Endocrine and Genetic Control of Seasonal Breeding in Sheep

A thesis presented in partial fulfilment of

the requirements for the degree of

Doctor of Philosophy

in Animal Science

at Massey University

ZHENZHONG XU

1991

PREFACE

The contents of this thesis represent original work conducted by the author under the supervision of Associate Professor M.F. McDonald, Professor S.N. McCutcheon and Dr. H.T. Blair, Department of Animal Science, Massey University.

Publications arising from this thesis are as follows:

1. Harris, P.M., Xu, Z.Z., Blair, H.T., Dellow, D.W., McCutcheon, S.N. & Cockrem, J., 1989. The effect of exogenous melatonin, administered in summer, on wool growth and testis diameter of Romneys. *Proceedings of the New Zealand Society of Animal Production*, **49**: 35-38. [Based in part on Chapter III]

2. Xu, Z.Z., McDonald, M.F., McCutcheon, S.N. & Blair, H.T., 1991. Seasonal variation in testis size, gonadotrophin secretion and pituitary responsiveness to GnRH in rams of two breeds differing in time of onset of the breeding season. *Animal Reproduction Science*, (In press). [Based on Chapter IV]

3. Xu, Z.Z., McDonald, M.F., McCutcheon, S.N. & Blair, H.T., 1991. Effects of season and testosterone treatment on gonadotrophin secretion and pituitary responsiveness to gonadotrophin releasing hormone (GnRH) in castrated Romney and Poll Dorset rams. *Journal of Reproduction and Fertility*, (In press). [Based on Chapter VII]

ABSTRACT

Xu, Z.Z., 1991. *Endocrine and Genetic Control of Seasonal Breeding in Sheep*. PhD thesis, Massey University, Palmerston North, New Zealand. 117pp.

A series of related experiments were conducted to investigate genetic variation in the pattern of seasonal changes in testis size, gonadotrophin secretion and pituitary responsiveness to an intravenous gonadotrophin releasing hormone (GnRH) challenge in rams during the transitional period from the nonbreeding to the breeding season, with the objective of identifying potential genetic markers in the ram for date of onset of the breeding season in the ewe.

Because the accurate measurement of changes in testis diameter was fundamental to the assessment of seasonality in rams used in the research programme, two preliminary trials were conducted to measure the reliability of this technique. In the first trial, testis diameters of 18 Romney rams were measured twice 1 h apart using a pair of dial calipers. Repeatability of measurements obtained from this trial was 0.90±0.05 (mean±SEM). In the second trial, testis diameters of 24 Southdown rams were measured before and after the animals were slaughtered. Pearson correlation coefficients between the live measurements and the diameter and weight of the dissected testes were 0.91 and 0.90 respectively ($P < 0.001$). An opportunity also arose to investigate the effects of continuous melatonin treatment for five weeks in early summer on testis growth in rams from the Massey University fleeceweight-selected (FW) and control (C) lines. The melatonin treatment significantly ($P < 0.001$) altered the pattern of seasonal variation in testis size. Compared with that of the untreated animals, testis growth of the melatonin-treated animals was significantly stimulated during the treatment period, but significantly depressed after termination of the treatment. The testis diameter of FW rams was significantly greater than that of C rams as the breeding season approached. There were indications that the FW rams were less responsive to melatonin treatment than the C rams. This trial also confirmed that seasonal changes in testis diameter could be detected using caliper measurements made on the live animals.

In the first major experiment, differences between rams (aged 2 years) of breeds with short (Romney) and long (Poll Dorset) breeding seasons in the pattern of seasonal variation in testis size, gonadotrophin secretion and pituitary responsiveness to an exogenous GnRH challenge were compared during the transitional period from the

nonbreeding to the breeding season. Testis size of rams of both breeds varied significantly during the trial period, with a significant breed difference in the timing and magnitude of this variation. Increases in testis size occurred earlier, but the magnitude of seasonal variation was smaller, in Poll Dorset rams. Overall, mean LH concentration was higher ($P < 0.05$) in Romney rams due mainly to a difference ($P < 0.001$) in the frequency of LH pulses. There was also an effect of sampling time ($P < 0.01$) and a significant ($P < 0.05$) breed x time interaction in LH pulse frequency. Mean FSH concentrations exhibited significant ($P < 0.01$) variation with sampling time and the increase in FSH concentrations occurred earlier ($P < 0.10$) in Poll Dorset rams. There was an effect of sampling time on both the peak ($P < 0.01$) and the total ($P < 0.05$) LH responses to the GnRH challenge, but no significant effects of breed or breed x time interactions were detected.

The between-breed differences in the pattern of seasonal variation in gonadotrophin secretion were shown to be due to breed differences in sensitivity to both the steroid-dependent and steroid-independent effects of season. In castrated Romney and Poll Dorset rams, depression by testosterone treatment of LH pulse frequency, basal and mean LH concentrations, mean FSH concentration and peak and total LH responses to exogenous GnRH was greater ($P < 0.01$) during the nonbreeding season than during the breeding season. Poll Dorset rams were less sensitive to testosterone treatment than Romney rams ($P < 0.05$). In rams not receiving testosterone treatment, LH pulse frequency was lower ($P < 0.05$) during the nonbreeding season than during the breeding season in the Romneys (15.8±0.9 vs. 12.0±0.4 pulses/8h), but not in the Poll Dorsets (13.6±1.2 vs. 12.8±0.8 pulses/8h).

In another experiment, the magnitude of differences between rams (aged 4 years) of the Romney breed in the pattern of seasonal variation in testis size, gonadotrophin secretion and the pituitary responsiveness to GnRH was studied. There were marked differences between Romney rams in the pattern of seasonal variation in testis size. Rams which had an early increase in testis size prior to the onset of the breeding season (the "early" group) had a greater magnitude of seasonal variation in testis size than those which had a late increase in testis size (the "late" group). The pattern of seasonal changes in testis diameter appeared to be repeatable from year to year. Differences between the groups in the pattern of seasonal variation in testis size were associated with group differences in endocrine function. Thus rams in the early group had a higher LH pulse frequency in March than those in the late group (4.4±0.4 vs. 1.7±0.3 pulses/8h, $P < 0.01$) and the seasonal increase in plasma FSH concentrations occurred earlier in the early group than in the late group. There were group differences in the pattern of seasonal variation in total LH response to the GnRH challenge.

The potential usefulness of testicular and endocrine parameters as predictors of genetic merit for date of onset of the breeding season was investigated in two progeny tests involving rams which had previously been studied for these parameters. The first progeny test involved rams of the Romney and Poll Dorset breeds. Results from this trial showed that, compared with straightbred Romney hoggets, a higher proportion of Poll Dorset cross hoggets reached puberty during the first breeding season (79.6 vs. 59.9%, $P < 0.05$). Poll Dorset cross hoggets also reached puberty earlier (13 May±3 vs. 22 May±3, $P < 0.10$) and at a younger age (264±3 vs. 276±3 days, $P < 0.10$) and had more oestrous cycles (2.7±0.2 vs. 2.0±0.2, $P < 0.05$) than Romney hoggets. Sire within breed had a significant ($P < 0.05$) effect on the number of pubertal oestrous cycles but not on the date of, or age at, onset of puberty. Liveweight at the beginning of the breeding season influenced both the proportion of hoggets reaching puberty during the first breeding season ($P < 0.01$) and the number of pubertal oestrous cycles ($P < 0.05$). There were significant effects of breed ($P < 0.001$) and sire within breed ($P < 0.05$) on the date of onset of the second breeding season. In the second progeny test, only rams of the Romney breed were studied. There was a significant ($P < 0.05$) sire effect on date of onset of the second breeding season but not on any of the measured pubertal oestrous parameters. While differences between Romney and Poll Dorset rams in the pattern of seasonal variation in testis size and gonadotrophin secretion were associated with breed differences in pubertal oestrous activity and date of onset of the breeding season, the within-breed correlations between the testicular and endocrine parameters in the rams and date of onset of the breeding season in their female offspring were low.

In conclusion, the present study has identified several physiological and endocrinological parameters in rams that might potentially be used as predictors of genetic merit for date of onset of the breeding season in ewes. Further, and more extensive, studies designed specifically to establish the genetic correlations between these potential parameters and date of onset of the breeding season are needed before these parameters can be incorporated into selection programmes. Finally, this study has also demonstrated that seasonality of rams is regulated by steroid-dependent and steroid-independent mechanisms similar to those operating in the ewe.

ACKNOWLEDGEMENTS

I want to express my sincere appreciation and gratefulness to my supervisors, Associate Professor M.F. McDonald, Professor S.N. McCutcheon and Dr. H.T. Blair, for their guidance and help during the experimental work and the preparation of this manuscript. It has been a great privilege to work with you and I certainly have benefited a great deal from those challenging and rewarding discussions that I had with you. Thank you very much for putting up with me for so long and for the encouragement extended to me when things seemed to have got out of control.

My special thanks are due to Professor S.N. McCutcheon for his great enthusiasm for my work and the tremendous help, both academic and personal, given to me during my stay in New Zealand.

I want to thank Mr D.L. Burnham, Ms C.M.C. Jenkinson, Ms J. Wickham, Mr M.L. Carter and the late Mr M.A. Wycherley for assistance with the field trials, which were often conducted under unpleasant weather conditions. Well done team!

To Miss M.F. Scott, Miss Y.H. Cottam, Miss F. Jackson and Mrs B.J. Purchas, thank you for your excellent technical assistance with blood sampling and laboratory analyses. Thanks are also due to Miss M.F. Scott for proof-reading the thesis.

Special thanks are due to Mr M. Hogan, Miss L. Watts, Mr T.G. Harvey, Mr W.B. Parlane, Mr K. Kilmister, Mr P.H. Whitehead and Mr L. Williams for providing and caring for the experimental animals and help with the experiments.

I want to express my appreciation to all those who volunteered to help with blood sampling: Jim Maas, Min Seok Hong, Yu Xian Sun, Muhammad Anwar, Guo Qiang Xing, Peter Walsh, Peter Morgan, Raymundo Rangel-Santos, Chris Roberts, Alfred Michel, Patrick Morel, Manoel Duarte and whomever else I may have forgotten to mention.

I want to thank Dr D.J. Garrick for advice on statistical analysis; Dr K.P. McNatty for help with setting up the LH and FSH assays; Dr K.R. Lapwood and Miss J. Candy for help with the testosterone analysis; and all the staff and postgraduate students in the

Department of Animal Science for friendship, support and understanding which made my stay in New Zealand an enjoyable and memorable one.

The materials used in the FSH and LH assays and the GnRH study were kindly donated by the National Hormone and Pituitary Program, and the National Institute of Diabetic and Digestive and Kidney Diseases, Bethesda, MD, USA.

I acknowledge the personal financial support received from the Education Commission of the People's Republic of China, the Macmillan Brown Agricultural Research Scholarship, and the New Zealand University Grants Committee Scholarship. This project was financially supported by the C. Alma Baker Trust and the Massey University Research Fund.

Finally, but most importantly, my very special thanks go to my wife, Jing, for her love and understanding, and for the many lonely days she suffered while I was completing this thesis overseas. I also want to specially thank my parents for their continued encouragement and support throughout my life, which played a very important part in my achievements so far.

TABLE OF CONTENTS

Page

Page

Chapter II: General materials and methods

Page

Chapter III: A preliminary study of the effects of exogenous melatonin, administered in early summer, on testis diameter of fleeceweight-selected and control rams

Chapter IV: Seasonal variation in testis size, gonadotrophin secretion and pituitary responsiveness to GnRH in rams of two breeds differing in time of onset of the breeding season

Page

Chapter V: Breed and sire effects on puberty and timing of the breeding season in sheep

Chapter VI: Differences in the pattern of seasonal variation in testis size, gonadotrophin secretion and pituitary responsiveness to GnRH in Romney rams and their relationships with oestrous activity in the rams' offspring

Page

LIST OF TABLES

Table **Page**

Appendix Tables

LIST OF FIGURES

Figure **Page**

Appendix Figure

LIST OF ABBREVIATIONS

The following abbreviations have been used in the text without prior definition:

Units:

°C	degree Celcius
h	hour
kg	kilogram
min	minute
mm	millimetre
ml	millilitre
ng	nanogram
μ	micron
μg	microgram
μl	microlitre

Hormones:

bTSH	bovine thyroid stimulating hormone
FSH	follicle stimulating hormone
GnRH	gonadotrophin releasing hormone
LH	luteinizing hormone
oFSH	ovine follicle stimulating hormone
oGH	ovine growth hormone
oLH	ovine luteinizing hormone
oPRL	ovine prolactin

Statistical:

SEM	standard error of the mean
NS	non-significant
+	$P < 0.10$
*	$P < 0.05$
**	$P < 0.01$
***	$P < 0.001$