THE ROLE OF PROLACTIN IN THE CONTROL OF OVINE LACTOGENESIS

A thesis presented in partial fulfilment

of the requirements for the degree of

Doctor of Philosophy

in Animal Science

at Massey University

Samuel Walter Peterson

1992

ABSTRACT

Peterson, S.W. (1992). *The role of prolactin in the control of ovine lactogenesis* . PhD thesis, Massey University, Palmerston North, New Zealand. 160pp

A series of trials was carried out to examine the role of prolactin (PRL) in the control of lactogenesis in New Zealand Romney x Border Leicester ewes. In addition, a study was made of differences in milk yields and plasma PRL concentrations between spring- and autumn-lambing ewes.

Daily subcutaneous injections of 2 mg CB154 inhibited PRL secretion and delayed lactogenesis. There were no consistent effects on plasma progesterone or insulin concentrations. CB154 treatment was more effective in reducing milk yield in twin-bearing than in single-bearing ewes when used for 20 days than for 9 days prepartum. The differential effects on milk yield cannot be explained by corresponding effects on plasma PRL or insulin concentrations. Circulating PRL during the period 20 to 10 days prepartum may have an important effect on milk yield in twin- but not single-bearing ewes.

Subcutaneous injections of 0.5 mg/kg live weight oPRL, administered on 2 consecutive days peripartum, to ewes treated with CB154 for 7 days prepartum, resulted in milk yields similar to those in control ewes and significantly ($P<0.01$) greater than those in ewes treated with CB154 alone. This indicated that oPRL prevented the CB154-induced reduction of milk yields and has established that the effect of CB154 on lactogenesis is mediated through suppression of PRL secretion and not by effects on some other hormone.

Injection of 10 mg oPRL directly into one mammary gland (via the teat duct) increased milk yields relative to the contralateral, bicarbonate-treated gland in CB154-treated ewes. The intramammary oPRL injection did not raise circulating PRL concentrations. Furthermore, the milk yields of bicarbonate-treated glands in ewes treated with bicarbonate only, did not differ from those of bicarbonate-treated glands in ewes treated with oPRL in the contralateral gland, demonstrating that there were no effects of oPRL, transferred via the circulation from the treated gland, on the contralateral gland. Glands treated with oPRL produced 15% ($P<0.05$) more milk than the bicarbonate-treated glands during the first 8 days of lactation and the difference was maintained throughout the 8-week lactation period, indicating that the oPRL had effected a permanent change in the ability of the gland to produce milk. It is concluded that PRL acts directly on the mammary gland without the need for a putative intermediate hormone, and that intramammary PRL concentrations during lactogenesis may have long-lasting effects on lactation.

The possibility was examined that dietary differences were responsible for seasonal differences in plasma PRL concentrations, milk yields, milk composition, lamb birthweight and lamb growth rate, observed in earlier trials. Mean plasma PRL levels were significantly ($P<0.01$) higher in spring- (192±38 ng/ml) than in autumn- (71±17 ng/ml) lambing ewes housed indoors under constant photoperiod (18L:6D) and fed the same diet. Milk yields were also significantly ($P<0.05$) higher in the spring- (2041±114 g/d) than in the autumn- (1563±109 g/d) lambing ewes over the 8 day lactation. Lamb growth rates (adjusted for birthweight, birthrank and sex of lamb) from birth to 8 weeks of age were significantly ($P<0.001$) higher in spring (282±12 g/d) than in autumn (225±15 g/d). The seasonal differences were confounded with corresponding differences in ewe live weight and it was not possible to determine whether dietary differences contributed significantly to the differences observed.

Two routes of oPRL supplementation were used to test the effectiveness of elevating peripheral or local levels of PRL in autumn-lambing ewes which, based on previous results, were expected to have low plasma PRL concentrations and milk yields relative to spring-lambing ewes. Administration of 10 mg supplementary oPRL directly into the gland or subcutaneous injection of 0.5 mg/kg oPRL did not increase the milk yields, or change the composition of milk, compared to controls. These results suggest that the circulating level of PRL, and the intramammary concentration of PRL, in autumn-lambing ewes are not limiting lactogenesis. Because the plasma prolactin concentration in the ewes was unexpectedly high, it was not possible to reach firm conclusions regarding possible effects of supplementary oPRL in ewes with naturally low plasma PRL concentrations. Nevertheless, the results indicate that raising the intramammary concentration of PRL around the time of parturition, in ewes with circulating PRL levels characteristic of normal spring-lambing ewes, does not enhance lactogenesis.

It is concluded that PRL is important to the complete initiation of lactogenesis in ewes, that it acts directly on the gland and that it is necessary for establishing the maximum potential of the gland to secrete milk.

ACKNOWLEDGEMENTS

I am extremely grateful to my supervisors, Associate Professor Duncan Mackenzie, Professor Stuart McCutcheon and Associate Professor Keith Lapwood for their expert assistance and guidance during this research programme. Their dedication to the task could not have been greater and the advice and encouragement they offered was invaluable.

I am equally grateful for the dedication, determination, patience and hard work of my milking team, Catriona Jenkinson, Yvette Cottam and Nicola Mason. The long hours, miserable conditions and the often trying behaviour of sheep were only tolerable due to the mutual support and good humour we shared. Thanks also to Nicky for mustering sheep.

Laboratory analyses were ably carried out by Janice Rumbal, Margaret Scott, Barbara Purchas, Fliss Jackson, Jane Candy and Jenny Mackenzie. Their perseverance in the face of large numbers of samples and assay development problems is much appreciated.

The willing assistance and co-operation of research-unit staff, Barry Parlane, Martin Chesterfield, Lynley Free, Mike Hogan, Tim Harvey, Annabel Whitton and Phil Whitehead is gratefully acknowledged.

A large team of graduate students, technical staff and other volunteers helped at various times with the more mundane tasks of cutting grass, feeding , cleaning and blood sampling. Thanks are due to Sean Beer, Dean Burnham, Mark Carter, Carolyn Clark, the late Merv Wycherly, Gabriele Leidecker, Sabine Muller, Jef Purchas (and Suzi), Beverley Thompson, Alvaro Wills, Jenny Wickham, Zhenzhong Xu and a succession of Dutch students, Ruud, Harrie, Bertus and Andries, who helped out for brief periods.

Thanks are due also to Dorian Garrick, Professor Al Rae and Associate Professor Hugh Blair for advice on statistical analyses, and to Jenny Edwards, Nasser Kaviani, Carlos Sosa and Hugo Varela-Alvarez for computing assistance. Other members of the Department of Animal Science were extremely helpful in discussions. Especial thanks to Steve Davis (DRC, Ruakura) for advice on mammary artery cannulation.

The generous donation of materials by Sandoz Pharma (Basle, Switzerland), Dr D.F.M van de Wiel ("Schoonoord" Research Institute for Animal Husbandry, Zeist, The Netherlands), the National Hormone and Pituitary Programme and the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, Maryland, USA) is gratefully acknowledged.

Thanks are due also for the secretarial assistance of Kathy Hamilton, Jill Jonasen, Judy Taylor, and Adele Walker in the preparation of funding applications, research reports and ethics applications.

The C. Alma Baker Trust, The Massey University Agricultural Research Foundation and The Massey University Research Fund provided financial support to the programme.

Finally, my love and thanks to Marjorie, my mother, and Catriona, my wife, who have encouraged and supported me during the many years of my formal education. This work is dedicated to them.

Ultimately, it is the pursuit of knowledge that has lead to this research programme. Often the search is tedious but I have found these two thoughts inspiring:

"There are things that are known and things that are unknown, and in between are the doors."
William Blake (1757-1827)

"If the doors of perception were cleansed, then all things would appear infinite."
Aldous Huxley (1894-1963)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

18L:6D	18 hours of light and 6 hours of darkness per day
BIC	sodium bicarbonate
bPL	bovine placental lactogen
bPRL	bovine prolactin
CB+BIC	group treated with CB154 plus bicarbonate
CB+PRL	group treated with CB154 plus ovine prolactin
CB154	2-bromo-α–ergocriptine mesylate
CB20	group treated with CB154 for a mean period of 20 days
CB9	group treated with CB154 for a mean period of 9 days
CIDR	controlled internal drug release (device)
Cl	chlorine
CV	coefficient of variation
cAMP	cyclic adenosine monophosphate
cDNA	complementary deoxyribonucleic acid
cRNA	complementary ribonucleic acid
DM	dry matter
d	day(s)
d.f.	degrees of freedom
E/S	group treated with subcutaneous ethanol/saline injections
EDTA	disodium ethylene diaminetetraacetic acid
GH	growth hormone
GRF	growth hormone-releasing factor
g	gram(s) or acceleration due to gravity
h	hour(s)
hGH	human growth hormone
hPL	human placental lactogen
IgG	immunoglobulin G
i.mam.	intramammary administration of oPRL via the intraductal route
i.u.	international units
i.v.	intravenous
K	potassium
kg	kilogram(s)
kPa	kilopascals
LPL	lipoprotein lipase
LWT	live weight
M	molar
ME	metabolisable energy

MJ	megajoules
mg	milligram(s)
min	minute(s)
ml	millilitre(s)
mRNA	messenger ribonucleic acid
Na	sodium
NIADDK	National Institute of Diabetes and Digestive and Kidney Diseases
ng	nanogram(s)
^{o}C	degrees Celsius
oGH	ovine growth hormone
oPL	ovine placental lactogen
oPRL	ovine prolactin
PGE2	prostaglandin $E_{2\alpha}$
PGF2α	prostaglandin $F_{2\alpha}$
PGFM	13,14-dihydro-15-keto-PGF, the main metabolite of $PGF_{2\alpha}$
PIF	prolactin release inhibiting factors(s)
PL	placental lactogen
PMSG	pregnant mares serum gonadotrophin
PRF	prolactin-releasing factor
PRL	prolactin
PRLsc	group treated with oPRL by subcutaneous injection
pg	picogram(s)
pH	hydrogen ion potential
RIA	radioimmunoassay
RPM	revolutions per minute
RRA	radioreceptorassay
r	correlation coefficient
rbST	recombinantly derived bovine somatotropin
rT_3	reverse triiodothyronine
SAM	S-adenosyl-methionine
SE	standard error of the mean
s.c.	subcutaneously
T_3	triiodothyronine
T_4	thyroxine
TSH	thyroid stimulating hormone
w/v	weight/volume