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STUDIES OF NEUROENDOCRINE MECHANISMS INFLUENCING
SEASONAL VARIATIONS IN SEMEN PRODUCTION AND PLASMA
HORMONE LEVELS IN RAMS

A thesis presented in partial fulfilment
of the requirements for the
Degree of Doctor of Philosophy
at Massey University

GRAHAM KEITH BARRELL
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Abstract of a thesis presented in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

STUDIES OF NEUROENDOCRINE MECHANISMS INFLUENCING SEASONAL VARIATIONS IN SEMEN PRODUCTION AND PLASMA HORMONE LEVELS IN RAMS

by Graham Keith Barrell

Many workers have shown that sheep are seasonal breeders with peak reproductive activity occurring during the autumn months. The initial experiment in this thesis was designed to define the seasonality of reproduction in rams of the N.Z. Romney breed as determined by repeated measurements of semen characteristics and of plasma hormone levels. These parameters were studied for 16 months in six N.Z. Romney rams on pasture, with five Merino and four Polled Dorset rams included for comparison.

Semen from all three breeds showed relatively regular seasonal changes in ejaculate volumes and seminal fructose levels with peak values being recorded during March. Likewise, monthly hormone levels varied in a regular manner with plasma LH, testosterone and prolactin concentration being elevated during the summer months. Many of the other semen parameters measured showed little tendency for seasonal variations, however a change in semen collection technique, from predominantly artificial vagina to entirely electro-ejaculation, may have masked some seasonal changes. All three breeds showed similar seasonal changes in the parameters studied although semen from the Polled Dorsets did not exhibit regular seasonal variations in fructose levels.
Further experiments were carried out to define the neuroendocrine mechanisms which regulate the seasonal reproductive changes in N.Z. Romney rams. Three olfactory bulbectomized rams, three cranial cervical ganglionectomized rams and four rams which had undergone both of these surgical modifications, were grazed together with the rams mentioned above. These surgical treatments disrupted the regular seasonal changes in plasma levels of LH and prolactin, but not, of testosterone. Spermatozoal concentrations in ejaculates from operated rams were higher than those from unoperated controls, whereas mean fructose concentrations were lower; however the pattern of seasonal changes in seminal fructose levels was similar in all groups of rams. Cranial cervical ganglionectomy reduced hydroxyindole-O-methyl transferase activity and cell volumes in the pineal glands, so it was concluded that disrupted seasonal patterns of changes in plasma LH and prolactin levels, plus the altered semen production in the surgically treated rams, resulted from modified pineal gland and/or olfactory system activity.

A preliminary investigation into the role of changes in daily photoperiod as the stimulus for seasonality of reproduction, was carried out by placing rams in light-controlled rooms at the time of the March equinox. Over the following nine months rams exposed to a phase-reversed annual lighting cycle showed earlier elevations of seminal fructose and plasma testosterone levels than rams on either the normal annual or a constant equinoctial lighting regime. In all three groups plasma prolactin levels were directly related to the length of daily photoperiod.

The findings of the above experiments were extended by a final study in which both pinealectomized and sham-operated rams were exposed to normal or reversed annual lighting cycles.
Effects of lighting on plasma testosterone and prolactin levels, and on seminal fructose levels, were diminished by pinealectomy. Autopsy data related to gonadal and accessory sex gland function showed significant operations x lighting regimes interactions, which supported the conclusion that in rams pineal gland function mediates endocrine and gonadal responses to changes in daily photoperiod.

Three short-term investigations of hormonal secretion profiles conducted during the latter experiment, showed that major fluctuations in the release of LH, testosterone, prolactin and cortisol occurred irregularly during the day. A nocturnal elevation of plasma prolactin levels was abolished by pinealectomy. These acute studies tended to confirm the findings of the latter experiment, but in particular they highlighted the pulsatile nature of hormonal secretion.
I wish to express my gratitude for the helpful advice and generous assistance provided by my chief supervisor, Dr K.R. Lapwood, who also proposed the area of research covered in this thesis.

Further thanks are due to Dr R.M. Greenway for his role as supervisor and provider of encouragement, and to Prof. R.E. Munford for generously providing facilities in the Department of Physiology and Anatomy and for his major contribution to the statistical analyses of the experimental data.

The financial assistance of the Veterinary Research Fund of Massey University is gratefully acknowledged, and the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, U.S.A. is acknowledged for provision of reagents for hormone assays. Dr G.D. Niswender of Colorado State University, U.S.A. is acknowledged for supplying antisera to testosterone and ovine-LH, so also is Dr L.E. Reichert, Jr, Emory University, U.S.A. for donating the highly purified ovine prolactin and LH for radioimmunoassay, and Prof. D.S. Flux for donating antisera to bovine prolactin. Dr D.C. Thurley and staff at Wallaceville Animal Research Centre, Upper Hutt, are thanked for performing cortisol assays.

I am indebted to Mr M.J. Birtles for carrying out the histological processing and to Mr T.G. Law for the photographs in this thesis. The enthusiastic and unflagging technical assistance of Mrs H. Carter, Mr R.N. Ward and Mr R.H. Telfer is warmly appreciated, and I wish to thank Mrs D.P. de Wiele, Mrs H.E. Walker and Miss C. Howell for their contributions to the preparation of this manuscript.
Finally, I thank my wife Rosalind and daughters, Sarah and Rebecca, for their encouragement, support and perseverance whilst I was engaged in these studies.

In these studies, the candidate made the major contribution to the conception and execution of the experiments. The chief supervisor assisted with the experimental design and in the development of surgical techniques and hormone assays. Otherwise, apart from the execution of cortisol assays and histological processing performed by others, and the development of an ovine-LH radioimmunoassay where the candidate's contribution was minimal, all remaining work described in this thesis involved the full participation of the candidate.
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