M pasture allowance level, while the same CIDR treatment for the ewes on the 1.6 M level either had no effect or reduced reproductive performance slightly. The mechanism responsible for the interaction between CIDR treatment and feeding level is not known. It is possible that it is a somewhat more complex mechanism than simply a luteal deficiency of progesterone caused by increases in the nutritional level is responsible. An insensitivity to progesterone may be involved, as the low fed ewe tended to respond to post-mating CIDR treatment, while the high fed ewes did not.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks and appreciation to my supervisor Dr. M.F. McDonald for his continued interest, guidance, support and advice during the experimental work and the preparation of this manuscript.

Special gratitude is extended to Mr. Hu Gao of the People's Republic of China who was of invaluable assistance throughout the entire duration that the field work was conducted. I also appreciate very much the invaluable help and encouragement and the many rewarding discussions that I had with Dr. S.N. McCutcheon during my studies here at Massey University.

Special thanks is extended to Mr. W.J. Parker for sharing his knowledge and experience and offering assistance with the nutritional aspects of this trial. Gratitude is also extended to: Dr. K. Lapwood and colleagues of the Department of Physiology and Anatomy for the advice and assistance with the progesterone assay; Mr. J.M. Rendel and Dr. D.J. Garrick for the helpful suggestions with the statistical analysis; the technical staff of Department of Animal Sciences physiology and nutrition labs for the help with the processing of samples; and the Department of Animal Science technicians and farm staff for the assistance and care given to the animals during the experiment.

The financial support in part from the Johannes August Anderson Scholarship is also acknowledged.

Special thanks is extended to Ms. J.L Wickham for the critical proof reading of this manuscript and her many helpful suggestions.

Thanks is also extended to all the post-graduate students and staff, both past and present, of the Department of Animal Science for their friendship, support and assistance, as well as sharing their knowledge and experiences with me.

Finally, a very special thanks is extended to my parents and family in Canada for their continued love, faith, encouragement and moral support for me during my studies here at Massey University.

TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	v
LIST OF TABLES.....	ix
LIST OF FIGURES.....	xi
LIST OF ABBREVIATIONS.....	xii
 Chapter I: Introduction.....	 1
 Chapter II: Literature Review.....	 3
1 The importance of a high reproductive performance.....	3
2 Determinants of reproductive performance.....	4
3 The impact of prenatal mortality.....	6
4 Factors affecting embryonic mortality.....	8
4.1 Embryo vigour.....	9
4.2 Maternal environment.....	10
4.3 Environmental conditions.....	12
5 Physiological control of implantation and embryonic survival.....	15
5.1 The oestrous cycle.....	15
5.2 Processes involved in implantation.....	16
5.2.1 Maternal recognition of pregnancy.....	16
5.2.2 Synchrony.....	17
5.3 Hormonal requirements for the establishment of pregnancy.....	19
5.4 Relationship between progesterone levels and conception rates.....	 20
6 Factors affecting progesterone concentrations.....	22
6.1 Number of corpora lutea (ovulation rate).....	22
6.2 Season.....	23
6.3 Stress.....	24
6.4 Nutrition.....	25
7 Physiological basis for nutritional effects on progesterone levels.....	 27
8 Effects of post-mating progesterone supplementation on early embryonic mortality.....	 28
8.1 Direct effects of progesterone supplementation on embryonic survival.....	 28
8.2 The indirect effects of luteal stimulation on embryonic survival.....	 33
8.2.1 HCG treatment.....	33

8.2.2 GnRH treatment.....	36
8.2.3 Melatonin treatment.....	38
9 Purpose and scope of the investigation.....	39
 Chapter III: Material and Methods.....	 41
1 Experimental design.....	42
2 Animals.....	43
2.1 Weighing of animals.....	43
2.2 Induction of oestrus and mating.....	43
3 Pasture characteristics and herbage mass.....	44
3.1 Experimental site.....	44
3.2 Determination of pasture cover.....	45
3.3 Herbage pasture composition and dry matter determination.....	45
3.4 Pasture rotation.....	45
4 Measurements of feed intakes.....	47
4.1 Estimation of voluntary intake.....	47
4.2 Sampling of the grazed sward for <u>in vitro</u> digestibility determinations.....	48
4.3 Determinations on <u>in vitro</u> digestibility.....	49
4.4 Faecal chromium analysis.....	49
4.5 Sheep intake estimation.....	51
5 Progesterone concentration.....	51
5.1 Blood sampling.....	51
5.2 Determination of progesterone levels.....	52
5.3 Progesterone assay.....	53
6 Reproductive information.....	53
6.1 Determination of ovulation rates.....	53
6.2 Pregnancy status.....	54
6.2.1 Returns to oestrus.....	54
6.2.2 Ultrasonic scanning for pregnancy determination.....	55
6.2.3 Lambing information.....	55
7 Statistical information.....	55
7.1 Analysis of variance (ANOVA).....	56
7.2 Repeated measures analysis.....	57
7.3 Discrete variable analysis.....	58
7.4 Analysis of frequency.....	58
7.5 Levels of statistical significance.....	58

Chapter IV: Results.....	59
1 Pasture assessment.....	59
2 Voluntary herbage intake.....	60
2.1 Liveweight of CRD ewes.....	62
3 Liveweights of all ewes.....	64
4 Synchrony in onset oestrus.....	66
5 Ovulation rate.....	69
6 Progesterone concentration.....	70
6.1 Levels from Days 9 to 14.....	70
6.2 Pooled progesterone concentration.....	73
7 Pregnancy rate.....	76
8 Embryo survival (lambs born per ovum shed and lambing percentage).....	78
9 Other reproductive traits.....	80
 Chapter V: General Discussion and Conclusions.....	81
1 Voluntary herbage intakes.....	81
2 Liveweight changes.....	84
3 Incidence of oestrus.....	86
4 Ovulation rate (OR).....	87
5 Progesterone concentrations.....	87
5.1 Daily progesterone concentrations.....	87
5.2 Pooling of blood samples.....	89
5.3 Age effects on progesterone concentrations.....	89
5.4 Effect of CL number on progesterone concentration.....	90
5.5 Nutrition and progesterone concentration.....	90
5.6 Progesterone and liveweight interaction.....	91
5.7 Exogenous CIDR treatment on progesterone concentrations.....	92
6 Reproductive performance.....	92
6.1 Age.....	92
6.2 Ovulation rate (OR).....	93
6.3 Feeding level.....	94
6.4 CIDR treatment.....	96
6.5 CIDR x feed level interaction.....	97
7 Further research studies.....	99
8 Conclusions.....	101
 Appendix.....	104
 References.....	108

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1: Summary of studies involving progesterone supplementation in cattle and their effect on pregnancy rate.....	29
2: Summary of studies investigating post-mating progesterone supplementation in sheep.....	31
3: Summary of studies involving human chorionic gonadotrophin (hCG) treatment in cattle and its effect on pregnancy rates....	34
4: Summary of studies involving the effects of a single injection of gonadotrophin releasing hormone (GnRH) on pregnancy rate in cows.....	36
5: Effects of a single injection of gonadotrophin releasing hormone (GnRH) on lambing performance in sheep.....	37
6: Paddock areas, grazing treatments and mean pasture heights.....	44
7: Pasture rotation used for the post-mating blood sampling and faecal collection during P-2 (Days 9-14).....	46
8: Schedule used for the daily collection of faecal collections and blood sampling during P-2.....	48
9: Descriptive assessment of pasture cover, height, composition and <u>in vitro</u> digestibilities for the three different pasture allowance levels.....	59
10: Predicted faecal outputs and voluntary herbage intakes of ewes during the two feeding periods.....	61
11: Percentage (and numbers) of ewes in each age group showing oestrus at each observation following CIDR removal.....	67
12: Effect of age, feeding level, CIDR treatment, ovulation rate and pregnancy status on pooled progesterone concentrations.....	73
13: Effect of age, feeding level, CIDR treatment, OR and significant interactions on the average pregnancy rate and the average pregnancy rate relative to the number of ovum shed.....	77
14: Effect of age, feeding level, CIDR treatment, OR and significant interactions on the embryo survival (represented by average percentage of lambs born per ovum shed and the lambing percentage) and the percentage of multiple births to the first mating.....	78

15: Effect of age, feeding level, CIDR treatment, cycle and number of lambs born on the mean birth weight, total litter weight, gestation length, lamb sex ratio and percent of multiple births.....	105
---	-----

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1: Embryo survival in sheep.....	9
2: General experimental plan and calendar of events.....	42
3: Liveweight changes for the 30 CID ewes during the experimental period (for the two-tooth (2T) and mixed-age (MA) ewes), as well as the significance levels.....	63
4: Liveweight changes for the total flock (n=206) during the experimental period (for the two tooth (2T) and mixed-age (MA) ewes), as well as the significance levels.....	65
5: The daily and cumulative incidence of oestrus following CIDR withdrawal (for the two age groups of ewes).....	68
6: Progesterone concentrations for pregnant versus non-pregnant ewes during Days 9-14 of the oestrous cycle (corrected for common ovulation rate and feed level).....	71
7: Progesterone concentrations for single versus twin ovulating ewes during Days 9-14 of the oestrous cycle (corrected for common pregnancy status and feed level).....	72
8: Scatter plot of the individual data points showing the relationship between the pooled plasma progesterone concentrations and liveweight for the two post-mating feeding levels.....	75

LIST OF ABBREVIATIONS

1	Single
≥2	Multiple
AI	Artificial insemination
BPR	Blood production rate
CIDR	Controlled internal drug releaser
CL	Corpus lutea
cm	Centimeter
-C	No CIDR treatment
+C	CIDR treatment
°C	Degree Celcius
CO ₂	Carbon dioxide
CPM	Counts per minute
Cr	Chromium
CRD	Controlled releasing device
CV	Coefficient of variation
d	Day
D	Day of oestrous cycle
DM	Dry matter
DMD	Dry matter digestibility
DOMD	Digestibility of the organic matter in the dry matter
DOMI	Digestible organic matter intake
EPM	Ellinbank pasture meter
FO	Faecal output
FSH	Follicle-stimulating hormone
g	Gram
GnRH	Gonadotrophin releasing hormone
H	High feeding or pasture allowance level
ha	Hectare
hCG	Human chorionic gonadotrophin
IM	Intra-muscular
IU	International units
kg	Kilogram
km	Kilometer
L	Low feeding or pasture allowance level
LH	Luteinizing hormone
LSM	Least squares mean
M	Maintenance
MA (>2)	Mixed age ewes

MCR	Metabolic clearance rate
ME	Metabolizable energy
mg	Milligram
MJME	Mega joules of metabolizable energy
ml	Milliliter
m ²	Square meters
n	Number
NA	Non-applicable
ng	Nanogram
NP	Non-pregnant
OM	Organic matter
OMD	Organic matter digestibility
OR	Ovulation rate
P	Pregnant
P-1	Period I
P-2	Period II
PGF 2 α	Prostaglandin F 2 α
PMSG	Pregnant mare serum gonadotrophin
ppm	Parts per million
SC	Sub-cutaneous
SEM	Standard error of the mean
2T (2)	Two-tooth ewes
ug	Micrograms
ul	Microliter
wt	Weight